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#### Early Investigations of Scurvy and the Antiscorbutic Vitamin

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# Historical

Scurvy is an ancient disease and its occurrence has been recorded from time to time, doubtfully since the period of Hippocrates (B.C. 500), and certainly from that of the Crusades to that of the First World War. In its long history it differs from some other nutritional deficiency diseases, notably beriberi and pellagra which are of recent origin. Beriberi dates from the second half of the nineteenth century and has attacked rice-eaters only since the introduction of modern methods of milling which remove the protective vitamin in the outer coats of the grain. Pellagra is somewhat older, being first recognized in Europe in the early part of the eighteenth century, its appearance coinciding with the introduction and cultivation of maize as a staple cereal in diet.

It is probable that in mediaeval England in the late winter and spring a considerable proportion of the population suffered from mild scurvy, or were at least in a pre-scorbutic condition, when it is remembered that the winter diet consisted chiefly of beer, bread, dried peas and beans, salted meat and cheese, with little if any fresh vegetables or other foods. It was, however, among sea-faring men on voyages far from land and separated from all fresh foods for long periods at all seasons of the year, that scurvy became the common and dreaded menace. Its ravages, as recorded in the history of sea travel during the last 400 years, were truly terrible. Nevertheless through the long and tragic story there can be traced evidence pointing unmistakably to the cause of the disease in a lack of fresh fruit and vegetables in the diet and to the means for its prevention and cure. The correct interpretation of this evidence, set out so admirably in the review of previous literature given in Lind's famous treatise, was obvious only to the more astute observers and many were the erroneous causes and cures recommended by other authorities almost up to our own times. Thus Krebel (1866), writing in the middle of the nineteenth century, after reviewing the records of scurvy available from the sixteenth century to his own day, stresses above all other causes the influence of climate and of stormy and rainy weather, although admitting the contributory effect of unwholesome food and badly regulated work and bodily exercise. False conclusions as to the cause of scurvy continued until the present century and were responsible for tragic consequences.

The following examples are selected to show the extent of the ravages due to scurvy at sea and the measures found successful for its prevention and cure. Vol. 12

The explorer Jacques Cartier, when wintering in 1535 on the shore of Newfoundland after his voyage from St Malo, records that by the middle of February not ten of his 110 seamen had escaped the disease. It was, however, cured by a decoction of fresh leaves, supposed by some to be the tips of the spruce fir, prepared under instructions given by the Indian natives of the district (Lind, 1753, p. 257). Admiral Sir Richard Hawkins tells us 'in twentie years since that I have used the sea I dare take upon me to give accompt of ten thousand men consumed with this disease'. He considered that a change from sea air to land air was most important to effect a cure, but added 'That which I have seene most fruitfull for this sicknesse, is sower Oranges and Lemmons' (Hawkins, 1662). There is also the instructive story of four ships which sailed from England in 1600 to establish the East India Company, in three of which 105 men died of scurvy on the voyage to Bombay. In the fourth ship, that of the Commodore, the crew received a daily ration of preserved lemon juice; they remained in good health and on arrival at port did all the unloading, as they alone were fit for work (Budd, 1840, p. 58). Bachstrom (1734), in his justly prized pamphlet, after reviewing the available evidence on the causes of scurvy, stated his belief that 'From want of proper attention to the history of the 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'. These foods, described as 'antiscorbutic' are declared to be alone its effective cure. It is to Bachstrom that we owe the dramatic tale of the helpless 'sailor in the Greenland ships . . . . so over-run and disabled with the scurvy' that his comrades put him on shore to perish. He having 'quite lost the use of his limbs ..... 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 fully recovered; and, upon his returning home, it was found to have been the herb scurvy-grass' (translations given by Lind, 1753, pp. 409, 411). Scurvy-grass (Cochlearia officinalis), whose habitat is the 'muddy sea shore', is a plant of the Cruciferae, a natural order which contains many of our common green vegetables, including the cabbage and the swede turnip, both of which possess a high antiscorbutic potency. An old religious tradition ascribed the food value of these crucifers to the arrangement of their four petals in the form of a cross.

In the squadron commanded by Lord Anson, which sailed round the world in 1740-4, scurvy took a terrible toll. In the 3 months of the voyage, March to June 1741, from Cape Horn to the Islands of Juan Fernandez, more than half the men on the *Gloucester* and on the Commodore's ship the *Centurion*, had died from scurvy, so that on the latter 'out of two hundred and odd men which remained alive  $\ldots$ ,' when 'wearing the ship in the middle watch  $\ldots$  the Lieutenant could muster no more than two Quartermasters and six Fore-mast men capable of working' (Anson, 1748, p. 154).

