Chapter 5

The Long, Rocky Road to Understanding Vitamins

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. The animal body is adjusted to live either upon plant tissues or the tissues of other animals, and these contain countless substances other than the proteins, carbohydrates, and fats. ... 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. ... I can do no more than hint at these matters, but I can assert that later developments of the science of dietetics will deal with factors highly complex and at present unknown. 

From F. G. Hopkins, The analyst and the medical man, 1906.

When popular columnist Anna Steese Richardson published advice to expectant mothers in 1916, her counsel reflected the entrenched nutritional dogma of her day. ‘Stoke the engine of your body with the right sort of coal’, she wrote, ‘Keep it clear of cinders and clinkers, cleanse it with pure water, renew the worn parts with rest... What is the right kind of coal? Food-stuffs classified according to their chemical properties. ... water, mineral matter, proteins, carbohydrates, and fats’ [1]. From the late-nineteenth century well into twentieth, proteins, carbohydrates, fats, and minerals were believed to be the food components essential to build and sustain human life. The limitations of that dogma were beginning to come to light, but scientific understanding of food was far from complete. Knowledge of what constitutes adequate nutrition was still only partial, in effect, a puzzle with pieces scattered around the globe and some not yet even suspected.

Certainly, the understanding of food and nutrition lagged far behind the other life sciences, and by no means had it taken the great leaps made in other areas of human health. Unlike the other sciences, comprehension of the vitamins and their role in maintaining health would not be arrived at by great breakthroughs, but, rather, through small, incremental steps, with some conjectures, mistaken conclusions, disappointments, and refutations along the way.

Ultimately, it was laboratory scientists experimenting with animals, not physicians attempting to heal human patients suffering the effects of vitamin deficiencies, who succeeded in identifying and fitting together the pieces of the nutrition puzzle. Recognition of the vitamins and how they function in sustaining life and health was
to come mainly through laboratory investigation using animal subjects. With animals, experimenters could control and manipulate conditions, when necessary making their subjects sick in ways that could not be done with humans. Most nutritional scientists conducted experiments with mice or rats, which were cheaper to acquire and maintain than larger animals and faster to show results. The small rodents could be had in large numbers at modest cost, had less variation among individuals, and enabled investigators to duplicate experiments to test the validity of results [2]. But even the breeding of rats for use as experimentation, which was essential in the study of vitamins, did not begin until after the turn of the twentieth century.

Moving Beyond Old Assumptions and Around New Certainties

The health-related science conducted in the decades before and just after 1900 was in many ways exceptionally fruitful. Studies in microbiology and the development of the germ theory, the work of Louis Pasteur in Paris and Robert Koch in Berlin, enabled the identification of the bacteria that cause anthrax, puerperal fever, cholera, plague, tuberculosis, leprosy, and many other diseases (textbox 5–1). Infectious microbes and toxins became widely implicated in the causation of disease, and antiseptic techniques were being developed. But certain common diseases, of which night blindness was but one, seemed not to conform to the germ theory. Although undeniably a revolutionary advance in many aspects of health care, the germ theory also created something of an obstacle to understanding vitamins and the ills caused by their lack.

Textbox 5–1. Right and wrong applications of the germ theory

During the 1854 London cholera epidemic, the physician John Snow (1813–1858) linked the cholera deaths to the use of contaminated water from the Broad Street pump in Soho. Snow persuaded the authorities to remove the handle from the Broad Street pump and was therefore credited with having averted further deaths [3]. But Snow’s understanding of the spread of cholera was ahead of its time, as what that of physician William Budd, who believed the disease was caused by a specific living organism found in the gastrointestinal tract and spread from person-to-person through contaminated drinking water [4]. Both physicians were defying the prevailing notion of the day. The spread of diseases such as plague, measles, cholera, and malaria was attributed to miasmas rising from decaying organic matter.

The concept that diseases are caused by infectious organisms or toxins produced by these organisms – that is, germ theory – supplanted the attribution of diseases to bad air by the late nineteenth century and became the reigning principle in pathology and public health. Physicians soon demonstrated, too, that diseases were spread by contagion, either by direct person-to-person contact or by mutual
contact with an intervening object or body (i.e. a vector). The French chemist Louis Pasteur (1822–1895) and the German physician-bacteriologist Robert Koch (1843–1910) further elaborated the germ theory of disease.

Germ theory gained acceptance in the late-nineteenth century and enabled the identification of the organisms responsible for anthrax [5], malaria [6], tuberculosis [7], cholera [8], leprosy [9], and diphtheria [10]. Given the productivity in determining the etiology of such diseases, the same line of inquiry into infectious diseases was often but mistakenly applied to the baffling diseases that in the early twentieth century were finally discovered to be caused by vitamin deficiencies. Such dead ends included the ideas that an intestinal infection caused scurvy, an unknown contagious disease caused rickets, and poor hygiene and sanitation facilitated an infectious disease that resulted in pellagra.

The growth of nutritional science in particular was stunted by accepted but insufficient theories. Many scientists in nutrition carried out their research within the given framework of food’s having four basic components, and focused in particular on proteins and calories. Leaders in this work included chemist Justus von Liebig at the University of Giessen, physiologist Carl von Voit at the University of Munich, physiologist Max Rubner at the University of Berlin, and biochemist Russell Chittenden at Yale University.

As with assembling anything that is still fragmentary, some pieces of the nutrition puzzle appeared to fit but in fact did not. Liebig, for example, held that, besides red cells, the two principal components of the blood are the nitrogenous substances, albumin and fibrin [11]. When a person eats meat, Liebig contended, her or his digestive juices convert the nitrogen-bearing proteins into albumins, which, in turn, are absorbed as components of blood; the albumen and fibrin become muscle. (He acknowledged that plant foods also contain nitrogenous substances such as fibrin, albumin, and casein, and these too are absorbed and integrated into tissues much as are those in meat.) When muscles exert force, according to Liebig, muscle tissues are consumed and broken down into urea, carbonic acid, and water. Thus, muscles consume themselves during exercise, but the proteins absorbed from foods constantly replace them. During the day, according to this theory, muscle tissues break down; they are reconstituted at night during sleep. Liebig assigned to starch, sugar, and other non-nitrogenous food-borne compounds the role of supporting respiration. The only true nutrients, Liebig held – that is, substances that warrant designation as essential components of food – are the nitrogenous substances that can form or replace active tissue. Simply stated, Liebig’s dogma identified proteins as the only source of muscular energy and physical activity as the only way to break down these substances. Liebig presented these ideas in 1842 in ‘Animal Chemistry, or Organic Chemistry in Its Applications to Physiology and Pathology’ [12].
Many scientists, but not all, accepted Liebig’s clever scheme as a great step ahead in understanding nutrition. But one critic was quick to point out that the theory was inconsistent with real life, in which sedentary businessmen lived on meat-rich diets, while hardworking laborers carried out their physical toils while living on high-carbohydrate, protein-poor diets [13].

Looking back in the context of Liebig’s theory at the night blindness suffered by sailors and soldiers (chapter 1, 4), the generally meat-rich military rations should have sufficed to render the men fit to carry out their duties, but clearly did not. The prevailing dietary dogma prevented physicians and scientific investigators from suspecting that night blindness was somehow related to a lack of something essential in servicemen’s diets, and even though nutritional research was gaining prominence, a theory of vitamins that would account for the problematic missing elements was not even on the horizon. The notion that disease could result from dietary deficiencies also ran counter to both the prevailing ideas regarding the nature of food and the infection/toxin theory of disease. At the same time, however, clinical observations and scientific experimentation had begun to suggest that certain diseases did not originate with germs or toxins but with food.

The most widely known of these illnesses – though none was yet recognized as caused by a vitamin deficiency – were scurvy, beriberi, rickets, and pellagra. By the turn of the twentieth century, the clinical features of these diseases were well recognized, and to some degree, so were the circumstances under which they developed. The dietary changes that could cure or prevent scurvy and beriberi were increasingly adopted. The dietary deficiencies that caused them – vitamin C in the case of scurvy, thiamin in the case of beriberi, vitamin D in the case of rickets, and niacin in the case of pellagra – had yet to be determined.

The clinical picture of what resulted from vitamin A deficiency, in contrast, was more incomplete and fragmentary. It consisted mainly of descriptions of night blindness among sailors, soldiers, and children in orphanages, and, in some instances, awareness that diarrhea and dysentery could be associated with night blindness. Bitot’s spots might be noted, also corneal ulceration, keratomalacia, and, ultimately, total blindness. And death rates among adults and children who presented these symptoms were extremely high. The only cures known, however, seemed to be ingestion of liver or cod liver oil, which was an empirical remedy familiar since antiquity – that is, it was known to work, but not how. The cabinet tenebreux also seemed to work in treating night blindness, but it was difficult to determine if a patient had truly been cured or was just tired of being shut up in dark closet for days on end. Any other remedy for night blindness, much less its cause, remained persistently elusive.

Characterizing vitamin A, from the initial glimmerings of its existence to its eventual isolation, purification and synthesis, was a long, much-interrupted process that, overall, took more than one hundred and thirty years. The 1816 dietary experiments of François Magendie in Paris, in which the investigator purposefully produced corneal ulceration in dogs by nearly starving them, yielded an early hint. In the next
generation, Charles Billard, attending severely undernourished infant orphans with corneal ulcers, recognized the relevance of Magendie’s observations and suspected a link between inadequate diet and corneal ulcers. But more than a half-century passed after Magendie and Billard’s time before significant progress was made toward a theory of vitamins – specifically, important steps made in a laboratory in northern Europe’s Baltic region.

Finally, studies were begun that forecast the intense, fast-paced period during which the vitamins finally came to be identified, characterized, and synthesized. Nicolai Ivanovich Lunin, a doctoral candidate studying chemistry in the laboratory of Gustav von Bunge (1844–1910) at the University of Dorpat in Estonia, showed in 1881 that adult mice could live in good health on milk. The mice in Lunin’s experiments did not survive on a diet consisting of the milk components caseinogens (milk proteins), milk-fat, milk-sugar, and the ash of milk (i.e. proteins, fats, carbohydrates, salts, and water). In publishing his results, Lunin stated that, ‘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 must be present in milk’ [emphasis added] besides caseinogens, fat, lactose, and salts’ [14]. Lunin is sometimes considered the first scientist to hypothesize that some uncharacterized substances essential for life were present in milk. French chemist Jean-Baptiste Dumas had made a somewhat similar observation during the Siege of Paris in 1871. It was von Bunge, however, and not Lunin (who had gone on to practice pediatrics), who pressed the matter.

Von Bunge reiterated the question in an influential textbook published in 1887: ‘Does milk contain, in addition to (protein), fat, and carbohydrates, other organic substances, which are also indispensable to the maintenance of life?’ [15]. A study by another von Bunge student, Carl A. Socin, of the different forms of iron in the diet, showed that mice fed only egg yolk (a rich source of vitamin A and iron), lived for nearly one hundred days, while mice fed an iron-poor diet with or without other forms of iron died within one month. 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 [16]. Although von Bunge remarked, ‘It will be useful to continue these investigations’, neither he nor his students explored this promising new territory much further; the professor’s main interest was the study of inorganic elements in nutrition.

