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History

Vitamine—vitamin. The early years of discovery

Louis Rosenfeld

In 1905, Cornelius Adrianus Pekelharing found that animals fed purified proteins, carbohydrates, fats, inorganic salts, and water would thrive only if small amounts of milk were added to the diet. He concluded that the milk contained some unrecognized substance that in very small quantities was necessary for normal growth and maintenance. In 1911, Casimir Funk isolated a concentrate from rice polishings that cured polyneuritis in pigeons. He named the concentrate "vitamine" because it appeared to be vital to life and because it was probably an amine. Although the concentrate and other "accessory food substances" were not amines, the name stuck, but the final "e" was dropped. In 1913 two groups discovered a "fat-soluble" accessory food substance. Initially believed to be a single vitamin, two separate factors were involved. One, effective against xerophthalmia, was named vitamin A; the other, effective against rickets, was named vitamin D. The factor that prevented scurvy was isolated in 1928. Known as "water-soluble C," it was renamed ascorbic acid.

INDEXING TERMS: accessory food substance • nutritional deficiency • indispensable dietary constituent

For many years, diseases had been vaguely known to result from some dietary deficiencies. Near the start of the 20th century, students of nutrition were investigating not deficiency diseases as such, but what were the components of a physiologically complete diet. They believed that a well-balanced diet need contain only a suitable amount of proteins, carbohydrates, fats, inorganic salts, and water. Advances in chemistry had made it possible to prepare a large number of these substances (proximate principles) as chemical compounds, and many investigations were undertaken to determine the quality and optimum amounts of these ingredients in an "average daily diet." There was a wide range from which to choose. However, animals fed these highly purified foodstuffs did not thrive or grow.

New York University Medical Center, Department of Pathology, 560 First Ave., New York, NY 10016.

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The earliest such study was by N. Lunin in Gustav von Bunge's (1844–1920) Laboratory in Basle (1881). He reported that young mice did not thrive on an artificial mixture of the purified components of milk (proteins, fats, carbohydrates, and salts) and consequently, that this synthetic milk diet lacked "unknown substances" without which life could not be sustained. This work was not followed up, attracted little attention, and was forgotten. Orthodox opinion preferred a simpler explanation for the nutritional failure of the experimental animals: The purified diets were so unpalatable and monotonous that loss of appetite, malnourishment, and death were inevitable.

In 1905, Cornelius Adrianus Pekelharing (1848–1922) of Utrecht carried out similar experiments with mice and purified foodstuffs, and got results similar to those of Lunin. If milk was given instead of water, the mice thrived upon the diet. He concluded that an unrecognized substance was present in milk, which, even in very small quantities, is important for nutrition, and without which the animal loses the ability to utilize the other components of its diet. The report, hidden in a Dutch medical journal, did not become widely known.

In 1884, efforts were being made to eliminate beriberi from the Japanese navy by giving the sailors increased amounts of meat, barley, and fruit. These dietary reforms were introduced by Surgeon General Kanehiro Takaki (1849–1915) and resulted in the eradication of beriberi from the navy. Clinical signs of beriberi primarily involve the nervous system, e.g., muscular atrophy and peripheral paralysis. Takaki correctly attributed the disease to a food deficiency, but mistakenly believed that sufficient amounts of protein prevented it.

Christiaan Eijkman (1858–1930; Fig. 1), a Dutch physician-physiologist, provided an impetus for further investigation working in the Dutch East Indies (Indonesia). In 1897, he discovered that the disease known as polyneuritis in animals and beriberi in humans could be induced in chickens and pigeons by a diet restricted to polished rice. The birds are unable to fly, walk, or even to stand. Cure and prevention was achieved by feeding them the unpolished rice or the rice polishings. For many years most medical authorities, influenced by the work of Pasteur, believed that a bacterium caused beriberi. Eijkman believed that the beriberi germ or toxin was in the polished

Fig. 1. Christiaan Eijkman.
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rice and was neutralized by "something" in the polishings.