The first recorded experiment on the cause and cure of scurvy is the famous human trial made by Lind during an outbreak on H.M.S. Salisbury to which he was attached as physician. To quote Lind's own description, '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 ..... 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 . . . . . 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 . . . . . .

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 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, 1753, p. 191).

To Bachstrom and Lind must be given credit for the correct recognition of scurvy as a nutritional deficiency disease and of the measures necessary for its prevention and cure, and to Captain James Cook is due credit for the successful demonstration of these measures in practice. During the 3 years at sea of his second voyage 'towards the South Pole and round the World', from 1772 to 1775, he lost only one man from sickness and that not from scurvy. He took every opportunity when touching land to obtain supplies of fresh vegetables and fruit. He believed in the virtues of celery and scurvy-grass and always had on board a good supply of Sauerkraut, which had been found beneficial in the Dutch navy. He took also a good provision of malt from which was brewed a 'sweet wort', served liberally to any man showing signs of scurvy; a 'rob' of oranges and lemons was also available (Cook, 1776). After this voyage Captain Cook was honoured with the award of the Copley Medal of the Royal Society, not, as might have been thought, for his navigational discoveries but, as stated by the President Sir John Pringle, for his success in maintaining so long a voyage without a death from scurvy among the men of his crew.

The ostensible purpose of this voyage, for which his ships were fitted out by the British Government, was to complete the discovery of the Southern Hemisphere, more especially of the continent believed to exist in the Antarctic regions. In this

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aim Cook did not succeed, but he concluded the account of this, his second voyage, in these words: 'But whatever may be the public judgment about other matters, it is with real satisfaction . . . . . that I can conclude this account with an observation, . . . . . that our having discovered the possibility of preserving health amongst a numerous ship's company, for such a length of time, in such varieties of climate, and amidst such continued hardships and fatigues, will make this voyage remarkable in the opinion of every benevolent person, when the disputes about a southern continent shall have ceased to engage the attention, and to divide the judgment of philosophers.' (Cook, 1777).

# Adoption of lemon juice as regular antiscorbutic in the British navy

After such an unequivocal result of his own experiment Lind devoted his energies to securing a regular issue of lemon juice in the Royal Navy, but it was not until 1795, after endless negotiations and the energetic representations of Sir Gilbert Blane and Dr Blair, the Commissioners for Relief of Sick and Wounded Seamen, that success was achieved (Blane, 1785, 1830).

Their arguments were reinforced by a recent experience on board H.M.S. Suffolk which in the previous year, 1794, had sailed from England to Madras. Each seaman had received 2/3 oz. lemon juice with 2 oz. sugar served daily in the grog. The voyage lasted 23 weeks with the loss of not a single man; the crew remained entirely free from scurvy (Budd, 1840, p. 58).

The scheduled allowance for the sailors in the Navy was fixed at 1 oz. lemon juice with  $1\frac{1}{2}$  oz. sugar, served daily after 2 weeks at sea, the lemon juice being often called 'lime juice' and our sailors 'lime juicers'. The consequences of this new regulation were startling and by the beginning of the nineteenth century scurvy may be said to have vanished from the British navy. In 1780 the admissions of scurvy cases to the Naval Hospital at Haslar were 1457; in the years from 1806 to 1810 they were two (Budd, 1840, p. 58).

It might be supposed that these happenings would mark the end of scurvy on sea voyages, but it was not so. Lessons were forgotten and mistakes were made continually. The fact, that the term 'lime-juice' was used indiscriminately for the Mediterranean lemon (*Citrus medica*, var. *limonum*) and for the West Indian lime (*Citrus medica*, var. *acidum*), led to tragic happenings of which the cause was only discovered many years later, when scurvy and antiscorbutics were being investigated experimentally.

