

## A Short History of Nutritional Science: Part 3 (1912–1944)<sup>1</sup>

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In the early years of the 20th century, the number of researchers engaged in nutritional work expanded enormously. Space does not permit more than an introduction to the proliferation of literature on any specific topic and it has unfortunately not been possible to fully distribute the credit to several groups of researchers working on the same problem. Also I have probably not given a due share to those publishing in languages other than English. When a development has been presented in a series of papers, I have tried to cite a relatively recent one because it is easier for readers to work their way back through the literature than try to go forward.

### *The vitamin era*

In 1912 Casimir Funk wrote that the antiberiberi factor, because of the different conditions under which it could be extracted from rice polishings and then precipitated, must be an organic base and therefore contain an amine group. He went on to suggest that pellagra, scurvy and rickets were caused by deficiencies of other yet to be identified factors. This was not original, but he then went on to hypothesize that these factors would all be found to have the same basic character, i.e., to be vital amines, for which he coined the name “vitamines” (3). It seems that he felt intuitively that they would be chemically similar in a manner analogous to the components of protein having different properties even though they were all amino acids. This was a leap of faith and not a correct prophecy, but some 10 years later he would claim that “the name contributed in no small measure to the dissemination of these ideas” (4). Another group wrote in agreement, “the name was a stroke of genius . . . such a captivating word . . . necessary to focus attention upon the possibilities of the field” (5).

Certainly “vitamins” (with the ‘e’ omitted after it was realized that they could not all be amines) formed a new vein of knowledge waiting to be mined and were to be the major topic of nutritional research for the next 30 years. Looking for example, at the first two volumes of *The Journal of Nutrition*, published from 1928–1930, we see that more papers (some 40%) dealt with this general topic than any other. In 1933 Leslie Harris, who had been given the responsibility of reviewing the previous year’s work in the field, pointed out that

nearly 1,000 papers had been published on vitamins with over 300 on vitamin D alone in just 12 months (6).

It is sometimes asked, “Who first had the idea of vitamins?” One can find tantalizing early quotations, but they were not followed up at the time. In 1804 Thomas Christie, a physician working in Sri Lanka, wrote, “The chief cause of beriberi is certainly a want of stimulating and nourishing diet . . . . However, giving ‘acid fruits,’ which I find of great value in scurvy has no effect in beriberi . . . . I can suppose the difference to depend on some nice chemical combination” (7,8). In 1830 John Elliotson lecturing at a London teaching hospital said that, “scurvy is a purely *chemical* disease . . . each part of the system is ready to perform all its functions, but one of the external things necessary for its doing so is taken away . . . the remedy for this state is fresh food” (9). In 1842 George Budd also lecturing in London added, “Scurvy is only one of a number of diseases due to specific dietary deficiencies, another is rickets and a third is characterized by a peculiar ulceration of the cornea” (10,11). Gerrit Grijns’ pronouncement already quoted in “A Short History of Nutritional Science: Part 2 (1885–1912),” (2) was probably the first clear statement, based on his own work, of the existence of an organic nutrient required only in small amounts.

### *Rats and mice fed purified diets*

Previously most work started with a diet that had been found in practice to be associated with a particular disease, and this type of work was to continue with important results that we shall consider shortly. However, from this point on, much of the productive work was to start from the bottom, so to speak, with “purified” diets, meaning mixtures prepared from only the major nutrients (protein, carbohydrates and fat) in forms as pure as possible, as well as minerals. These diets would be deficient in all of the hypothetical vitamins, and the task would be to find what combinations of supplements would restore them to promoting good performance. McCollum explained, “The older practice of experimenting with combinations of natural foodstuffs is not searching enough in character to reveal any of the fundamental principles of nutrition, or to . . . lay the foundation of a system of feeding based upon scientific principles” (12).

### *The work of E. V. McCollum*

To begin with I will follow the progress in the field through the work of McCollum and his volunteer assistant Marguerite Davis at the Wisconsin Agricultural Experiment Station attached to the University of Wisconsin; they were certainly

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leading the pack in the early years (13). It is interesting in view of the importance of their discoveries that McCollum's young colleagues at Yale told him that he was foolish to accept a position in which he would be working in nutrition since, "the subject was already worked out, with nothing remaining to be discovered!" (14). He had been recruited from Yale, as has been previously related (2), to work on the single-grain experiment with cattle that had been producing dramatic and inexplicable results.

