Original Communication

The Discovery of the Vitamins

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Abstract: The discovery of the vitamins was a major scientific achievement in our understanding of health and disease. In 1912, Casimir Funk originally coined the term “vitamine”. The major period of discovery began in the early nineteenth century and ended at the mid-twentieth century. The puzzle of each vitamin was solved through the work and contributions of epidemiologists, physicians, physiologists, and chemists. Rather than a mythical story of crowning scientific breakthroughs, the reality was a slow, stepwise progress that included setbacks, contradictions, refutations, and some chicanery. Research on the vitamins that are related to major deficiency syndromes began when the germ theory of disease was dominant and dogma held that only four nutritional factors were essential: proteins, carbohydrates, fats, and minerals. Clinicians soon recognized scurvy, beriberi, rickets, pellagra, and xerophthalmia as specific vitamin deficiencies, rather than diseases due to infections or toxins. Experimental physiology with animal models played a fundamental role in nutrition research and greatly shortened the period of human suffering from vitamin deficiencies. Ultimately it was the chemists who isolated the various vitamins, deduced their chemical structure, and developed methods for synthesis of vitamins. Our understanding of the vitamins continues to evolve from the initial period of discovery.

Key words: beriberi, discovery, pellagra, rickets, scurvy, vitamins, xerophthalmia

Nutrition at the beginning of experimental physiology

François Magendie (1783–1855), the pioneer of experimental physiology, laid the foundations for nutrition research in the early nineteenth century. Magendie conducted his research in Paris at a time when philanthropists were seeking ways to feed the poor. Chemists discovered that gelatin could be extracted from leftover bones. A possible solution to hunger was at hand: people of means could consume the meat, while the poor would receive a gelatin broth. However, the poor revolted against the unappetizing broth. The authorities appointed a committee, the Gelatin Commission, to evaluate gelatin.

Magendie advocated an experimentalist approach in which facts were established by direct observation. He raised two questions that paved the way for research on the vitamins and for nutrition in general: Are foods that do not contain nitrogen (i. e., proteins) nutritious? Is gelatin a complete source of protein in the diet? In 1816, Magendie conducted studies in which he fed dogs nothing but sugar, gum arabic, or other foods that did not contain nitrogen [1]. The dogs lost weight, developed corneal ulcers, and subsequently died, a condition that resembles what is now known as human vitamin A deficiency. Charles-Michel Billard (1800–1832) reported similar corneal ulcers in abandoned infants under his care in Paris and remarked that the eye lesions resembled those in Magendie’s malnourished dogs [2]. After ten years of research, the Gelatin Commission concluded that gelatin was not a complete food. Some surprises came in regard to previous assumptions about food. “As so often in research, unexpected results had contradicted every reasonable expectation,” reported Magendie, “Have we not above all made it evident that science is still in its first steps in every aspect of the theory of nutrition?” [3].

By the late nineteenth century, the prevailing dogma held that there were four essential elements of nutrition: proteins, carbohydrates, fats, and minerals. Most work focused on proteins and calories. Justus von Liebig (1803–1873), and Carl von Voit (1831–1908), Max Rubner (1854–1932), and Russell Chittenden (1856–1943) were among the most influential proponents of these ideas. Different proteins were considered of equal value in nutrition. Likewise, fats – whether from lard, butter, or cod-liver oil – were considered interchangeable in nutrition.

From the germ theory of disease to nutritional deficiencies

The concept that diseases are caused by infectious organisms or toxins produced by these organisms – that is, germ theory – became the reigning principle in science. Louis Pasteur (1822–1895) and Robert Koch (1843–1910) were influential proponents of the germ theory of disease. Investigations identified the organisms responsible for anthrax, malaria, tuberculosis, cholera, leprosy, and diphtheria. Other diseases such as scurvy, beriberi, rickets, and pellagra – considered by some to be infections – continued to baffle scientists.

In the Dutch East Indies, Christiaan Eijkman (1858–1930) observed that a polyneuritis, the equivalent of human beriberi, developed in chickens fed rice that had been polished of its bran [4]. Eijkman concluded incorrectly that the starch in polished rice carries a toxin, while the bran neutralizes the toxin [5]. Gerrit Grijns (1865–1944) continued the investigations of Eijkman but concluded that beriberi was caused by the lack of a vital component in the diet: "There occur in various natural foods, substances, which cannot be absent without serious injury to the peripheral nervous system." [6]. Grijns eventually convinced Eijkman that beriberi was caused by a nutritional deficiency. The contributions of Grijns were overlooked, most likely because he published his findings in Dutch.

The origins of the vitamin theory

In 1906, Frederick Gowland Hopkins (1861–1947) articulated what is now known as the "vitamin theory" during a speech given in London. Hopkins hinted at some dietary studies he had conducted that suggested: "…no animal can live upon a mixture of pure protein, fat, and carbohydrate, and even when the necessary inorganic material is carefully supplied the animal still cannot flourish." He continued: "Scurvy and rickets are conditions so severe that they force themselves upon our attention; but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend upon *unsuspected dietetic factors* [emphasis added]…" [7].