# Experimental study of scurvy

The first experimental investigations of scurvy, with use of animals, are those of Holst and his colleagues on guinea-pig scurvy (Holst & Frölich, 1907, 1912). They were undertaken at the request of the Norwegian Government, concerned at the repeated occurrence of cases of so-called 'ship beriberi' in the Norwegian navy and mercantile marine. Holst & Frölich showed that withdrawal of their customary fresh green food from the diet of guinea-pigs and subsistence on a complete cereal ration, caused a fatal disease which was considered to be the analogue of human scurvy. The signs, which included loss of weight, loosening of teeth, haemorrhages in all parts of the body and severe bone lesions, closely resembled those of the human disease. In the course of their comprehensive research Holst & Frölich demonstrated that a daily ration of fresh cabbage, carrot, dandelion leaves, sorrel, cranberries or fresh fruit juices had a protective and curative action when added to the diet of oats, rye or rice. The antiscorbutic potency was found to be greatly reduced if the vegetables were dried, a fact that had been demonstrated under tragic circumstances in previous human experience by the impotence of dried herbs and vegetables to prevent or cure scurvy in armies in the field (Kramer, 1737; Smart, 1888). The effect of prolonged cooking processes in lowering the value of ordinary vegetables was shown, while the relative stability in sorrel and fruit juices was correctly attributed to their acid reaction. Holst & Frölich

further showed that the antiscorbutic principle was insoluble in alcohol unless acid-

ified, or in light petroleum, and that it passed freely through a dialysing membrane. During the First World War, at the instance of our army authorities, who needed guidance in dealing with scurvy outbreaks among our troops in the Middle East, the experimental study of scurvy was undertaken at the Lister Institute. The prime aim was to gain quantitative information about the distribution of the antiscorbutic principle or vitamin, as it was now recognized to be, among different vegetables and fruits, and to discover, if possible, some potent and stable source which would be suitable for transport. The work was pursued along the lines employed by Holst & Frölich and their results were abundantly confirmed. The basal diet of grain for the guinea-pigs was, however, modified by inclusion of autoclaved milk which, although deprived of antiscorbutic value, retained fatsoluble vitamins and contributed other important nutrients. The consequence was that the young animals were maintained in good health and continued to grow until the onset of scurvy, which could thus be studied, uncomplicated by the state of inanition which followed consumption of the unsupplemented ration of grains. By using several groups of animals receiving graded doses of the foods tested it was attempted to establish the minimum protective dose for each food, so that a rough comparison could be made of their relative antiscorbutic value. It was thus possible to establish the special value of cabbage and the swede turnip and to determine the extent to which the value was lost in drying by different methods or by cooking at different temperatures for different lengths of time.

Use was made of monkeys as well as guinea-pigs. Monkeys are very troublesome laboratory subjects but have certain advantages. The signs of scurvy resemble those in human beings even more closely in monkeys than in guinea-pigs. Their requirements are rather less than those of guinea-pigs so that they can be used to determine the value of weak antiscorbutic materials, such as milk, for which the necessary doses are too large to be taken by the smaller animal.

In the course of the trials at the Lister Institute it was shown that, weight for weight, raw cabbage leaves were about the equal of fresh lemon juice, the minimum daily protective doses for guinea-pigs being 1.5 g and from 1.5 to 2.5 g respectively.

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Raw swede juice was about as potent as raw cabbage,  $2 \cdot 5$  ml. being needed; of raw carrot juice about 20 ml. and of cooked potato juice 20 g were required, while 20 g of grapes and 20 ml. raw beet-root juice gave only slight protection (Chick & Rhodes, 1918). Raw cabbage leaves lost about 7% of their value when heated in water for 20 min at from 90° to 100°, but about 90% during 60 min at from 70° to 80°, showing that short periods of cooking at a higher temperature caused less destruction than longer times at a lower one. The temperature coefficient of the destructive process was low and calculated to be only about 3.0 for an increase in temperature of from 30° to 40° (Delf, 1918). This fact was opposed to the view that the loss on heating was due to heat-denaturation of a substance having the nature of a protein or enzyme, but was in accord with the observation of Holst & Frölich, that the antiscorbutic principle was a substance of relatively low molecular weight and could pass through a dialysing membrane. The effect of drying at a low temperature, 37°, on the antiscorbutic vitamin also was tested on cabbage leaves which, when dried and kept for from 2 to 3 weeks, were found to lose over