At roughly the same time in England, Frederick Gowland Hopkins (1861–1947) played a key role in becoming one of the early scientists to experiment with feeding animals isolated food components. In time, Hopkins would attain the status of giant in the field of biochemistry and become a Nobel laureate. Originally trained as a chemical analyst, Hopkins began his professional life as a scientific toxicologist and built a reputation as an expert witness in poisoning homicides. He conducted chemical analyses for such celebrated cases as the trials of Adelaide Bartlett (husband
died of chloroform poisoning), Florence Maybrick (husband died of arsenic poisoning), and Israel Lipski (neighbor died of nitric acid poisoning). Changing course quite early in his career, Hopkins went to the University of London and studied medicine at Guy's Hospital. At age thirty-seven, he was invited by the physiologist Michael Foster to teach and develop chemical physiology at Cambridge University.

Hopkins was interested in the chemistry of proteins, and at age forty in 1901 he isolated the amino acid, tryptophan (Glossary) [17]. The isolation of tryptophan was an extraordinary early achievement, made especially remarkable by the fact that, as Hopkins noted, he '...went to Cambridge without any training as a specialized bio-chemist' [18]. Unlike many colleagues of his generation, he had never spent time as a visitor in a German scientist's lab nor, for that matter, at the side of any master. Undeterred by his unconventional professional beginnings, Hopkins voiced objection to the idea, derived from classical cytology, that protoplasm was the living substance of cells. Many of Hopkins's colleagues believed that molecules of food and oxygen enter this mysterious complex, lose their identity there, and then emerge as recognizable substances such as urea and carbon dioxide. But taking a biochemist's view, Hopkins regarded the notion of protoplasm much as Pasteur and the other microbiologists regarded the obscure concept of spontaneous generation (i.e. the concept that life can arise from inanimate matter). Both protoplasm and spontaneous generation became increasingly improbable. Accordingly, Hopkins saw no reason why the chemical reactions inside a cell itself should be any different from chemical reactions observed in the laboratory [19]. He was also skeptical of the prevailing view that the food value of diets was based upon their energy and nitrogen values. ‘Recent advances in physiology’, he declared, ‘seem to justify a fresh attack upon this subject (following) somewhat different lines’ [19].

With colleague Edith Willcock, Hopkins fed carbohydrates, fats, and minerals to mice, thus demonstrating that tryptophan was an 'essential' amino acid. (The so-called ‘essential’ amino acids that are indispensable for life are those that the body cannot synthesize.) The only source of protein in the experimental diet was zein, the plant protein derived from maize that contains no tryptophan. Hopkins and Willcock's mice died unless tryptophan was added to the diet. Their study was the first to show that a specific amino acid was necessary, i.e. 'essential', in nutrition and led the way to identification of other essential amino acids [20].

A month before the 1906 publication of the Hopkins-Willcock amino acid findings, Hopkins, as Examiner in Pharmacology and Therapeutics at the Institute of Chemistry, spoke before the Society of Public Analysts at Burlington House in London. His lecture, titled ‘The Analyst and the Medical Man’, asserted that, 'the doctor and the analyst share between them... almost the entire burden of the maintenance of public health.' He then turned to the subject of dietetics, ‘...in which the medical man is the recognised authority, charged with instruction of the public, but for a scientific knowledge of which he depends largely on the chemical physiologist and the analyst'. He acknowledged that, as 'public analysts' – i.e. scientists – the members of the
society played a role in protecting against the adulteration of foods and maintaining a safe food supply. He then presented new findings about the composition of food itself – facts that were ‘. . . less known and seemingly academic. I believe, however, that my theme, which is that the influence of minimal qualitative variations in dietaries, will one day become recognized as of great practical importance’.

Seeking an example, Hopkins reviewed the tryptophan study, in which he had found that ‘a group in the protein molecule may serve some purpose in the body other than that of forming tissue or supplying energy’. Future analysts would be asked to do more than measure the total protein of a particular food; they would have to undertake the more difficult task of what Hopkins referred to as ‘discriminative analysis’. His studies had led him to conclude that, ‘. . . 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. The animal body is adjusted to live either upon plant tissues or the tissues of other animals, and these contain countless substances other than the proteins, carbohydrates, and fats’.

From there, he looked at the broad subject of diet and some of the widely known but not yet understood deficiencies:

Physiological evolution, I believe, has made some of these well-nigh as essential as are the basal constituents of the diet. . . The field is almost unexplored; only is it certain that there are many minor factors in all diets of which the body takes account. . . In diseases such as rickets, and particularly in scurvy, we have had for long years knowledge of a dietetic factor; but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure. They are, however, certainly of the kind which comprises these minimal qualitative factors that I am considering. . . 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. . . I can do no more than hint at these matters, but I can assert that later developments of the science of dietetics will deal with factors highly complex and at present unknown [21].

The research underlying much of this address remained unpublished for a matter of years, during which illness and a severe injury caused a hiatus in Hopkins’s career. Moreover, even as he recovered, the teaching responsibilities that came with his appointment as Science Tutor dominated his time. Shortly before he reached fifty, however, Hopkins was made a Fellow of Trinity College and Praelector in Biochemistry, a position once held by his mentor, Michael Foster. This post carried no teaching obligations, so he could finally work in this biochemistry laboratory without interruption.

In a critically important experiment carried out during this period, Hopkins observed that young rats grew poorly when fed a basal ration of protein, starch, cane sugar, lard, and minerals. With the addition of just a little milk to their diets, however, the animals grew normally. Hopkins published these remarkable findings in 1912 in the *Journal of Physiology* [22]. The unknown factors in milk that supported life were present in ‘astonishingly small amounts’. A graph included in the paper shows the comparative growth patterns (fig. 5.1). After eighteen days of the experiment,
Hopkins switched his subjects’ diets so that the rats receiving the basal diet plus milk now received no supplementary milk; their growth subsequently stopped within about thirty days. Those receiving the basal diet alone began growing poorly within the first eighteen days, but after receiving milk each day starting on day eighteen, they grew rapidly. This study marks a critical early advance in the identification of a class of what Hopkins called food’s ‘accessory factors’.

Connecting the ‘Accessory Factors’ and the Vitamin Deficiency Diseases

Hopkins's work added significantly to the early evidence of the existence of vitamins and would eventually receive national and international recognition. But it was by no means specific or definitive. Nor did Hopkins establish whether there were many of these vital substances in food or just one. The biochemical nature of his ‘accessory factors’ and how they figure in human health was far from being determined. Efforts to understand a handful of familiar, debilitating diseases led investigators on paths toward the elusive substances in food that, when missing, could cause such trouble.

Beriberi

In the Netherlands, work parallel with Hopkins’s was in progress in pursuit of a specific objective: how to prevent or cure beriberi. A result of thiamin deficiency, beriberi causes numbness or tingling in the extremities, difficulty walking sometimes to the point of leg paralysis, sometimes heart failure, and often death. In the late-nineteen century, beriberi was especially common in the Dutch East Indies, hence

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**Fig. 5.1.** Growth curves of mice in experiments of Frederick Gowland Hopkins (1912) [22]. Lower curve shows growth of 8 male rats with the basal diet, indicated by open circles (○). At day 18 (vertical broken line), the 8 rats were given 3 ml of milk per day, indicated by dark circles (●). Upper curve shows the growth of 8 similar rats receiving the basal diet plus milk. At day 18, these 8 rats were given the basal diet only with no additional milk.
a major concern for the Dutch government, which ruled a large portion of the area. The Dutch government formed a commission to study beriberi and appointed bacteriologist Cornelius Pekelharing (1848–1922) as its director. On the way to the East Indies, the commission stopped at Robert Koch's bacteriology laboratory in Berlin. The Dutch physician, Christiaan Eijkman (1858–1930), on sick leave after a severe episode of malaria, was augmenting his medical training in Koch's lab while recuperating. Pekelharing was impressed with Eijkman's talents and arranged for the younger physician to join the beriberi project.

The team’s initial investigations – carried out in two rooms in a military hospital in Batavia (today's Jakarta) – involved autopsies of beriberi victims. The work included attempts to find suspected disease-causing bacteria in the cadavers’ blood. Blood from beriberi victims was injected into various animals to determine whether the transferred blood would cause the experimental subjects to develop the disease. In the course of these studies, Eijkman observed that a polyneuritis, the equivalent of human beriberi, developed in chickens – specifically, in chickens fed grains of rice that had been polished of their bran sheathing ('silverskin') [23]. Eijkman concluded – incorrectly, but consistent with the dominant germ/toxin theories of disease the time – that the starch in polished rice carries a toxin, while the silverskin neutralizes the toxin or prevents its formation [24]. Only later did further findings from the Dutch East Indies prompt Eijkman to change his view on the causation of beriberi.

The beriberi problem, however, was not finally solved until a third, considerably younger, Dutch investigator, Gerrit Grijns (1865–1944), came to work in Java. Grijns continued the investigations begun by Pekelharing and Eijkman, but with an open mind. Grijns concluded – correctly – that beriberi was caused not by the presence of something pernicious but by the absence of something essential: the experimental chickens’ diet lacked a vital component (textbox 5–2). Furthermore, Grijns observed – again, correctly – that the deficiency caused neurological damage. Publishing his findings in 1901, he wrote, 'There occur in various natural foods, substances, which cannot be absent without serious injury to the peripheral nervous system' [25]. Going further, Grijns observed that mung beans were potent in curing polyneuritis in chickens.

Textbox 5–2. Beriberi and ‘water-soluble B’

As with night blindness and vitamin A, the correlation between beriberi and thiamin took many years to piece together. Nearly a half century passed between Eijkman’s initial observations in Indonesia of a possible cause of beriberi and the identification and characterization of thiamin, a B complex vitamin – known during part of the interval by Elmer McCollum’s term, ‘water-soluble B.’ In 1916, McCollum and a graduate student, Cornelia Kennedy (1881–1969), worked with pigeons to study polyneuritis (the avian form of beriberi); this disease was caused by lack of ‘water-soluble B’ [26].
In 1902, another Dutch colleague, Dirk Johann Hulshoff-Pol, conducted a controlled trial with patients in a mental hospital in Java. With some three hundred patients housed in twelve pavilions, Hulshoff-Pol tested three different dietary interventions along with their regular polished rice diet. The patients were divided into four groups, which were subdivided for housing into three pavilions and maintained as follows:

- The regular rice diet only (the control group),
- The regular rice diet plus 300 grams per day of green vegetables,
- The regular rice diet plus 150 grams per day of mung beans, and
- The regular rice diet only, plus periodic disinfection

After nine months, none of the patients who had received mung beans developed beriberi, but 33% of the control group and 42% of the added-green-vegetables and disinfection groups did develop the disease. The addition of regular disinfectant fumigations was to test whether the disease was actually carried by cockroaches. The results showed that cockroaches were not to blame. The addition of mung beans to the diet effectively prevented beriberi [30].

Other dietary human trials were soon to follow in southeast Asia, including a notable experiment in 1909 conducted with two road crews in a remote rainforest on the Malay Peninsula [31]. Laborers eating white rice developed beriberi; those who ate brown rice did not. The relationship between new cases of beriberi and the type of rice consumed was consistent when the diets of two road crews were switched.