In 1901, Gerrit Grijns (1865–1944; Fig. 2), Eijkman's assistant in Java, continued the studies. He was probably the first to have a clear conception of beriberi as a deficiency disease and to attempt to isolate the protective and curative component from foods. Grijns proposed that the disease was caused by a nutritional deficiency, or the lack of a specific natural substance found in certain foods.

Casimir Funk (1884–1967; Fig. 3), a chemist, regarded the Eijkman factor in beriberi as a definite organic chemical substance, one of several whose inclusion in trace amounts in an otherwise adequate diet was responsible for the cure or prevention of deficiency diseases such as beriberi, scurvy, rickets, and pellagra. In 1911 Funk isolated a pyrimidine-related concentrate from rice polishings that was curative for polyneuritis in pigeons *[1, 2]*. His concentrates were primarily nicotinic acid—not effective for beriberi but later shown to cure pellagra—contaminated with the antiberiberi factor *[3, 4]*. His analyses indicated that the concentrate contained nitrogen in a basic form and was probably an amine. Since it appeared to be vital to life, Funk named it "vitamine" *[5]*. Although they were not amines, the name stuck and has been applied to a whole series of substances found in foods, independent of their chemical structure.

In 1920, Jack Cecil Drummond (1891–1952; Fig. 4) suggested that, since there was no evidence to support Funk's original idea that these indispensable dietary constituents were amines, the final "e" be dropped, so that the resulting word vitamin would conform with the standard scheme of nomenclature, which permits "a neutral substance of undefined composition" to have a name ending in "in." Drummond also recommended that the "somewhat cumbrous nomenclature" then in use (fat-soluble A, water-soluble B, water-soluble C) be dropped, and the substances be referred to as vitamins A, B, C, etc., until their true nature was identified *[6]*.

Fig. 2. Gerrit Grijns.
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Hopkins and Accessory Food Factors

Frederick Gowland Hopkins (1861–1947; Fig. 5), the father of British biochemistry and a major contributor to bio-

Fig. 3. Casimir Funk.
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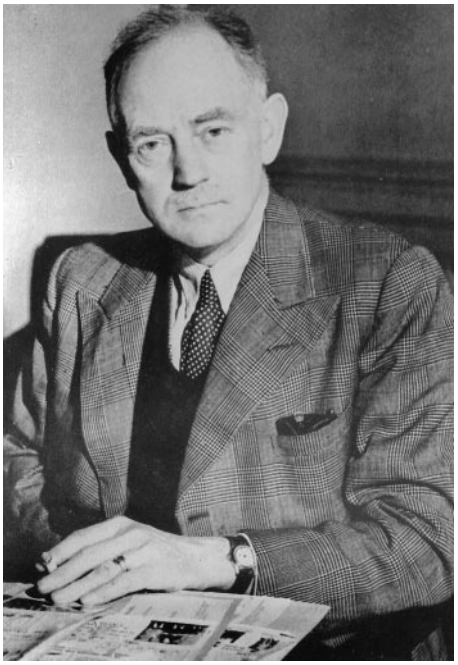

Fig. 4. Jack Cecil Drummond.
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chemical thought and to experimental biochemistry, firmly established the existence of vitamins. He opposed the vitalist thinking of many of his contemporaries. For him the nature of protoplasm was not mysterious but something accessible to the experimental approach. He entered Guy's Hospital Medical School at the age of 27 and distinguished himself in chemistry. After qualifying, he worked for several years in the medical school as a laboratory technician by day and as a clinical chemist in a privately owned clinical research laboratory in the evenings.