boiling water before being dried the residual value was greater. These experiments, confirming the previous work of Holst & Frölich, added fresh evidence to the experience of those who had found dried herbs and vegetables useless for prevention of human scurvy. Kramer (1720, 1737), early in the eighteenth century, when chief surgeon with the Austrian army in Hungary in the Turkish War and confronted with scurvy among his troops, received from Vienna in response to his appeals for help, a large and varied supply of dried 'antiscorbutic' herbs. They proved useless and thousands perished. The incident was in due course forgotten and is yet another example of the way in which many conclusive facts concerning the cause and cure of scurvy were continually set aside with tragic consequences. Lind records 'The latest proposal to the Lords of the Admiralty was a magazine of dried spinage prepared in the manner of hay. This was to be moistened and boiled in their food. To which it was objected by a very ingenious physician (Dr Cockburn), 'that no moisture whatever could restore the natural juices of the plant lost by evaporation, and, as he imagined, altered by a fermentation which they underwent in drying' (Lind, 1753, p. 184). As late as the First World War, the public were urged by well-meaning authorities to send dried and preserved vegetables to prisoners of war in German hands and a soup cube consisting of yeast extract issued officially as a beriberi preventive to our troops in the Middle East, was 'reinforced' with dried herbs in the expectation that it might prevent scurvy also.

93% of their original value (Delf & Skelton, 1918); if they were plunged into

Antiscorbutic value of germinated seeds. An important result obtained by Fürst in Holst's laboratory was the discovery that dry cereals or legumes (oats, barley, peas, beans, lentils), which have no antiscorbutic properties, develop them during germination (Fürst, 1912). The same fact had been anticipated more than 100 years earlier when in 1782 a 'Mr. Young of the Navy' recommended that, although we 'cannot have a kitchen garden at sea ..... beans and pease and barley and other feeds brought under the malting or vegetation process, are converted into the state

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of a growing plant, with the vital principle in full activity . . . . . and cannot fail to supply precisely what is wanted for the cure of scurvy' (Curtis, 1807, p. 41).

The observations of Fürst were extended and confirmed at the Lister Institute (Chick & Hume, 1917). After soaking in water for 24 h at room temperature and germinating for 48 h at room temperature, dry lentils and peas were found to possess an antiscorbutic value which, though less than that of citrus-fruit juice or of cabbage or swede turnip, equalled that of green (runner) beans or potatoes and was greater than that of carrot or beetroot (Chick & Delf, 1919).

In the dry state legumes are eminently suited for transport and can conveniently be converted to a fresh vegetable where and when needed. In the First World War germinated haricot beans were used successfully for cure of mild scurvy in Serbia by Wiltshire (1918) and at Archangel by Stevenson (1920).

The above facts supply a scientific basis for Captain Cook's conviction of the antiscorbutic value of his sweet wort, freshly brewed from the supplies of malt he always took on board his ships, and also for the esteemed value of other fermented liquors used by native tribes. The kaffir beer which is made from partly germinated sorghum and maize has been found an efficient preventive of scurvy among South African labourers (Delf, 1921).

Lime juice and lemon juice. In the First World War the reports of medical officers concerning outbreaks of scurvy among our troops in the Middle East stressed a belief that the preserved lime juice regularly served out was quite ineffective as a preventive. This view was confirmed by the experimental examination of army and navy official samples, in which no measurable antiscorbutic value was detected, and tests made on both guinea-pigs and monkeys of the juice expressed from fresh limes showed it to possess only about one-quarter of the potency of fresh lemon juice (Chick, Hume & Skelton, 1918). Lemon juice preserved in various ways was found to retain a considerable proportion of its original potency (Davey, 1921).

The suspicion that preserved lime juice was not an effective antiscorbutic was not new but had existed for many years, since an Arctic expedition, which in 1875 left England under Sir George Nares to discover the North Pole, had suffered grievously from scurvy within a few months, although well supplied with lime juice. The Admiralty commission appointed to inquire into the cause of the disaster arrived at no satisfactory conclusion. The problem was the more perplexing since a previous Arctic expedition dispatched in 1850 in the *Investigator*, under McClure, to search for Sir John Franklin, had enjoyed comparative immunity from scurvy in spite of great hardship during 2 years in Arctic waters. The clue to the discrepancy was discovered only 40 years later in a scrutiny of Admiralty records carried out by Smith (1918, 1919a,b). The earlier expedition was supplied with the preserved juice of Mediterranean lemons, the later one with that of West Indian limes. The change was made by the Admiralty about the year 1860, and it 'is from the date of the change from lemons to limes that a change begins in the historical evidence as to its value' (Smith, 1919a,b).