**Rickets**

Rickets, a common condition in children in Northern Europe and northern North America, was another disease that challenged the infection/toxin theory. A result of abnormally low mineralization and mechanical strength in developing bones, rickets is caused by lack of vitamin D; vitamin D is found in oily fish, in egg yolk, and, like vitamin A, in cod liver oil. Deformities such as bowlegs and knock-knees in the weight-bearing long bones are characteristic of rickets in children. Vitamin D is also generated in the skin through direct exposure to sunlight. Thus, both a deficient diet and lack of exposure to sunlight can contribute to rickets.

Observations made in a London zoo suggested that rickets might be caused by a dietary deficiency. In 1889, John Bland-Sutton, a surgeon at Middlesex Hospital and
prosector at London's Regents Park Zoological Garden, reported on studies of rickets in monkeys, lions, and other animals [32]. The lion cubs had the classic bone deformities of rickets. Before an effective treatment was found, the misshapen cubs were removed from the exhibits and kept out of public view. Bland-Sutton surmised at first that the rickets was caused by a deficiency of fat in the diet. He recommended that, in addition to their usual diet of lean meat, the lion cubs be fed cod liver oil and crushed bones. The results were dramatic: the prescribed diet cured the rickets.

Bland-Sutton's findings were consistent with the empirical observations of the many pediatricians who for some time had been using cod liver oil to treat rickets in children [33]. In the early 1880s, Max Kassowitz, a pediatrician in Vienna, had introduced cod liver oil and phosphorus called ‘Kassowitz formula’ for the treatment of rickets in children [34].

**Scurvy**

Although long known to be preventable by including lemons and other specific foods in the diet, scurvy remained an enigma from a scientific standpoint – i.e. that it resulted from vitamin C deficiency – well into the twentieth century. A host of symptoms can be associated with scurvy, including softening of gums and loss of teeth, hemorrhages in the skin, weakness, and lower extremity edema. 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 [35]. Holst had studied with Koch in Berlin and visited the Grijns laboratory in Batavia to gain insights from the beriberi studies in progress there. When he returned to Oslo, however, Holst was frustrated in his attempts to produce scurvy in chickens and pigeons, and he decided to work with Frölich using guinea pigs as experimental subjects.

The guinea pig was a fortuitous choice, since guinea pigs, like humans (and unlike dogs, rats, mice, and birds), require vitamin C as an essential nutrient. The use of the guinea pig was a departure from the usual practice in German and French laboratories of using dogs for feeding studies. However, the guinea pig was known as a children's pet in the early 1900s, and its space and food requirements were minimal compared with dogs' [36]. Holst and Frölich showed that guinea pigs fed grain, groats (the hulled grains of cereals), and bread developed scurvy, but those for which fresh cabbage or fresh potatoes were also provided did not. After reviewing the epidemiological and clinical data on scurvy in humans and their own experimental results, Holst and Frölich concluded: ‘...epidemiological facts speak in favor of the opinion that the described disease in guinea-pigs is identical with human scurvy’.

**Night Blindness**

The piecemeal clinical picture of night blindness caused by of vitamin A deficiency (see previous chapters) finally came together between 1896 and 1904, when Japanese physician Masamichi Mori described more than fifteen hundred children with hikan
– that is, xerophthalmia [37]. Mori had studied medicine at the Mie Prefectural Medical School and the Tokyo University and gone on to work in Germany and Switzerland before returning to Mie Prefecture to practice surgery. The children Mori described had night blindness, Bitot’s spots, corneal ulceration, keratomalacia, and diarrhea. The death rate among them was high. Most were between ages one and four and one-half, and many came from poor families living in mountainous regions, where the diet completely lacked milk and fish. Once under medical care, the children were given cod liver oil daily, and this 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.

**Pellagra**
Pellagra, a disease characterized by skin lesions, diarrhea, wasting, neurologic and psychiatric disturbances, and high mortality, was known in parts of Europe. Pellagra is caused by a lack of niacin in the diet. Foods that are rich in niacin include red meat, liver, fish, poultry, legumes and cheese. The appearance of pellagra coincided with the cultivation of maize, and it became a common problem in southern and western France by the early nineteenth century. Persons afflicted with pellagra were mostly poor peasants who ate primarily corn, since they could not afford a more diverse diet. Physicians advanced several theories to explain the causation of pellagra, including the consumption of too much maize, an intoxication caused by eating moldy maize, and, following the doctrines of Liebig, that corn lacked sufficient protein [38]. Although the exact cause of pellagra was not identified at the time, the physician Théophile Roussel advocated changes such as increased animal husbandry and substitution of other cereal crops for corn that eventually led to the disappearance of pellagra from France [39]. By the time pellagra was largely forgotten in Europe, it made a new appearance among poor sharecroppers in the southern United States early in the twentieth century (detailed below).

**Finding an Elusive Panacea in Milk**
Polished rice and beriberi cured with whole grains in southeast Asia, rickety London lions and Japanese babies with dry, ulcerated eyes cured with cod liver oil, Norwegian guinea pigs cured of scurvy with cabbage – all these findings became familiar to European and American life scientists in the early 1900s (table 5.1). The dogma of only four essential food components was losing its grip on investigators interested in nutrition and health. The infection/toxin theory of disease, too, was beginning to seem inadequate to explain all illnesses. Nutrition scientists were warming up for the race to characterize the elusive food components that Hopkins grouped as ‘accessory factors’. Wilhelm Stepp in Strasbourg, Thomas Osborne and Lafayette Mendel at Yale, and Elmer McCollum (ultimately) at Johns Hopkins University, Marguerite Davis, and Harry Steenbock at the University of Wisconsin – all of them went to work
feeding isolated food substances to assorted animals in hope of identifying what, for a while, came to be called ‘vitamines’ (textbox 5–3).

**Textbox 5–3. A family name for the ‘accessory factors’**

With scientists in far-flung academic centers on both sides of the Atlantic closing in on F. G. Hopkins’s ‘accessory factors,’ the still-elusive quarry needed a collective name. In 1912, Polish-born biochemist Casimir Funk proposed ‘vitamine,’ from the Latin *vita* for life and ‘amine,’ referring to the presumed chemical nature of the substances [40]. (Amines are a class of nitrogen-containing organic compounds derived from ammonia [NH₃]; the term provides the basis for the phrase ‘amino acid’). McCollum had suggested a nomenclature of ‘fat-soluble A’ and ‘water-soluble B.’ In 1920, Jack Drummond, a biochemist and vitamin researcher at University College, London, proposed the term be shortened to vitamin. From the strict standpoint of chemistry, not all the ‘vitamines’ were chemical amines. Drummond also suggested dropping McCollum’s designation of ‘fat-soluble A’ and ‘water-soluble B.’ Instead, he recommended, ‘the substances should be spoken of as vitamin A, B, C, etc. This simplified scheme should be quite sufficient until such time as the factors as isolated, and their true nature identified’ [41]. The new nomenclature, when Drummond proposed it and ever since, has been widely adopted in the scientific community and beyond.

### Table 5.1. Observations of deficiency diseases by 1910

<table>
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<tr>
<th>Disease</th>
<th>Characteristics</th>
<th>Clinical observations</th>
<th>Animal observations</th>
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<tbody>
<tr>
<td>Scurvy</td>
<td>bleeding gums</td>
<td>cured or prevented with lemons, oranges, onions, green leafy vegetables</td>
<td>experimental scurvy produced in guinea pigs; disease prevented by adding cabbage to diet</td>
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<td></td>
<td>hemorrhages in skin</td>
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<td></td>
<td>high mortality</td>
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<tr>
<td>Beriberi</td>
<td>numbness, tingling in extremities</td>
<td>cured or prevented with milk, brown (unpolished) rice, mung beans</td>
<td>experimental beriberi produced in chickens and pigeons (avian polyneuritis); disease prevented by adding brown rice to diet</td>
</tr>
<tr>
<td></td>
<td>difficulty walking</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>high mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rickets</td>
<td>bone deformities</td>
<td>associated with bad hygienic conditions and lack of exercise</td>
<td>rickets in lion cubs in London zoo cured with cod liver oil</td>
</tr>
<tr>
<td>Xerophthalmia</td>
<td>Bitot's spots</td>
<td>cured or prevented with cod liver oil</td>
<td>conjunctivitis and corneal ulceration in rats fed a limited diet</td>
</tr>
<tr>
<td></td>
<td>corneal ulceration</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>blindness</td>
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<td></td>
<td>high mortality</td>
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</tbody>
</table>
A young medical graduate working in chemistry in Germany, Wilhelm Stepp (1882–1964), conducted studies that suggested the existence of fat-soluble substances essential for life. He mixed flour with milk and formed it into dough – he called it milk-bread – and fed it to experimental baby mice, which grew normally to adulthood. But when Stepp extracted any fats present in his milk-bread using alcohol and ether and fed the resulting paste to mice, the animals could not survive beyond three weeks. When the extracted substance was added back to the fat-free paste, the mice survived normally. Stepp’s conclusion, published in 1909: there is some fat-soluble substance that is essential for survival [42]. In further studies published two years later, Stepp noted that ‘certain lipid substances present in milk (that are) soluble in alcohol-ether, are indispensable for the survival of mice’ [43]. After these valuable findings, Stepp turned his attention to the practice of medicine.

As a new generation of life scientists was reaching maturity, the center of biochemical research was undergoing a geographic shift, from Europe to the United States. Thus, one of the most influential early associations in biochemistry was housed not in France, Germany, or Great Britain but in the United States. Starting in 1909, Thomas Osborne (1859–1929) and Lafayette Mendel (1872–1935) – one from a socially prominent New England family, the other born to German Jewish immigrants in New York State – had a long, highly productive scientific collaboration in New Haven, Connecticut. Their work together yielded more than one hundred published papers.

Osborne had a long association with Yale, where he did his undergraduate and graduate studies and worked as an instructor. Osborne soon joined the staff of the Connecticut Agricultural Experiment Station, which was located about a mile north of the Yale campus. He initiated a project to investigate the proteins in plant seeds. His early work refuted a half-century-old thesis of Justus von 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 [44]. To analyze the proteins of different seeds, Osborne applied chemical techniques to determine the seeds’ amino acid composition. His conclusion: the proteins of seeds are specific substances with distinguishing amino acids, and even among closely allied species, seed proteins have pronounced differences.

Osborne’s next question: how influential are the different proteins in nutrition? Osborne would assess the relative nutritive properties of proteins in partnership with his friend, Lafayette Mendel. Mendel’s lab was housed in the Sheffield-Town mansion, an Italianate structure with towers and cupolas, in which the old drawing room served as a lecture hall and the art gallery a large, somewhat makeshift laboratory. Here Mendel, a devoted and demanding teacher, supervised graduate students. They, in turn, kept their experimental animals in various rooms (fig. 5.2). In time, the laboratory sprawled into the mansion’s tower and crannies in the basement [45].