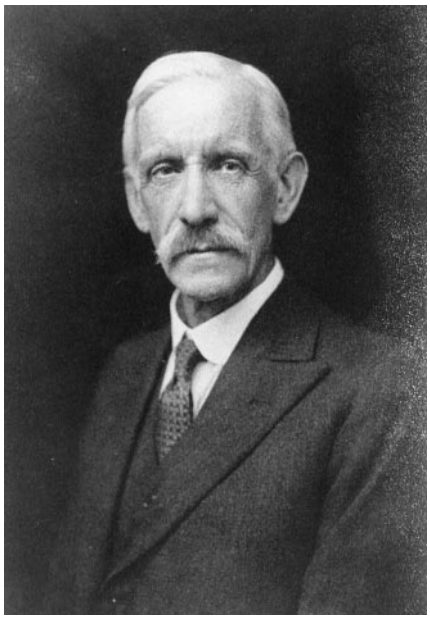

Fig. 5. Frederick Gowland Hopkins.
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As a result of his interest in uric acid, along with his early training and experience as an analyst, Hopkins developed a new and superior method for its determination in urine (1892) *[7]*. Although eventually superseded by colorimetric and other methods, Hopkins's procedure remained the most accurate and reliable for several decades. Together with Sidney W. Cole, Hopkins tracked down the glyoxylic acid impurity in the glacial acetic acid reagent responsible for the already well-known Adamkiewicz reaction of proteins. Consequently, they modified the reaction by replacing the acetic acid with a solution of glyoxylic acid. Their work led to the discovery and isolation of tryptophan (1901) *[8, 9]*, and to the finding that it was essential for growth. In 1921, Hopkins isolated and named glutathione, a tripeptide. He also discovered the enzyme xanthine oxidase.

In 1898, Hopkins joined the physiology staff at Cambridge University, but not until 1911, when he was almost 50 years of age, was he able to devote the greater part of his time to the development of biochemistry at the university and to his own research. In 1914, Hopkins became chairman and the first professor of biochemistry at Cambridge, and the new department became a magnet for biochemists. After Hopkins introduced biochemistry into the natural sciences curriculum at Cambridge in 1935, elementary courses in biochemistry became widespread in English universities.

In 1912, Hopkins published what is perhaps the best known of his works: "Feeding Experiments Illustrating The Importance of Accessory Factors In Normal Dietaries" *[10]*. He had been impressed by the conflicting results in the nutritional studies of other workers. Reasoning that there was more to an adequate diet than the types of amino acids in the protein, he concluded that normal food must contain some unknown component that was lacking in a basic synthetic diet made up of a mixture of purified protein (casein), carbohydrate (starch), and fats (lard), with mineral salts and water. For some unexplained reason, young rats fed on such diets failed to grow and even lost weight unless they were given small amounts of milk daily. Hopkins reasoned that milk contained "accessory food factors" that are required only in trace amounts but are indispensable for normal growth and maintenance.

Apparently there were physiological values in natural food products not indicated by the ordinary methods of chemical analysis and not included in total energy values that were absolutely essential to growth, maintenance, and general well-being. The chemical nature of these physiological values remained a mystery. Consequently, although Hopkins's paper, and Funk's review a few months earlier, focused attention on the "vitamine question," the very existence of vitamins continued to be in doubt.

Discovery of Vitamins A, D, and C

The effect of the small additions of milk observed by Lunin, Pekelharing, and Hopkins was soon recognized to be due to the action of more than one essential substance. Independent investigations in the US provided evidence for another growth factor. In 1913, Lafayette Benedict Mendel (1872–1935) of the Sheffield Scientific School (affiliated with Yale University) and Thomas Burr Osborne (1859–1929) of the Connecticut Agricultural Experiment Station in New Haven discovered a "fat-soluble" accessory food substance that was clearly distinct from the "water-soluble" factor revealed in the beriberi studies. Their finding resulted from the comparison of two diets of purified components fed to white rats. One diet contained dried whole milk and the other, dried skim milk. The substitution of butter for some of the lard in the "skim milk" diet prevented the loss of weight and eventual death of the rats and demonstrated that butter contains a trace amount of some fat-soluble organic substance that is essential in nutrition of this animal.

Unluckily for Osborne and Mendel, Elmer Verner McCollum (1879–1967) and Marguerite Davis at the University of Wisconsin reported a similar observation with rats fed "the ether extract of egg or of butter" 3 weeks before the Osborne and Mendel paper was received for publication *[11, 12]*. Both papers appeared in the same volume of the journal. McCollum and Davis were credited for the discovery of the first accessory food substance to be recognized as a vitamin, which they called "fat-soluble A." Both teams had shown by controlled animal experiments that certain fats contain a factor essential for nutrition, whereas others do not *[11, 12]*.