There were previous expressions of the "vitamin theory" prior to Hopkins. Nicolai Lunin (1853–1937) conducted studies with mice and concluded: "Mice can live quite well under these conditions when receiving suitable foods (e. g., milk), however, as the above experiments demonstrate that they are unable to live on proteins, fats, carbohydrates, salts, and water, it follows that *other substances indispensable for nutrition* [emphasis added] must be present in milk…" [8]. His mentor, Gustav von Bunge (1844–1920) reiterated in 1887: "Does milk contain, in addition to [protein], fat, and carbohydrates, other organic substances, which are also *indispensable to the maintenance of life?* [emphasis added]" [9]. A study by another von Bunge student, Carl A. Socin, demonstrated that there was an unknown substance in egg yolk that was essential to life, and he raised the question of whether this substance was fat-like in nature [10].

Perhaps the earliest articulation of the "vitamin theory" came from the French chemist, Jean Baptiste Dumas (1800–1884). During the Siege of Paris (1870–1871), many infants and toddlers died when the city was cut off from the milk supply of the countryside. Some opportunists tried to manufacture an artificial substitute for cows' milk, but this artificial milk failed to sustain the infants. Many children died. Dumas pointed out: [Since no] conscientious chemist can assert that the analysis of milk has made known all the products necessary for life… we must renounce, for the present, the pretension to make milk… it is therefore always prudent to abstain from pronouncing upon the identity of these *indefinite substances employed in the sustenance of life*, *in which the smallest and most insignificant traces of matter may prove to be not only efficacious, but even indispensable* [emphasis added]….The siege of Paris will have proved that we… must still leave to nurses the mission of producing milk" [11].

Further studies with milk

Wilhelm Stepp (1882–1964) conducted an important but overlooked study in which he mixed flour with milk and formed it into dough, which he called milk-bread; the dough supported growth in young mice. After the milk-bread was extracted with alcohol and ether, mice could not survive beyond three weeks on the extracted milk-bread. When the substance extracted with alcohol and ether was added back to the extracted milk-bread and fed to mice, they survived normally. Stepp concluded that a fat-soluble substance was essential for life [12], thus: "certain lipid substances present in milk, soluble in alcohol-ether, are indispensable for the survival of mice" [13].

In 1912, Hopkins showed that young rats did not grow well when fed a basal ration of protein, starch, cane sugar, lard, and minerals. After a small amount of milk was added to the basal ration, they had normal growth. The unknown factors in milk that supported life were found in "astonishingly small amounts" and were termed "accessory factors" by Hopkins [14]. Casimir Funk (1884–1967) proposed the term "vitamine" instead of "accessory food factors" in 1912 for the deficient substances in the food as related to beriberi, scurvy, and pellagra [15]. Soon these unknown factors in foods became synonymous with both "vitamine" and "accessory food factors".

New challenges to old dogmas

At Yale University, Thomas Osborne (1859–1929) refuted the half-century-old thesis of Liebig that four forms of plant protein – vegetable albumin, plant gelatin, legumin or casein, and plant fibrin – were identical to four animal proteins with similar names [16]. Osborne concluded that proteins of seeds are specific substances with distinctive amino acids, and even among closely allied species, seed proteins have pronounced differences. Another theory was soon overturned. German scientists believed that chemical analysis rather than actual feeding experiments could establish the nutritional value of foods. Edwin B. Hart (1874–1953) challenged this idea through what later became known as the "single grain ration experiment" conducted at the University of Wisconsin. Hart fed cows a diet of corn, wheat, oats, or a combination of the three, and according to the German theory, the cows should have fared the same. The results showed otherwise, as the corn-fed cows grew better and were much more healthy than the cows that were on wheat, oats, or a mixture [17].

Fats are not equivalent in supporting life

Another major dogma of the time was that all fats were equivalent in nutritional value.

This notion was challenged by Elmer McCollum (1879–1967) and his assistant Marguerite Davis (1887–1967) at the University of Wisconsin, who showed that young rats on a diet of casein, lard, lactose, starch, and salts grew normally if an ether extract from butter or egg yolk (ether mixed with either butter or egg yolk to chemically extract substances that were soluble in ether) was added to the diet. If a similar ether extract of lard or olive oil were added, the animals died. They concluded: "Our observation that ether extracts from certain sources improve the condition of animals on such rations, strongly supports the belief that there are certain accessory articles in certain food-stuffs which are essential for normal growth for extended periods" [18]. Osborne and his colleague Lafayette Mendel (1872–1935) reported that rats fed a basal diet of isolated proteins, starch, lard, and "protein-free" milk grew normally for about sixty days but then declined and died. The addition of butter or replacement of lard with butter in the diet allowed normal growth in young rats. They concluded: "In seeking for the 'essential' accessory factor we have, therefore, been led first to supply the cream component, in the form of butter…it would seem, therefore, as if a substance exerting a marked influence upon growth were present in butter…" [19]. In contrast to the prevailing dogma, the fat in butter or egg yolk was not equivalent in nutritional value to the fat in lard or olive oil.