Osborne and Mendel conducted feeding experiments with rats at the Connecticut Agricultural Experimental Station to determine the nutritive value of purified
proteins. After some initial frustrations, including a fire that destroyed their lab in 1910, they developed for their rats a basal diet of isolated food substances consisting of casein, sugar, starch, lard, and minerals. With these elements combined, this diet served quite well to support life in rats, and it enabled the investigators to study and compare the effects of adding various seed proteins. Initial experiments revealed difficulty in maintaining adult rats for longer than about two to three months either on the basal diet or on diets to which seed proteins were added. Powder made from dried milk produced the distinctive results of maintaining the health of young rats, but when the milk powder was replaced with casein and mineral salts, the rats died [46]. The team then developed protein-free milk by removing the casein and other proteins, filtering the resulting solution, and drying it. This protein-free milk powder became an important component of their initial studies, which they summarized in the 1911 monograph, ‘Feeding Experiments with Isolated Food-Substances’ [2]. They continued their studies, acknowledging in 1912, ‘. . .with respect to the actual requirements of fat on the part of the healthy organisms there is at present almost no definite information available. Fats are, of course, commonly found present in greater or lesser abundance in every dietary; but to what extent they represent an indispensable need of the animal remains to be learned’ [47].
This gap was soon to be filled not only by work in Osborne and Mendel’s own laboratory but also from the lab of Elmer McCollum (1879–1967). A native of Kansas, where he did his undergraduate work, McCollum, too, pursued the PhD at Yale, where he studied organic chemistry. After completing his doctoral thesis in 1906 on the chemistry of the pyrimidines, McCollum paid a call on the man he described as ‘the outstanding teacher and investigator in physiological chemistry in America,’ Lafayette Mendel [48]. As no academic positions were open, Mendel invited McCollum to spend the next academic year as a post-doctoral student in his laboratory. McCollum made the most of that year, attending lectures in biochemistry and learning new analytic methods. At the end of McCollum’s post-doc year, Mendel arranged for his protégé to work in the Department of Agricultural Chemistry at the University of Wisconsin. McCollum’s new mentor would be biochemist Edwin B. Hart (1874–1953).

At the time, the generally accepted method for analyzing food, devised by German scientists, was based strictly on chemical analysis of the food. Hart and his colleagues were skeptical of this approach. Instead, they sought to evaluate the foods by actually assessing how the foods affected animal growth. Hart’s main project consisted of testing on cows single-item versus multiple-item diets, specifically, either maize, wheat, or oats, or a mixture of the three. According the German system of food analysis, these four diets were chemically equivalent. McCollum’s first assignment was to record the cows’ food intake and then analyze their milk and urine. In his second year in Hart’s ‘single grain ration experiment’ McCollum was joined in his duties by Harry Steenbock (1886–1967).

Like McCollum a farm boy, Harry Steenbock had grown up in Wisconsin, where he did his undergraduate work at the Wisconsin College of Agriculture in Madison. Before graduating in 1908, Steenbock was one of just two students to attend McCollum’s first lectures at the University of Wisconsin on the biochemical aspects of nutrition. McCollum and Steenbock spent many hours preparing large quantities of different feeds for Hart’s cows. The different diets produced dramatically different results, McCollum recalled decades later in his autobiography. ‘The wheat-fed cows were small of girth and rough-coated. They were all blind, as shown by the lead color of the eyes and by their inability to find their way about. . . (T)he corn-fed cows were, by standards of animal husbandry, in excellent condition’ [49].

During this time, McCollum followed developments in animal and plant chemistry in Europe by reading the ‘Yearbook on the Progress of Animal Chemistry’ (Jahres-Bericht über die Fortschritte der Tier-Chemie). Thus, he knew of the experiments of Hopkins, Lunin, Socin, Stepp, and Pekelharing, which involved feeding different diets to mice or rats to determine what constituted the simplest diet. This gave McCollum the idea of experimenting with rats instead of cows [50]. With his own six dollars, he purchased a dozen albino rats and started a breeding colony in 1908. His colleagues at the agricultural experiment station in Wisconsin frowned on the notion of raising rats, which were universally considered pests. But McCollum persisted. McCollum’s early attempts to feed rats isolated and purified food substances were, in his words, ‘of a bungling
nature', because he had difficulty ascertaining the purity of the isolated dietary components [51]. At first, he thought the rats failed to thrive because the rations were not palatable [52]. But the care and feeding of McCollum's experimental rats began to change with the arrival of Marguerite Davis. Born in Racine, Davis had gone to California for her undergraduate work, but after graduating, she returned to Wisconsin. In July 1909, Davis presented herself at McCollum's lab and requested to study biochemistry there. McCollum was relieved to turn over to Davis the management of the rat experiments, as he was mostly occupied with Prof. Hart's single-grain-ration cow studies.

Hart's project ended, inconclusively, in 1911 [53]. Why the corn diet was so superior to other diets for the cows' health and growth remained unexplained. But to continue further work with cows, given their breeding pattern and lifespan, would probably have taken another four years. While Hart's experiment was winding down, McCollum's other main associate, Steenbock, also ceased to figure importantly in McCollum's experimental work. Steenbock left Wisconsin temporarily to seek further training in New Haven and Berlin. Only later, in 1914, would Steenbock return to Madison to complete his doctoral studies.

Although McCollum continued with Davis to pursue his investigations of isolated food substances in rats, his only publications in 1911 and 1912 were related to nutritional studies conducted with Hart and other colleagues using cows, pigs, and hens. In July 1913, McCollum and Davis reported in the *Journal of Biological Chemistry* that different fats were not equal in value for rats' growth – a finding that ran counter to the prevailing nutritional dogma that diverse fats had similar nutritional values. On a diet of casein, lard, lactose, starch, and salt, young rats grew normally so long as an ether extract from butter or egg yolk was added. (The ether extract was prepared by mixing ether with butter or egg yolk to dissolve out the fat-soluble substances.) The rats died, however, if a similar ether extract from lard or olive oil were added. The investigators concluded that, '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' [54]. The differences in rats fed butterfat and those not fed butterfat could be dramatic (fig. 5.3). Many years hence, when looking back at this experiment, McCollum somewhat magnified the significance of its findings (textbox 5–4).

**Textbox 5–4. Discovery and a Distorting Mirror Of Hindsight**

In much later writings, Elmer McCollum stated that he had discovered vitamin A with his 1913 study [54]. He also maintained that, 'this observation was promptly verified by Osborne and Mendel' [55]. McCollum based this claim on his observation that the unidentified factor, which only later was identified as vitamin A, was fat-soluble. Thus, McCollum's responsibility for the identification of vitamin A was, to say the least, an exaggeration [56].
Socin (in 1891) and Stepp (in 1911) had long since suggested or demonstrated the fat-soluble nature of this unknown substance. The fat-soluble substance in butter and egg yolk in their experiments actually contained three vitamins: A, D, and E – three of the many ‘accessory factors’ that were far from identification in 1913. More incremental steps over many years had to be taken before these vitamins were separated, the chemical structure of vitamin A was described, and vitamin A was isolated, purified, crystallized, and synthesized. There is little justification to a claim that any single one of these many steps represented ‘The Discovery of Vitamin A’.

The august Nobel committee in Stockholm exercised the caution of specificity when it used the word ‘discovery’ in awarding its 1929 Prize in Physiology or Medicine to Frederick Gowland Hopkins ‘for his discovery of the growth-stimulating vitamins’ and his Dutch colleague, Christiaan Eijkman, ‘for his discovery of the antineuritic vitamin’ [57]. Apparently more inclined than McCollum to give credit where it was due, Hopkins acknowledged that the 1881 study of Nicolai Ivanovich Lunin had implied the existence of the ‘accessory factors’. He also paid respect to Cornelis Pekelharing, who in 1905 had conducted studies in Utrecht showing that milk contained unknown ‘essential substances’. Pekelharing’s experiments, though definitely relevant and earlier than Hopkins’s, won little recognition outside the Netherlands in his own time because he published his work in Dutch, as did Gerrit Grijns [58].

Later, in 1937, when the Nobel Committee presented the Swiss chemist Paul Karrer with the Nobel Prize in Chemistry for describing the chemical structure of vitamin A, they further clarified the awarding of the 1929 Nobel Prize: ‘The other half of the Nobel Prize in Medicine was awarded in the same year to Hopkins in recognition of his discovery of the vitamins of growth, that is, the substances necessary for the growth of the animal body – contained for instance in milk – and of which one of the most important has now been identified with vitamin A’ [59]. This declaration left no doubt that the Nobel Committee had firmly closed the door to any other nominations for the ‘discovery of vitamin A’.

McCollum’s colleagues in New Haven, meanwhile, were coming to similar conclusions. In the August 1913 issue of the Journal of Biological Chemistry, Osborne and Mendel reported that rats fed a basal diet of isolated proteins, starch, lard, and milk with all its protein extracted grew normally for about sixty days, but then they declined and died. The addition of butter or replacement of lard with butter in the diet allowed normal growth in young rats. Osborne and Mendel concluded, ‘In seeking for the “essential” accessory factor we have, therefore, been led first to supply the cream component, in the form of butter. . . (I)t would seem, therefore, as if a substance exerting a marked influence upon growth were present in butter. . . ’ [60].
In the next year, Osborne and Mendel reported results from tracking down a clue that had surfaced several years before in Basel, Switzerland. In 1909, ophthalmologist Paul Knapp had reported that rats fed ‘purified’ diets of protein, carbohydrates, fats, and minerals not only grew poorly but also showed increased susceptibility to infection and eye problems. Specifically, Knapp’s rats on the basal diet developed conjunctivitis and corneal ulceration, and before long, they died. When milk was provided, however, the subjects’ eye disease and deaths were averted, leading Knapp to surmise that something in milk might be preventing eye lesions [61]. In 1914, Osborne and Mendel tested Knapp’s proposition about milk. Specifically, they showed that rats on their basal diet developed inflamed eyes and diarrhea, but they recovered when cod liver oil or butterfat was added to their diet [62].

Meanwhile, Elmer McCollum too, arrived at similar conclusions. In 1917, he noted that, ‘blindness results if the animals are permitted to go without this dietary essential or with an inadequate supply for a sufficient time’ [63]. The elusive ‘accessory factors’ were sufficiently close to being identified that McCollum started identifying them with letters of the alphabet (textbox 5–3). In the following year, he wrote, ‘Xerophthalmia and polyneuritis are abundantly demonstrated to have their origin in the lack of a sufficient amount of the fat-soluble A and water-soluble B, respectively, in the diet’ [64].

The eye disease that civilian physicians saw in malnourished infants and children, that military doctors described in servicemen, and that scientists were inducing in experimental animal subjects proved to be the same disease. Its cause was lack of what McCollum termed ‘fat-soluble A’, which also became known as the ‘anti-xerophthalmia vitamin’.

**Obstructions, Chicanery, and Perseverance**

With F. G. Hopkins’ ‘accessory factors’ still unproven, diehard proponents of the four-basic-components food dogma stood their ground, pointing to possible flaws...
in the experimentation being done in the pursuit of accessory factors. A particularly acerbic voice was to be heard from Breslau, Germany. Franz Röhmann (1856–1919), a prominent investigator and staunch defender of Justus von Liebig, asserted, ‘The assumption that some unknown substances are indispensable for growth is a convenient device for explaining experiments that result in failure – a device that becomes superfluous as soon as the experiment succeeds’ [65]. Röhmann illustrated his case: he fed mice what he called a purified diet of proteins, carbohydrates, fats, and minerals, and not only did the mice grow to maturity, but also they produced healthy young [66]. In 1917, however, Osborne and Mendel discredited Röhmann’s conclusion by noting that the commercial casein preparation Röhmann had used was impure and contaminated with other vitamins [67].