"Fat-soluble A" was first believed to be a single vitamin capable of curing xerophthalmia and rickets. Cod-liver oil was first used as a therapeutic agent in the 1770s. The beneficial effect of fish liver oils in the treatment of rickets, osteomalacia, generalized malnourishment, and certain eye conditions was widely recognized by the middle of the 19th century, but no satisfactory explanation accounted for its superiority over other edible fats. In 1922, McCollum et al. *[13]* showed that cod-liver oil aerated at the temperature of boiling water for 12 to 20 h retained its antirachitic activity in rats, but was ineffective against xerophthalmia. In addition, these properties were unequally distributed in certain foods. Apparently, two separate factors were involved. The factor effective against rickets later was named vitamin D. The Wisconsin workers found that when cod-liver oil is saponified, the vitamin remains in the nonsaponifiable fraction; therefore, it is a sterol.

Meanwhile, advances were being made in the study of scurvy, probably the first disease to be definitely associated with a food deficiency. Scurvy was common in northern Europe and for centuries was the scourge of sailors on long voyages when fresh food was not available. The symptoms of scurvy are weakness, anemia, pain in the joints, and hemorrhages from the mucous membranes of the mouth. The gums are particularly affected by swelling, redness, and ulceration. In 1753, James Lind (1716–1794), a British naval surgeon, wrote *Treatise on the Scurvy* and reported the effective use of orange and lemon juice in preventing scurvy in sailors and urged this as a standard part of the diet. In 1795, the government finally added lemon juice to the ration of the British sailor.

In 1907, two Norwegians, Holst and Frolich, produced a condition in guinea pigs comparable with human scurvy by feeding them a cereal diet and eliminating fresh animal and vegetable foods. The addition of the restricted foods to the diet cured the surviving animals *[14]*.

The name "water-soluble C" was initially proposed by Drummond *[15]* in 1919 for the antiscorbutic factor. Albert Szent-Gyorgyi (1893–1986) isolated this substance in 1928 during enzyme research and renamed it ascorbic acid. Szent-Gyorgyi received the Nobel Prize for physiology or medicine in 1937 for his discoveries with special reference to vitamin C.

What soon followed the work of Hopkins, McCollum and Davis, Osborne and Mendel, and others was a complete revolution in the science of nutrition. Largely through Mendel's work, nutrition was transformed from empiricism to a clearly recognized branch of biochemistry founded upon scientific principles. The American Institute of Nutrition was formed in 1933. As for Hopkins, he was knighted in 1925 and in 1929 shared the Nobel Prize in physiology or medicine with Eijkman "for their discovery of the growth-stimulating vitamins."

Year by year, additional factors were discovered and shown to be necessary for prevention of one kind of disorder or another in humans or animals. Several of these substances were also needed as growth factors by microorganisms. Synthesis in the laboratory provided a product identical in properties and physiological effect with the "natural" vitamin, and gave rise to a new growth industry of "nutritional supplements"—an idea subject to much criticism and controversy.

The claim is often made that healthy individuals eating a well-balanced diet do not need vitamin supplements. However, the public, increasingly aware of the benefits of vitamins as heralded by commercial advertisements and publicity in the news media, purchases these preparations in single and multiple combinations—often at levels far surpassing the recommended dietary allowance (RDA) for daily intake published by the National Academy of Sciences. Excessive use of some vitamins, which is more common in affluent societies, can cause vitamin imbalance. Vitamin poisoning may occur, especially involving overdose of vitamin A.

Federal agencies, reference laboratories, and industrial manufacturers are responsible for analyzing vitamin content of foods. Manufacturers are required to list on packages the vitamin content of processed foods, especially for A and C. Milk is fortified with vitamins A and D, and breads and other wheat products are enriched with the B-complex vitamins. Vitamin supplements are pre-

scribed for expectant mothers and often for elderly patients. Multivitamins are included in the total parenteral nutritional mix for patients unable to consume oral feedings. Although deficiency of a single vitamin is relatively uncommon in humans, it can occur as a result of an inborn error of metabolism or from an unusual restriction in dietary intake. More frequently, complex deficiencies may result from food fads or as complications from diseases affecting food absorption, as well as in nutritionally deficient areas of the world. Deficiencies may also arise from large losses of blood, from hemodialysis, after gastrointestinal surgery, as a consequence of the use of certain drugs, or following certain types of treatment such as radiation or chemotherapy.