The growing distrust, during the latter part of the nineteenth century, of the

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value of lime juice as a preventive of scurvy paved the way for new theories as to its cause. Observations were put forward by some to show that the cause lay in bacterial infection and by others in poisoning by tainted meat. The latter theory was supported by the experience of the Jackson-Harmsworth Expedition to Franz-Josef Land in 1894-7. The sledging party carrying no lime juice, but eating large amounts of fresh bear meat, kept in good health, while a crew left with the ship and subsisting on canned and salted meat developed scurvy, although taking a daily ration of lime juice (Jackson & Harley, 1900a,b). The National Antarctic Expeditions in 1901 and 1910 were equipped and provisioned on the basis of the tainted meat theory and there can be little doubt that Captain Scott and his comrades were suffering from scurvy when overtaken by exhaustion and death. The experience of Augustine Courtauld, of the British Air-Route Expedition to Greenland in 1930-1, forms a striking contrast. He endured an isolated existence for 7 months on a diet which contained no vitamin C but was supplemented by a dessertspoonful daily of a carefully concentrated and preserved lemon juice. He remained in perfect health and, on his return to England, the sample of lemon juice was found to have retained its original antiscorbutic potency (Medical Research Council, 1932, p. 256).

# Infantile scurvy

Infantile scurvy may be considered a disease of modern times. It was unrecognized until artificial feeding became a common practice in the latter part of the nineteenth century. In the preparation of the baby's ration of cow's milk, modified in various ways, the food was usually heated, often for prolonged periods, to avoid the possibility of milk-borne infections. As the heat sterilization became more efficient, the incidence of infantile scurvy increased. The use of cereals and proprietary foods as a substitute for milk was often a contributory cause.

Breast-fed babies appear to be amply protected. Milk is, however, a weak antiscorbutic and, for children fed on heated or dried cow's milk, an extra source of vitamin C is indispensable. The use of orange juice is now the accepted rule, and scurvy in infants and young children, at least in western countries, is a thing of the past.

The disease was first described as a clinical entity by Cheadle (1878, 1879), and by Barlow (1883, 1894), and its nature disclosed. Its pathology was shown to be similar to that of scurvy and it was found to be amenable to the same preventive and curative treatment with fruit juices and green vegetables. It is, indeed, the manifestation of scurvy in a young growing organism, where the lesions in the developing bony tissues are more severe than in the adult skeleton. Infantile scurvy is an intensely painful complaint, often associated with rickets, and has serious consequences in stunted growth and delayed development (Chick & Dalyell, 1921).

Infantile scurvy, known in Germany as the *Barlowsche Krankheit*, was reported in babies fed on pasteurized milk during the early years of the present century from Berlin by Neumann (1902a,b) and Heubner (1903) and from New York by Hess & Fish (1914). Outbreaks have often occurred in times of war or other emergency, when the milk supply in cities has been interrupted and young infants have been nourished on cereal gruels, or when the scanty amount of milk available has of necessity been heated once or oftener to prevent souring during transit. Scurvy was a common occurrence in children's homes and hospitals in Vienna during and following the First World War. Among babies the cause was traced to scarcity of milk, combined with repeated heating of the small supply available; among older children to shortage of fresh fruit in the diet and to methods of prolonged cooking of the small ration of vegetables. In lack of oranges and lemons, swede juice was found to be a useful preventive for young infants (Chick & Dalyell, 1920, 1921).

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In these notes an attempt has been made to follow the study of the cause of scurvy from its early beginnings. They show the uncertainty inseparable from conclusions drawn from clinical evidence alone. They show further how the problem becomes more clear, the evidence more trustworthy and the investigation speeded up when adequately controlled experiment with a susceptible animal becomes possible.

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### The Discovery and Chemistry of Vitamin C

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This gathering, to sharpen and deepen our appreciation of a great Scottish scientist, is made especially significant by meeting within the halls of his Alma Mater. We, as guests of the University of Edinburgh, count it a privilege to share in paying tribute to a renowned alumnus. And in like spirit, we share the hope that sons in future years will honour both themselves and James Lind by seeing as clearly and labouring as diligently as did he (Lind, 1753).

As a biochemist who has had a measure of fellowship with other students of vitamin C in relation to human health, comparative physiology, and food processing, I could not escape (even if I wanted to) a sense of inspiration and deepened friendships from being here.

Other speakers will review the early literature on scurvy, but beyond the reach of manuscripts, one point is intriguing to a student of nature: did the loss of one or more genes that permit biological synthesis of the antiscorbutic vitamin, without disrupting other structures essential to survival, occur only once, or more often in nature? Assuming a common heritage of man and other surviving primates, can it be that we, among all the higher forms of animals and plants, share the single successful experiment with guinea-pigs, in the process of evolution?

From the records of primitive man's wide knowledge of the necessity of antiscorbutic foods (e.g. the American Indian, as reported by Cartier (Biggar, 1924)),