Four major problems did indeed muddy experimentation with animal subjects, leading to broad and confusing variations in results and misinterpretations of data. First, the so-called ‘purified’ diets of protein, carbohydrate, fats, and minerals were often impure and could, as Osborne and Mendel said of Röhmann’s work, be contaminated with different vitamins. Second, the experimental animals varied from species to species in their need for certain vitamins; unlike humans, mice, rats, chickens, pigeons, and dogs can synthesize their own vitamin C and do not require it in the diet, but guinea pigs do require vitamin C. Third, different animals’ ability to store certain vitamins in their tissues was not well understood, hence difficult to take into account. (Not until later did scientists become aware that the liver can store large but varying amounts of vitamin A, and this capacity was not calculated into interpretation of the results of experimental deprivation of dietary vitamin A [68].) And fourth, until large-scale breeding colonies were established, the individual animals purchased from suppliers could differ significantly from one another. The biochemist Casimir Funk (1884–1967), for example, reported in 1916 that 80% of the rats acquired for an ongoing experiment arrived at his laboratory with defects [69]. Such technical difficulties in performing experiments and interpreting results could impede progress and fuel rivalries and animosities.

Mendel articulated the problem caused by impure experimental diets in a 1914 lecture before the Harvey Society of New York: ‘It is not unlikely – to speak conservatively – that there at least two ‘determinants’ in the nutrition of growth. One of these is furnished in our ‘protein-free milk’ which insures proper maintenance even in the absence of growth. . . another determinant is furnished by these natural fats (butterfat, cod liver oil, or egg fat). . . both are essential for growth when the body’s store of them (if such there be) becomes depleted’ [70]. As though to corroborate Mendel’s cautionary note, McCollum and Davis began to realize in 1915 that the commercial lactose preparation they were providing as a ‘purified’ sugar in their basal diet was in fact impure [71]. An unidentified water-soluble factor was associated with the lactose [72]. Likewise, Mendel’s own experiments, conducted with Osborne, came under question when it became apparent that the ‘protein-free milk’ they used was contaminated with other growth-supporting substances; the unknown contaminant turned
out to be thiamin, the B complex vitamin missing in patients with beriberi [73]. In fact, all four researchers had inadvertently tainted their rats’ ‘purified diets’ with small amounts of thiamin.

While researchers in the United States doggedly searched for the causes of diseases, a British colleague turned his attention towards enigmatic remedies. Cod liver oil and butter had proven excellently but incomprehensibly effective in curing night xerophthalmia and rickets. But what did they contain that produced this salutary effect? At the request of the Medical Research Council, Edward Mellanby (1884–1955), Professor of Pharmacology at the University of Sheffield and former student of F. G. Hopkins, began to study rickets in dogs. Mellanby fed puppies a basal diet of milk (<200 ml/day), rice, oatmeal, and salt, or milk and bread. The addition to the basal diet of certain foods, such as cod liver oil, butter, or extra milk (500 ml/day), could prevent rickets in young puppies, whereas meat protein, casein, linseed oil, or yeast did not (fig. 5.4) [74]. Mellanby published the conclusion in 1918–1919 that rickets was a nutritional deficiency disease, and he attributed it to ‘a diminished intake of an anti-rachitic factor which is either fat-soluble A, or has a somewhat similar distribution to fat-soluble A’ [75]. His data did not exactly fit the idea that the factor, when missing, was what McCollum would term ‘fat-soluble A; Mellanby therefore qualified his statement about the distribution of this unknown factor in foods as possibly being similar to ‘fat-soluble A.’

McCollum, meanwhile, was about to do an about face. Since biochemist Casimir Funk, like Mellanby working in England at the time, had suggested that deficiencies of ‘vitamines’ were the cause of beriberi, scurvy, and pellagra, McCollum prepared to study scurvy in his albino rats. After several experiments, McCollum made a startling declaration in 1917: scurvy was not a nutritional deficiency disease [76], despite rather overwhelming evidence to the contrary. McCollum and his research assistant, William Pitz, fed both rats and guinea pigs a diet of protein, sugar, butterfat.
(containing ‘fat-soluble A’) and wheat germ (containing ‘water-soluble B’). The rats – which, unknown to McCollum do not require vitamin C – survived in perfect health, but the guinea pigs developed the typical symptoms of scurvy.

After testing additional diets and conducting pathological, germ-theory-based investigations, McCollum concluded that scurvy is not a nutritional deficiency at all; rather, it is caused by a bacterial infection brought on by extreme constipation. ‘The significance of this interpretation is far reaching,’ he pronounced. ‘It removes from the list one of the syndromes (scurvy) which has long been generally accepted as being due to a dietary deficiency.’ McCollum continued to adhere to this idea for some time, perhaps because, if the rat could not be used to generalize about human dietary deficiencies, this might cast doubt on all the work he had done with ‘fat-soluble A’ and ‘water-soluble B’ [77].

In retrospect, McCollum’s reversal might be construed as personally adversarial. While research was progressing quickly in the field of ‘vitamines’, personal disagreements and rivalries were beginning to surface. In 1917, at the invitation of Professor William Howell, McCollum moved from Wisconsin to Johns Hopkins University in Baltimore to head the Department of Chemical Hygiene at Johns Hopkins’s newly founded School of Hygiene and Public Health. A cloud of accusations of ethical impropriety and professional misconduct accompanied McCollum’s departure from Madison and was aired in print in the journal Science [78].

Upon McCollum’s departure, all the research notebooks in the Wisconsin agricultural station disappeared. His former supervisor, Professor Hart, wrote a letter to Science titled ‘Professional Courtesy’ [79] that drew attention to the missing notebooks. Among them were those of Harry Steenbock, who was considered McCollum’s ‘ace student’ and a ‘brilliant’ upcoming scientist [80]. Steenbock had worked with McCollum since 1915, ‘on the understanding that opportunity would be given Steenbock to develop some independent problems which he could work out separately from McCollum, if he so desired’ [81]. Before leaving Wisconsin, McCollum had reassured Dean Harry L. Russell that he would ‘leave the records in such shape so that all Station material could be utilized’.

With McCollum now departing, Russell was worried about the future of the laboratory work, and he recorded his meeting with McCollum in his diary. Russell had cause to be concerned, as he wrote in his diary, ‘Steenbock reported to me earlier in the day the conversation which he had had with McCollum in which McCollum was averse to giving him much of any information, said that from now on they were scientific rivals’ [82].

Steenbock’s research plan and data from his missing notebooks soon appeared in the Journal of Biological Chemistry – in a paper published by McCollum [83]. The paper was based on laboratory work carried out entirely by Steenbock and included wording taken verbatim from Steenbock’s notebooks. But Steenbock name did not appear even as a co-author, nor had McCollum sought Steenbock’s permission to publish his data.
McCollum responded to the ethical criticisms raised by the letter in *Science* with a dismissal. ‘We do not feel that a reply to the charges contained in his statement is necessary, further than to say that the work referred to was *planned* (emphasis added) entirely by one of us (McCollum) and was carried out by Mr. Steenbock. . . (N)othing better can be done than to leave the public to judge for itself on the basis of the research records of all concerned as to the probable responsibility for the *planning* (emphasis added) of this work’ [84]. Steenbock, in turn, pointed out that the Wisconsin Agricultural Experiment Station required that all scientific manuscripts be subject to review and approval by the director of the station, i.e. McCollum’s onetime mentor, Edwin Hart. In violation of the policy, McCollum submitted two papers for publication without approval [85]. In a second letter to *Science*, again not responding to the specific allegations, McCollum gave a long explanation about how he alone built up the line of experimentation and the rat colony. He expressed the belief that his work had benefited large groups of malnourished people. He further claimed, ‘during my stay at the University of Wisconsin nobody had anything to do with independent work with my rat colony. . . there is no property right in research or its results so long as it is incomplete and not protected by patent’ [86].

Finally, McCollum took a parting shot at his one-time colleagues at Wisconsin. ‘A few prefer to attempt to bring into disrepute some investigator who has opened up a new field of research when he has reached a point where much further work remains to be done. . . in the hope that they may thereby so discredit him that his work will be interfered with, with a view to making possible the reaping of a harvest of opportunity which his absence from the field would make possible’. A former staff member at Wisconsin recalled, ‘(T)his friction developed because Steenbock had become ambitious too and it was an impossible situation to have two forceful men in the same department. Especially when one of them, Steenbock, had occupied the position of subordinate before the antagonism developed’ [87]. Steenbock, who had an impeccable reputation for honesty, wrote to Russell that he only wanted to set the record straight: ‘I am not making capital out of this affair, but I do want to submit some facts and then quit’ [88].

Given the tension that had grown up, McCollum’s former colleagues at Wisconsin had been ‘most pleasantly relieved by his departure’ [89]. Despite their former collaborative relationship, Hart described McCollum as ‘a poor operator on a team’, tempering this criticism by noting that McCollum was ‘a capable individualist’ [90].

McCollum went beyond removing all the research notebooks, including those of other investigators’ ongoing studies. As a final act of sabotage, he released all the albino rats from their cages in the animal colony at the Wisconsin Agricultural Experiment Station laboratories. Steenbock had to spend two full months trapping enough of the rats to restart the animal colony and resume his research program [91].

In New Haven, Mendel was certainly aware of the personal conflict that was brewing, since he had mentored both McCollum and Steenbock. Still collaborating with Osborne on their research, Mendel steered clear of the controversy, however. The work on which
they were focused was bearing particularly valuable fruit: their 1918 studies identified the liver as the storage organ for vitamin A. To do so, they fed rats different organs and tissues from pigs, and thus found that liver was an extremely potent source of vitamin A [92]. At the time, scientists were beginning to appreciate the significance of the liver as the storage organ for vitamin A. The time it took for vitamin A deficiency to appear in their experimental animals depended upon how much vitamin A was stored in their livers.

During his first year in Baltimore, McCollum worked on a nutrition textbook and established another breeding colony of rats with which to continue his investigations. The book, published in 1918 and titled *The Newer Knowledge of Nutrition: the Use of Food for the Preservation of Vitality and Health*, included the author’s criticism of the work of his colleague, Casimir Funk. McCollum asserted that his own studies had disproved the existence of some so-called deficiency diseases and that some of these diseases were more likely attributable to an improper proportion of proteins, carbohydrates, fats, and minerals:

What has been said... regarding the special dietary properties of the different food-stuffs which go to make up the diet of civilized man, and the dietary habits of those classes of people who suffer from the diseases which have come to be recognized as being due to faulty diet, make it easy to see that there has become fixed in the minds of students of nutrition and of the reading public, an altogether extravagant idea regarding the importance of the substances which Funk gave the name 'vitamines.' Of the diseases which Funk considered due to lack of unidentified substances of this nature, namely beriberi, scurvy, pellagra, and rickets, but one, beriberi, has been shown to be due to this cause. ... Pellagra, scurvy and rickets do not belong in the same category with beriberi, and there do not exist 'curative' substances of unknown nature for these diseases. The individual is predisposed to the development of these syndromes by faulty diet, but the faults have been shown by the biological method for the analysis of the individual food-stuffs or their mixtures, to reside in maladjustments, and unsatisfactory quantitative relationships among the now well-recognized constituents of the normal diet. They are to be sought in the quality and quantity of the protein, the character and amount of the inorganic constituents. ... [93].