Analysis of Vitamins

In 1926, Carr and Price *[16]* introduced the reaction of vitamin A with antimony trichloride in chloroform, in which the blue color produced soon reaches a maximum intensity and then rapidly fades or changes to other colors. Under carefully controlled conditions the blue color persists long enough to make accurate readings possible.

Chemical methods for determining vitamin C are based upon the reducing properties of the vitamin and include titration procedures with various oxidizing agents. In 1937, Roe introduced a color reaction with 2,4-dinitrophenylhydrazine to determine vitamin C. In 1943, Roe and Kuether *[17, 18]* further developed the method and applied it to analyses of blood, plasma, and urine. Vitamins A and C were the only ones commonly determined in the clinical chemistry laboratory. However, the infrequent number of requests for these tests make it convenient to refer them to the reference laboratories where vitamin A is analyzed by HPLC. Vitamin C continues to be analyzed by modifications of the method with 2,4-dinitrophenylhydrazine, but fluorometric and HPLC techniques have also been used.

The Reality of Vitamins

Paul Karrer (1889–1971), in his Nobel Prize lecture for chemistry in 1937 for his investigations on carotenoids, flavins, and vitamins A and B_2, stated that "scarcely ten years have elapsed since the time when many research scientists doubted the material specificity of the vitamins and were of the opinion that a special state of matter . . . was the cause of the peculiar vitamin effects which had been observed" *[19]*. Similar doubts had been expressed earlier in the discussion about what the enzymes "really are." These exchanges were part of the tug of war between mechanists and chemists that recurs on numerous occasions. The former see all physiological events as mechanical processes, whereas the latter explain all vital phenomena in essentially chemical terms. The controversy originates in the search for answers that would flow from one simple and universal concept and ends in the recognition that neither of the opposing ideas alone can provide the answer.

During the 1930s interest in vitamins grew, and chemical methods were sought to replace the very slow and laborious assays involving animals. When the determination of vitamin A was readily achieved by an ultraviolet measurement in the range of 320–330 nm, at least five photometers were developed specifically for this assay. They used line emission sources that were not applicable to the majority of ultraviolet analyses. In 1940 the two most popular spectrophotometers were made by Cenco and by Coleman. They used an incandescent tungsten source that barely reached the ultraviolet region. Scientists who wanted ultraviolet photoelectric instruments had to build their own.

In early 1940, Arnold Beckman and his colleagues recognized that the DC amplifier designed for the pH meter could also be used with vacuum-type phototubes. The company, whose major products were pH electrodes and meters, began a spectrophotometer development program that in 14 months resulted in the model DU Quartz Photoelectric Spectrophotometer *[20, 21]*.

The design of the DU was carefully thought out. A prism monochromator was selected in preference to a grating to minimize stray light. The instrument featured variable slits, a hydrogen lamp source for the ultraviolet, and an incandescent automotive headlight bulb (operated at reduced voltage for stability) for the visible region. Two phototubes were used, one for the ultraviolet, the other for the visible.

The introduction of the DU in 1941 ensured the end of absorptiometry by means of the spectrograph with its dependence on the tedious, inconvenient, and imprecise processing and measurement of photographic plates. Now for the first time an ultraviolet and visible absorption spectrum could be obtained with relatively inexpensive instrumentation and within a reasonable time, even though point-to-point readings were required. The DU greatly accelerated method research in the visible and ultraviolet range. The DU met a need and was an immediate success. It remained unsurpassed in its field for 35 years.

The contribution of industrial scientists to the development of clinical chemistry has been one of the characteristics of American science and may be traced to Arnold O. Beckman, the founder of Beckman Instruments (Fullerton, CA). Although the two instruments for which he is best known, the Model G pH meter and the DU spectrophotometer, were not designed specifically for clinical chemical applications, they subsequently led to widespread use in acid-base studies and photometric measurements of many kinds.

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