Scientific misconduct by Elmer McCollum

In his later writings, McCollum claimed that he discovered vitamin A with his study in 1913, and that "this observation was promptly verified by Osborne and Mendel" [20, 21]. This version of events is often accepted in the hagiography that surrounds McCollum. However, McCollum's contention to have "discovered" vitamin A is based upon his observation that the unidentified factor was fat-soluble. Others had priority: Socin suggested that this unknown substance was fat-soluble in 1891 [10], and Stepp demonstrated that there was a factor that supported growth and concluded correctly that it was fat-soluble in 1911 [13]. In addition, the fat-soluble substance found in butter and egg yolk actually contained three vitamins: vitamins A, D, and E, which were yet unidentified in 1913.

In 1917, McCollum left the University of Wisconsin for a new position at the Johns Hopkins University. His departure was clouded by accusations of academic misconduct that were aired in public in the journal *Science.* McCollum stole the research notebooks of his colleagues at Wisconsin, including the notebooks of his perceived rival, Harry Steenbock (1886–1953) and subsequently published Steenbock's work in two articles in the *Journal of Biological Chemistry.* McCollum violated university policy by publishing without approval of the station head and dean, and he sabotaged their animal experiments by releasing all the animals from their cages [22].

McCollum later reflected upon the controversy of the purloined notebooks and unauthorized publications in his autobiography with the prevarication: "unfortunately I failed to say that they were published with permission of the Director, as was customary in experiment station bulletins." [21]. McCollum's account is directly contradicted by the "black book" diaries kept by Harry Russell, correspondence of Edwin Hart, Director of the Agricultural Experiment Station to Mendel, editor-in-chief of the *Journal of Biological Chemistry*, and a telegram from Russell to Steenbock in which he verified that he did not give McCollum permission to publish the papers [22].

Clinical observations of vitamin deficiencies

Masamichi Mori (1860–1932) made an early seminal description of vitamin A deficiency in fifteen hundred children in rural Japan. Cod liver oil proved to be an effective treatment for both the eye lesions and diarrhea. Contrary to the view of many physicians, Mori concluded that the disease was not infectious but rather was caused by the lack of fat in the diet. Joseph Goldberger (1874–1929) conducted rigorous epidemiological studies that showed pellagra was due to a defective diet rather than an unknown infection [26]. One of the most influential woman scientists in the early history of vitamins was Harriette Chick (1875–1977), a nutritionist at the Lister Institute. Chick served on the Accessory Food Factors Committee of Great Britain. She conducted important clinical investigations of rickets in Vienna following World War I at a time when many still believed that rickets was an infectious disease [27].

The importance of animal models

Fundamental insights into vitamin deficiencies came through studies with specific animal models: pigeons and chickens for beriberi, mice and rats for vitamin A deficiency, guinea pigs for scurvy, and dogs for rickets and pellagra. Most animals are able to synthesize vitamin C and cannot serve as models for vitamin C deficiency. In 1907, two Norwegian scientists at the University of Cristiania (Oslo), bacteriologist Axel Holst and pediatrician Theodor Frölich, developed an animal model that enabled a breakthrough in the study of scurvy [28, 29]. Edward Mellanby (1884–1955) made important steps towards the identification of vitamin D when he showed that a fat-soluble substance was responsible for rickets in studies conducted with dogs [30–32].

Isolation, description of structures, and synthesis of vitamins

Barend C. P. Jansen (1884–1962) and Willem F. Donath (1889–1957) crystallized thiamin in 1926 [33]. Robert Williams (1886–1965) described the chemical structure of thiamin and its synthesis in 1936 [34,35]. Albert Szent-Györgi (1893–1986) isolated a substance "hexuronic acid" [36], that was later confirmed as vitamin C by Charles Glen King (1896–1988) [37] and Szent-Györgi [38]. Norman Haworth (1883–1950) described the chemical structure of vitamin C and its synthesis in 1933 [39]. In 1936, Adolf Windaus (1876–1959) described the structure of both vitamin D_2 and, cholecalciferol, or vitamin D_3 [40, 41]. Harry Holmes and Ruth Corbet crystallized vitamin A in 1937 [42]. Conrad Elvehjem isolated nicotinamide from liver concentrates and used it to cure blacktongue in dogs, thus showing that niacin was the "anti-pellagra vitamin" [43]. Otto Isler synthesized vitamin A in 1947 [44].

The importance of basic research

The period of discovery of the vitamins paved the way for the development of dietary allowances, fortification of foods with vitamins, vitamin supplementation, and wider recognition of nutritional deficiency disorders. This succinct review has dealt with the five vitamins involved in scurvy, beriberi, rickets, pellagra, and xerophthalmia. Further details regarding the discovery of each of the numerous vitamins and associated Nobel Prizes has recently been presented in a special issue [45].

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