In his book (which sold fourteen thousand copies in the first three years), McCollum advocated greater consumption of what he termed 'protective foods' – milk, eggs, and leafy vegetables. The 'newer knowledge of nutrition' of the title and the basis of his recommendations came mostly from McCollum's nearly three thousand feeding experiments with his rat colony. But the book was not kindly received by some of his colleagues working in the field. In it, McCollum mentioned his own name nearly seventy times and, feigning a kind of remote objectivity, referred to himself in the third person. Few of the dozens of major investigators contributing to the 'newer knowledge' received more than passing mention, including F.G. Hopkins, Osborne and Mendel, Stepp, and his own one-time collaborator, Steenbock (see also textbox 5–4).

Even as McCollum dispensed from on high his advice on healthful eating, pellagra began to appear in certain populations in the southern part of the United States. Known previously in southern Europe and familiar in medical circles everywhere for its symptoms (the '4Ds,' dermatitis, diarrhea, dementia, and death), pellagra made an
unwelcome arrival in the United States and an aggressive advance in the South. With nearly sixteen thousand cases reported from eight states between 1907 and 1911, pellagra reached epidemic proportions, giving rise to considerable anxiety. The US Surgeon General asked Joseph Goldberger (1874–1929), an infectious disease specialist then working for the National Health Service, to investigate the causation of pellagra and allocated nearly one-fourth of his budget to the problem.

At the time, pellagra was widely believed to be an infectious disease, but it did not fit the picture of a contagious disease, and Casimir Funk said as much. Working counter to the common belief and following Funk’s conviction, Goldberger conducted epidemiological investigations showing that pellagra was associated with the diet that predominated among the southern poor, namely, salted pork fat, corn bread, and molasses. Observing groups of employees and inmates incarcerated in prisons, asylums, and orphanages where pellagra was present, Goldberger found that even workers whose jobs brought them into close contact with pellagra victims—including himself—never contracted the disease [94]. He went on to demonstrate that pellagra could be eliminated by providing milk, eggs, fresh meat, beans, peas, and oatmeal in the diet [95]. At the same time, other studies were coming to opposite conclusions. The Thompson–McFadden Pellagra Commission, a research group funded by two philanthropists, conducted its own investigations in mill villages in the cotton belt in the south and concluded that pellagra was indeed an infectious disease [96].

Using more rigorous dietary methods than the commission’s, Goldberger conducted further epidemiological investigations in the mill villages. Unlike the commission, he showed that households that consumed more lean meat, milk, butter, cheese, and eggs had lower risk of pellagra [97]. Furthermore, to test whether pellagra was an infectious disease, Goldberger and other volunteers (including his wife) went so far as to inject themselves with blood, nasal secretions, ground-up skin lesions, urine, and feces from pellagra patients: none of the volunteers developed the disease [98]. Goldberger’s conclusion: ‘On the whole. . . the trend of available evidence strongly suggests that pellagra will prove to be a “deficiency” disease very closely related to beriberi’ [99]. Sound though his evidence was, however, Goldberger’s conclusions did not meet with total acceptance.

On April 25, 1919, McCollum read a paper before the American Philosophical Society in which he criticized Goldberger’s work, finding fault with most of the latter’s studies and the very idea that pellagra could be caused by a lack of an unknown dietary substance. In the face of overwhelming evidence from epidemiological observations and intervention studies in humans, McCollum still would not accept that pellagra could be attributable to the lack of an unknown dietary substance. McCollum sided with the Thompson–MacFadden commission’s view and declared that, ‘pellagra is caused by an infectious agent’ [100]. He took the occasion to reassert his exclusive confidence in animal experimentation and warned against drawing conclusions from dietary experiments with human adults. His position: ‘(W)e must be guided
in human nutrition by the results of animal experimentation, in which the conditions can be made sufficiently rigid to bring into stronger contrast the faults of certain types of diets as contrasted with others. McCollum and his colleagues had failed to produce pellagra in rats; hence, pellagra could not be a dietary deficiency disease.

Despite the self-assurance implied by this proclamation and others, McCollum grew increasingly nervous about his reputation. The scientific grapevine was aquiver with comment on his misbehavior while leaving Wisconsin. The assessment of his colleagues not only about his scientific accomplishments but also about his character could potentially make the difference between recognition and high regard, and failure. The selection process for a Nobel Prize involved collecting the opinions of eminent scientists in a candidate's field – in McCollum's case, it could mean the opinion of highly respected Thomas Osborne in New Haven.

In Spring 1919, McCollum wrote with some urgency to Osborne's close collaborator and his former mentor, Lafayette Mendel [101]:

Dear Doctor Mendel,

I want to thank you for the copy of the collected reprints which you sent me recently. I prize this set which has been accumulating year by year very much. I shall keep you supplied with a set of my papers from time to time.

There is a matter which has caused me much concern lately and about which I have finally decided to ask your advice. It has come to me from several sources that Dr. Osborne has said some very hard things about me, and I am at a loss to know why he should do so. To be sure we have disagreed with you on certain matters in our research, but I have confined my criticism entirely to specific points of a highly technical nature, and, I believe, have had a basis for such criticism in experimental work in which I have firmly believed. I have never made uncomplimentary remarks about either of you, nor have I felt inclined to do so. I have always valued the friendship of both of you and have repeatedly mentioned your research in my public lectures and never in any instance have I verbally criticized it in any way. It has seemed to me that progress in science must be our goal and that in our technical articles we must frankly seek to place before our readers what we conscientiously believe to be the facts and their correct interpretation. This has been possible for me without in any way letting personal feeling enter into my attitude.

The latest one to report this unpleasant attitude on the part of Dr. Osborne was Dr. Howell. He did not tell me just what he said but indicated that it was so serious that he felt that I should take the matter up with Dr. Osborne and come to some sort of an understanding. Naturally I cannot rest until I have made an effort to set some matters right. Will you be so kind as to tell me frankly what you know about the situation, and advise me as to what I should do. I want to do the right thing by all, but it is a most serious thing for me to have accusations such as I have been told of made by a man who is held everywhere in such high regard as is Dr. Osborne.

With kindest regards,

Sincerely yours,

E. V. McCollum

Mendel was known for his kindness and had a reputation as a keen judge of character [102]. When new academic positions opened up across the country, Mendel was often consulted regarding his opinion of the possible candidates. Mendel was concerned for the success of all of his students, and his own students and fellows were highly sought after by other universities. He responded to McCollum a few days later [103]:
My Dear McCollum

I have your letter of May 20th in which you ask my advice regarding an unpleasant situation that seems to have arisen. At the outset I wish to emphasize what I feel confident is clear to you, namely, that I myself dislike exceedingly to be concerned in any way with personal differences, fancied or real, that are likely to impair the friendships which I value. My preference is to stand aloof from controversial discussions. However, in the present instance I presume that you have approached me in a spirit of personal friendship which I cannot disregard. Respecting of my own attitude you are perhaps aware that I have urged your advancement or appointment to scientific posts on more than one occasion. I shall therefore reply frankly, in the belief that you will read this unwilling expression of opinion in the light of correspondence between good friends and with a recognition of its confidential character.

You write: 'Will you be so kind as to tell me frankly what you know about the situation, and advise me as to what to do.'

I myself have never heard Osborne say anything detrimental to your character; in fact, outside of our own laboratory I cannot recall having heard him discuss you otherwise than incidentally. This does not mean that he and we have not often discussed your work, your results, methods and mode of presentation. On the contrary your papers have formed a frequent topic of conversation, as might be expected. Sometimes one or several of us have taken vigorous exception to some aspect of your work; often we have sought to reconcile apparent discrepancies between our results, and not infrequently your papers or certain groups of experiments or certain generalizations have called forth undiluted praise.

I believe, however, that Osborne has been frankly outspoken with respect to one aspect of your attitude towards the subject of nutrition – and in this he has not stood alone. From year to year your publications have revealed what seems to be a growing studied indifference to the contributions of other persons to the development of the science. The climax was reached in your recent book which (at least, so it intimated) seemingly makes you alone responsible for the newer progress in nutrition. It has been regarded by some as an ungenerous presentation that is oblivious to much that cannot be attached to your own valuable work. I am writing here in an impersonal way, for I have never reviewed or discussed your book in public. Hence you may know that I am not the author of any published notices thereof. That in the direction indicated your book has created an unfortunate impression elsewhere is apparent from reviews which doubtless you have seen. Under the circumstances I ought perhaps to tell you in confidence that comparable criticism have been repeated to me as coming from others, including even your own colleagues and former students. The upshot of this is that the impression of a somewhat self-centered viewpoint of the physiology of nutrition on your part is not confined to New Haven.

The tone of some of your published experimental criticisms, which have doubtless been worded strictly within the limits of journalist correctness, has sometimes disclosed (or has been interpreted to exhibit) a desire to belittle others in unnecessary ways. Science grows in part by correcting and supplementing earlier work, not primarily by disparaging it. I must confess that some of your pronouncements seem extremely cocksure, even to me.

I do not know what Dr. Howell has said to you. If I were to advise you, dear McCollum, as you request in your letter, it would be in this spirit: Your work has aroused widespread interest and approval. Keep it up vigorously. But if you are convinced that the judgment of various other workers and students to which I have alluded may have some justification, however slight, bear it in mind in the future, and be as tolerant, as generous and as sympathetic in your presentations as you very rightfully expect others to be of your own contributions. I am certain that you should gain true friends thereby. Imbued with this spirit, I should regard past incidents as closed.

With assurance of my personal regard, I am.

_Lafayette B. Mendel_
McCollum appears to have given some consideration to Mendel’s advice. In subsequent editions of his book, he made more frequent mention of other investigators, though often in the context of his harsh criticism of their experiments. But McCollum continued to avoid citing important work of former colleagues at Wisconsin (textbox 5–5), prompting Hart to write to Mendel about these omissions: ‘Evidently the University of Wisconsin is persona non grata among some of our explorers in biological chemistry. . . it is all very interesting in depicting the character of men, even scientists’ [104].

Textbox 5–5. The lone wolf who never lost his bite

Elmer McCollum pursued his research at Johns Hopkins University in a style that his former dean Allen W. Freeman characterized as ‘a lone wolf who liked to have assistants, usually women, rather than co-workers’ [105]. McCollum felt that teaching interfered with his research. He gave only one lecture a week and complained that, ‘A man in research has to have time for thought and reflection’ [106]. His aversion to teaching translated into a sparse generation of graduate students and post docs to carry on nutritional research [107].

In 1957, McCollum published A History of Nutrition: the Sequence of Ideas in Nutrition Investigations. In it, he reiterated his claim to have ‘discovered’ both vitamins A and D [108]. Despite its appearance of comprehensiveness, the book has some obvious omissions, particularly if the work involved a Nobel Prize given to his colleagues. Missing are references to Paul Karrer’s descriptions of the chemical structures of carotene and vitamin A, the important description of the chemical structure of vitamin D by the Nobel laureate Adolf Windaus, and the chemical description of vitamin C by Norman Haworth. He dismissed or disparaged colleagues’ work, including the pivotal experiment of F.G. Hopkins of 1912, writing, ‘Hopkin’s [sic] experiments did not advance knowledge beyond what had been [already] proven. . . ’ [109]. He belittled Harry Steenbock’s 1924 discovery that ultraviolet irradiation of foods generated vitamin D, noting – incorrectly – that Steenbock had merely ‘confirmed’ other investigators’ findings [110].

Lafayette Mendel’s Far-Flung Progeny and His Legacy

Contrary to Edwin Hart’s quip that Wisconsin had been made an unwanted presence in biochemistry, the work in progress in the lab of his junior colleague Harry Steenbock was putting Madison front and center in the field. In 1918, on the basis of rat experiments, Steenbock published the Journal of Biological Chemistry the first in a remarkable series of papers titled ‘Fat Soluble Vitamine’. Early work on ‘fat-soluble A’ dealt mostly with the vitamin A that occurs in foods from animal sources, that is, pre-formed vitamin A [111]. The primary sources of preformed vitamin A are milk, butter, cheese, liver, and cod liver oil. In fact, however, fat-soluble vitamin A also occurs
in three related carotenoid molecules (alpha-carotene, beta-carotene, and beta-cryptoxanthin, known as provitamin A carotenoids) that are present in dark green leafy vegetables, and orange and yellow fruits and vegetables such as carrots, mango, and papaya. In April, at a meeting of the American Society of Biological Chemists in Baltimore, Steenbock announced the novel and important idea that the vitamin A levels in foods were related to the amount of yellow pigment present. In a series of papers on carotenoids, Steenbock showed that carrots and yellow sweet potatoes are sources of vitamin A, whereas white tubers such as rutabagas, parsnips, and white potatoes contain no vitamin A, nor do sugar beets (table 5.2) [112]. Yellow maize, but not white maize, contains vitamin A [113]. Dark leafy greens are rich sources of the vitamin [114]. (That the predominant blue-green of chlorophyll conceals yellow pigments was already known [115]. The yellow pigment of these leafy greens becomes evident in the fall, when the chlorophyll fades and no longer masks the yellow.) In fact, the yellow and orange hue of foods was found to be a good approximate indicator of vitamin A potency. For example, red palm oil, which is intensely deep red, is rich in vitamin A [116]. Oranges, in contrast, have only modest amounts [117].

A noticeable inconsistency arose from the observations of Steenbock and others: while yellow pigment is associated with high-potency vitamin A, cod liver oil, which is colorless, is also an extremely potent source of vitamin A. So the obvious question: If color does not offer a reliable way to detect the presence of vitamin A and its potency, what is a good index? To find an answer to that question, scientists had to determine exactly what constitutes the enigmatic ‘fat-soluble A’.

In 1926, a color test was developed that allowed scientists to measure the amount of vitamin A present in solutions [118]. Antimony trichloride gave a deep blue color reaction that could be measured consistently. Soon the antimony trichloride color reaction and other methods, such as passing different wavelengths of light through solutions, were applied to contrast the differences between carotene and vitamin A (table 5.3). The controversy over the relationship between carotene and vitamin A

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**Table 5.2. Vitamin A content of foods is linked to yellow color**

<table>
<thead>
<tr>
<th>Vitamin A potency of foods</th>
<th>Active (yellow)</th>
<th>Inactive or low (white)</th>
</tr>
</thead>
<tbody>
<tr>
<td>butter</td>
<td>casein</td>
<td></td>
</tr>
<tr>
<td>egg yolk</td>
<td>egg white</td>
<td></td>
</tr>
<tr>
<td>cod liver oil</td>
<td>lard</td>
<td></td>
</tr>
<tr>
<td>yellow corn</td>
<td>white corn</td>
<td></td>
</tr>
<tr>
<td>carrot</td>
<td>parsnip</td>
<td></td>
</tr>
<tr>
<td>sweet potato</td>
<td>white potato</td>
<td></td>
</tr>
<tr>
<td>red palm oil</td>
<td>cottonseed oil</td>
<td></td>
</tr>
<tr>
<td>apricot</td>
<td>apple</td>
<td></td>
</tr>
</tbody>
</table>

---
continued until 1928, when Elisabeth, Baroness of Ugglas, and her husband, Hans von Euler-Chelpin, at Stockholm University showed that carotene could cure vitamin A deficiency in rats [119]. In 1929, Thomas Moore at Cambridge University demonstrated that carotene could be converted to vitamin A. Moore fed crystalline carotene to rats and showed that the vitamin A concentration increased dramatically in the liver [120].

Another unsolved mystery still remained, however. Mellanby had noted in his rickets experiments with dogs that the data suggested that the unknown causal dietary factor had a distribution in foods that was somewhat similar to but not identical to that of vitamin A. ‘Fat-soluble A’ seemed resistant to heat alone at temperatures up to 120°C, but it was not indestructible. F. G. Hopkins had shown in 1920 that a combination of aeration (i.e. bubbling air through a liquid) and heat could oxidize ‘fat-soluble A’ and thus destroy its biological activity [121]. Two years later, McCollum and colleagues found when working with rats that if cod liver oil were aerated and heated, it no longer cured xerophthalmia but promoted calcium deposition in bone in rats with rickets. They thus showed that while cod liver oil consisted of ‘fat-soluble A’, which cures xerophthalmia, the oil also contains another fat-soluble substance, which plays a role in bone growth [122]. The latter fat-soluble substance was initially called the ‘anti-rachitic factor’, or simply ‘X’, and it eventually became known as vitamin D [123].

The distinction between vitamins A and D became more apparent with further rickets investigations. Sunlight deprivation was thought to play a role in rickets, and in 1919 a German pediatrician had shown that ultraviolet light cured children of rickets [124]. In that same year, Mendel received a letter from the British nutritionist Harriette Chick (1875–1977), who was the first woman appointed to London’s Lister Institute of Preventive Medicine. Chick worked as assistant to the director, Charles Martin, and established a group to study beriberi and scurvy. She served as Secretary to the Accessory Food Factors (Vitamines) Committee, which had been established in 1918 with F.G. Hopkins as chair. At the time this was the world’s only formal group devoted entirely to vitamin research. In her letter, Chick informed Mendel, ‘I have just arrived in Austria on behalf of the Medical Research Committee to study the

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Table 5.3. Differences in carotene and vitamin A to the biochemist

<table>
<thead>
<tr>
<th>Carotene</th>
<th>Vitamin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>deep red color</td>
<td>colorless</td>
</tr>
<tr>
<td>synthesized in plants</td>
<td>formed in the animal body from dietary carotene</td>
</tr>
<tr>
<td>ultraviolet light</td>
<td>ultraviolet light</td>
</tr>
<tr>
<td>absorption bands: 492, 462, 437, 348, 280 nm</td>
<td>absorption band 328 nm</td>
</tr>
<tr>
<td>reaction with antimony trichloride: dull blue</td>
<td>reaction with antimony trichloride: vivid blue</td>
</tr>
</tbody>
</table>
“deficiency” diseases here and if possible to help in treatment, especially in cases of children. Rickets is terribly bad and scurvy seems to occur every winter. Of course shortage of milk is the real difficulty. . . and their diet after leaving the breast is very short and in some cases appears to be devoid of fat soluble A. It appears that seventy to ninety percent of the children between one and five years have rickets and many have terribly severe cases’ [125].

The conditions Chick described were the result of cataclysmic upheaval in Europe. By the end of World War I and the fall of the Austro-Hungarian Empire, when Hungary had ceased to export food to Austria, hunger in Austria was reaching a critical level. Food for Vienna’s population of 2.3 million was minimal [126]. The job of Chick’s committee was to provide expertise on the nutritional needs of war-affected civilians. At first, Chick and her colleagues encountered skepticism at the University of Vienna’s children’s hospital. The director, Clemens von Pirquet, later wrote that he had ‘little expectation that it would lead to results of much practical value. . . I was of the opinion that a vitamin deficiency in our ordinary diet was a very exceptional occurrence. . . (W)ith regard to the etiology of rickets I held the view that it was an infectious disease, widely prevalent in this part of Europe. . . ’ [127]. Deeply troubling reports of rickets among Vienna’s children were reaching the world (fig. 5.5). Infants with corneal ulceration, keratomalacia, and blindness, too, were common [128]. Relief efforts began in earnest.

In 1920, Pirquet also wrote to Mendel on the progress of the nutritional investigations, which had given rise to a large and productive research enterprise. ‘Besides the 300,000 children we are now feeding,’ Pirquet wrote, there were ‘100 university
professors who deserve feeding at least as much as the children’ [129]. Pirquet reserved one-fifth of the hospital for rickets research. Under the auspices of the Accessory Food Factors Committee, Chick and her colleagues conducted a series of trials. They found that cod liver oil prevented or cured the children’s rickets, and that the same result could be achieved by exposing the children to a mercury vapor lamp, which emits ultraviolet light. Whether from a mercury lamp or direct sunlight, ultraviolet light generates vitamin D in skin that is exposed to the light. The committee’s conclusion: rickets was caused both by lack of certain foods and by inadequate exposure to sunlight [130]. (One of Chick’s later achievements was development of standards for vitamin requirements; textbox 5–6).

**Textbox 5–6.** Finally, vitamins find a place on the international map

In June 1931, the first International Conference on Vitamin Standards was held in London under the auspices of the League of Nations (forerunner of the United Nations). Improving health on a multinational scale was one of the tasks the league undertook, and, by holding the vitamin standards conference, the league was making a tacit statement that the field of vitamins had reached maturity and that vitamins and physical wellbeing were fundamentally linked.

That dual recognition translated into a call for measurement units and a set of standards as to how many units of each known vitamin were essential to maintaining good human health. Thus, the conference was charged with finding standards and recommending measurements units for ‘fat soluble A, antirachitic vitamin D, antineuritic vitamin B, and antiscorbutic vitamin C’ [131]. Edward Mellanby chaired the conference, which convened scientists from Great Britain, Sweden, Denmark, the Netherlands, Norway, France, Germany, and the United States. Many of the investigators who had figured prominently in the vitamin research of the previous two decades including were present, including Elmer McCollum, Harry Steenbock, Harriette Chick, Jack Drummond, and two Nobel laureates, Adolf Windaus and Hans von Euler-Chelpin (see fig. 5.6).

The committee laid the fundamental groundwork for work that eventually led to the determination of the required daily allowances of the different vitamins. The most important initial step was to agree on how the vitamins were to be measured and to exchange reference materials (solutions or compounds with known amounts of vitamins) between the different laboratories around the world. This process was something akin to agreeing on a common currency to be used by all groups.

During this period of fruitful vitamin research, investigators throughout the western world turned to Mendel for advice – Hopkins in Cambridge, England; Hart in Wisconsin; McCollum in Baltimore; Chick in London; Pirquet in Vienna, and many others. In his thoughtful and generous style, Mendel advanced the research by
inspiring other, younger scientists. He characterized the worst and the best research environment in a letter to a colleague and the University of Chicago. ‘(I)t means a lot to an institution to have a considerable number of young men who are growing up to their possibilities. One reason why we have had few such conditions is that our heads of departments are as a rule too self-centered and autocratic. The ideal ‘chief’ is a good promoter of talent in men’ [132]. Acting on his convictions, Mendel gave his students opportunities to meet and be inspired by leaders in the field. He and Hopkins had a long and warm friendship, which is reflected in the notebook of a colleague: ‘When Dr. F. Gowland Hopkins of Cambridge University visited the old [Yale] laboratory. . . Dr. Mendel told his students that Dr. Hopkins would be there and that they might have an opportunity to meet him if they would ‘hang around’. Needless to say everyone hurried through his work to be ready to meet the great British pioneer in nutrition. One student, hurrying down the circular stairway with a
tray full of bottles and glassware, stumbled and fell with a great crash just as Mendel and Hopkins came up the winding stairs underneath. Dr. Hopkins remarked jovially, ‘I see you have designs on my life,’ immediately dissipating the embarrassment of the very red-faced student’ [133].

In the spring of 1924, Mendel received a letter from Wisconsin with the news that Steenbock had discovered that the vitamin D concentration of certain foods could be increased by irradiating the foods with ultraviolet light. ‘I am writing you this,’ Steenbock began, ‘on the assumption that as my former instructor you are personally interested in my professional welfare and I accordingly invite your criticism’ [134]. Mendel replied that his onetime protégé’s as yet unpublished work:

. . . (has) awakened my ardent enthusiasm and filled me with pleasure. I congratulate and compliment you on the prospect of a tremendously important discovery. Furthermore, I appreciate very much the cordial spirit that you have shown in taking me into your confidence, so to speak, in connection with what you have been doing. I hope that you will clinch the essential finding regarding the effect of the radiation on non-potent fats so that there can be no question of any accidental error in reaching your conclusion; and then you should make sure that you are not deprived of the credit of the discovery to which you are entitled in connection with this investigation [135].

On September 5, 1924, Steenbock published a paper in *Science* that announced his findings [136].

With rickets highly prevalent in many parts of the world, Steenbock’s process for increasing the vitamin D content of foods could be widely and lucratively applied in the food industry. Steenbock recognized the commercial implications and arranged to meet with the dean and president of the University of Wisconsin to urge them to seize the economic advantage and seek the patents for his process. Being either frugal or shortsighted, the university refused to pay for the patent applications, so Steenbock took the matter into his own hands and, for USD 660, hired an attorney to secure the patents. The Quaker Oats Company offered Steenbock USD 1 million for rights to the patents. But Steenbock believed that his professor’s salary was sufficient for himself and that his university and scientific research should be the beneficiaries [137].

At Steenbock’s insistence, the Wisconsin Alumni Research Foundation was eventually incorporated. Steenbock spent ten dollars to assign his patents to the foundation and requested no share of the royalties; his patents eventually earned the foundation some USD 14 million. Resolutely modest and unassuming, Steenbock returned to his lab, working with Hart and other colleagues, to continue a highly productive career. He made seminal scientific contributions, including further new insights into vitamins D and A, nutritional anemia, and vitamin E [137], while his vitamin D discoveries earned millions for the University of Wisconsin.

Not until 1931 was the chemical structure of vitamin A finally described. This was accomplished by Paul Karrer (1889–1971), an organic chemist in Zurich, who, the year before, had deduced the correct chemical structure of beta-carotene (Karrer was later awarded a Nobel Prize in Chemistry in 1937 for discovering the structures of
vitamin A and beta-carotene) [138]. Six years later, Harry Holmes and Ruth Corbet at Oberlin College had crystallized vitamin A [139]. A full decade had passed by the time Otto Isler and colleague chemists at the Swiss pharmaceutical company Hoffmann-La Roche synthesized vitamin A [140]. The crystallization and synthesis were critical to understanding vitamin A, and these final steps paved the way for the production of pure preparations of vitamin A that could be used for vitamin preparations and the fortification of foods for the public.

The relationship between vitamin A deficiency and night blindness was finally solved definitively through the work of George Wald (1906–1997), a biochemist at Harvard University. Before working at Harvard, Wald had spent time in the biochemistry laboratory of Otto Meyerhof in Heidelberg. Wald had posited that rhodopsin, the ‘visual purple’ in the retina (see Chapter One), was related to the carotenoids; he based this theory on the absorption spectrum of rhodopsin. (When lights of different wavelengths pass through a compound in solution, the pattern of the light absorption yields information that can serve in deducing the structure of the compound.) The opportunity to test this supposition presented itself in Heidelberg. On a summer day when most of the lab personnel were on holiday, a shipment of three hundred frogs arrived, but no one seemed to be there to experiment with them. A lab assistant who, like Wald, had stayed behind to work was about to release the frogs when Wald stopped him [141]. With this windfall of study subjects, Wald was able to examine the frogs’ retinas under diverse light conditions. In solutions of the visual purple, he found high concentrations of vitamin A [142].

Wald detected a novel carotenoid that was yellow in color, which he termed retinene. Retinine, he found, was present in retinas from animals with dark-adapted eyes. Conversely, he found high concentrations of vitamin A in retinas that had been exposed to light. These observations led Wald to proposed that the retina undergoes a visual cycle in which light causes the purple of rhodopsin to turn bright orange, which, in turn, fades to a yellow color (‘visual yellow’) consisting of retinene plus the protein opsin [143]. The visual yellow then loses color, becoming ‘visual white’, which is rich in vitamin A. He proposed that rhodopsin is regenerated from vitamin A plus opsin or retinene plus opsin [144].

These observations by Wald brought to completion the long line of investigation that began with early observations relating night blindness and lack of vitamin A [145]. The characterization of vitamin A, from the hints of its existence shown by Magendie in 1816 to its isolation, description of chemical structure, and eventual synthesis in 1947, was an excursion down a tortuous road that took nearly one hundred and thirty years to complete.
References

Nobelstiftelsen (1938) Le Prix Nobel en 1937. Stockholm, Imprimerie Royale, P. A. Nordstedt & Söner, p. 35. When the Nobel Prize was awarded to Hopkins and Eijkman for the discovery of the vitamins, an editorial in Time Magazine (November 11, 1929) noted: ‘Many a US nutritionist declared last week, without carping at the Nobel award to Professors Hopkins and Eijkman, that, if a future Nobel Prize for vitamin research is made, it should go to Professor Elmer Verner McCollum, 50, head of the department of chemical hygiene at Johns Hopkins School of Hygiene & Public Health.’


Hart (1918); Series No. 9/11/13–2, Box 1, Folder 11. Steenbock, H. Letter to H. L. Russell, March 12, 1918.


Series No. 9/1/1/12–1, Box No. 4. Russell diaries, ‘Dr. McCollum’s Work,’ April 26, 1915.

Series No. 9/1/11–2, Box No. 4. Russell diaries, ‘Completion of McCollum’s Work,’ June 11, 1917.


Steenbock, H. (1918) Professional courtesy. Science 47, 535–536. In recounting the episode of the two disputed papers in his autobiography, McCollum alleges: ‘Fortunately I failed to say that they were published with permission of the Director, as was customary in experiment station bulletins,’ [McCollum (1967), p. 150]. On the contrary, Russell’s confidential notes of his meeting with McCollum on June 11, 1917 taken in his ‘black book’ diaries shows that he did not give permission for McCollum to publish the papers (Russell kept meticulous notes of his meetings with various faculty members in his diaries). Russell later sent a telegram to Steenbock on April 3, 1918 that reiterated the lack of authorization: ‘McCollum agreed to submit results of Wisconsin work to me prior to publication did not give him permission to publish biological chemistry papers’ [Series No. 9/11/13–2, Box 1. Folder 10. Russell, H. L. Telegram to H. Steenbock, April 3, 1918]. In addition, Hart took the extraordinary step of writing to Mendel, editor of the Journal of Biological Chemistry, to inform Mendel that McCollum specifically violated University of Wisconsin policy and published the papers without the required Station review or approval [MS1146. Accession 1998-M-099. Box 1, Folder 1, Hart, E. B. Letter to L. B. Mendel, November 16, 1917].


DeLuca, H. E-mail communication to the author, June 22, 2009. Professor Hector DeLuca did his doctoral work under the supervision of Harry Steenbock from 1951–1954 and heard the account of McCollum’s departure from the University of Wisconsin directly from Steenbock, who he says was ‘extremely honest.’ The episode is also consistent with the published research record. Steenbock was quick to publish his data, but he was only able to submit his first paper in the ‘Fat-Soluble Vitamin’ series on July 1918, nearly one year after the reported disruption of their research with albino rats.


McCullom (1957), pp. 217–218, 281. In addition to claims for the ‘discovery’ of vitamins A and D, claims were made elsewhere that McCullom also ‘discovered’ vitamin B. For example, see Herriott, R. M. (ed.) (1953) Symposium on nutrition: the physiological role of certain vitamins and trace elements. Baltimore, The Johns Hopkins Press, p. xv.

McCullom (1957), p. 211.

McCullom (1957), p. 284. Other conspicuous omissions from McCullom’s book include the work on alcoholic fermentation by Arthur Harden and Hans von Euler-Chelpin for which they received the Nobel Prize in 1929. McCullom introduces a false reference to dispute the priority of Joseph Goldberger in his studies of pellagra (pp. 303–304). McCullom often distorted the sequence of investigation or omitted their findings, especially that of his former competitors, in A History of Nutrition.

Preformed vitamin A consists of animal sources of vitamin A such as that found in liver, cod liver oil, egg yolk, butter, and cheese, in contrast to pro-vitamin A carotenoids found in plant-source foods such as dark green leafy vegetables and orange and yellow fruit and vegetables.


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McCullom (1957), p. 284. Other conspicuous omissions from McCullom’s book include the work on alcoholic fermentation by Arthur Harden and Hans von Euler-Chelpin for which they received the Nobel Prize in 1929. McCullom introduces a false reference to dispute the priority of Joseph Goldberger in his studies of pellagra (pp. 303–304). McCullom often distorted the sequence of investigation or omitted their findings, especially that of his former competitors, in A History of Nutrition.

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129 MS1146, Series 1, Box 1, Folder 11. Pirquet, C. Letter to L. B. Mendel, October 17, 1920.


137 Schneider, H. A. (1973) Harry Steenbock (1886–1967) – a biographical sketch. Journal of Nutrition 103, 1233–1247. Steenbock lived together for many years with his parents in the family home in Madison. His father and mother died in 1942 and 1946, respectively, leaving Steenbock alone in the family home for the first time, at age sixty. The University of Wisconsin trustees finally persuaded him to accept part of the royalties from his patents; they introduced a standard policy of providing part of the royalties for other faculty members who filed patents. Upon his death, Steenbock bequeathed millions of dollars from his own estate to various philanthropies, including an academy that fostered the interest of young students in science.


145 Wald's contribution to the understanding of vitamin A and rhodopsin in the visual cycle in the retina was acknowledged in 1967 with a Nobel Prize.