### Searching the literature

McCollum had already mastered German and began with a careful reading of the earlier European work summarized in Maly's *Jahresbericht über die Fortschritte der Tier-Chemie*, having bought the 37 volumes describing work published between 1870 and 1907. He found there descriptions of 13 experiments in which efforts to maintain small animals on purified diets had all failed. The influential professor Von Bunge believed that the elements iron and phosphorus needed to be part of organic complexes in order to be absorbed, and he attributed the failures of these diets to the complexes having been decomposed by the purification procedures rather than to lack of any unknown nutrient (15–17). In any case the studies had not been followed up.

Cornelis Pekelharing, whose work as the leader of the Beriberi Commission in Indonesia has been previously described (2), also reported that mice would not thrive on a simplified diet of casein, egg albumin, rice flour and minerals. He buried his brief announcement in a 1905 paper on a different subject, but made it clear that the mice were deficient in something that he could supply to them in whey (i.e., milk with the fat and casein removed). He added that he would say no more about it because he could not identify the missing factor. Indeed his work remained generally unknown until translated into English 20 years later (18) and McCollum was not aware of it. Strangely, Gowland Hopkins, Professor of Biochemistry at Cambridge University, said something very similar in the following year. Toward the end of an obscure published lecture we find, "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. . . . 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 . . . that I am considering." Many years later in his Nobel Prize speech, he said that he had based his remarks on studies he had made with mice in 1906–1907, but like Pekelharing had thought that they would not be taken seriously until he had identified the missing factor (19,20). He was probably the first to associate the deficiencies of a purified diet with human disease conditions.

It is interesting that McCollum had also failed to find any reference to the important work of Eijkman and Grijns in studying the disease produced by feeding chickens white rice and the latter's conclusion that it was caused by deficiency of a relatively unstable water-soluble organic compound (2).

In 1909 a Swiss ophthalmologist, who had seen in another trial the mention of eye lesions appearing in rats fed a purified diet, repeated the experiment and identified the xerophthalmia and keratomalacia that he saw with conditions found in human subjects that had proved responsive to cod liver oil (21,22).

### McCollum's first trials

It had been speculated at this time that animals needed to be supplied with phosphorus in the form of nucleic acids. McCollum set out to test this by using vegetable proteins prepared without any significant phosphorus compounds attached. He fed three rats a mixture of the plant proteins edestin (from hemp seed) and zein (from corn) at levels providing 12–18% protein in powdered diets that also included 5% butter fat and 8% minerals as well as starch and cane sugar, all mixed with a little finely divided cellulose and water, and dried at 38°C. The rats, initially weighing 100–170 g, all lost weight and showed little appetite after one week. He tried adding different flavorings, e.g., banana, bacon etc., which restimulated the appetite for a day or two, and concluded that the lack of palatability was the limiting factor with such diets. However, from phosphorus balance data he concluded that rats could manufacture their own nucleic acid without the need for either organic phosphorus or purines (16). In Connecticut Osborne and Mendel confirmed this last point in 1911 (23). These workers were primarily interested in being able to compare the nutritional values of a variety of isolated proteins tested in diets with no other protein source present. However, in order to do that they needed a basal protein-free mixture that would meet all the other needs of young rats, and that brought them willy-nilly into the vitamin field.

### Factors "A" and "B"

McCollum could now use the phosphoprotein casein as his purified protein source without worrying about its phosphorus content and he experimented to improve his mineral mix. In 1913 he reported with Marguerite Davis that rats fed diets containing 12–18% casein, 20% lard, 20% lactose, 6% minerals and starch, would grow well for 8 to 14 weeks but then stop and in some cases lose weight. However, growth would restart if the rats were given 1 g of ether extract of egg on alternate days. In the following year they reported that butterfat, but neither olive oil or cottonseed oil, would also stimulate rats to grow again. They also reported that if butterfat were saponified and the emulsion shaken with olive oil, the olive oil was then active in restarting rat growth, which suggested that the active factor was nonsaponifiable and remained fat soluble (24). Again the need for a fat-soluble substance was confirmed by the Connecticut group who also reported the high potency of cod liver oil (25).

By this time, Hopkins had argued that the two American groups had only been able to obtain growth with their diets because the casein and lactose were insufficiently purified from a water-soluble "growth factor" (26,27). McCollum and his colleagues looked into this and agreed, concluding that rats needed both a fat-soluble "Factor A" and a water-soluble "Factor B" that was identical to the antiberiberi factor deficiency which developed in chickens and pigeons fed white rice (28).

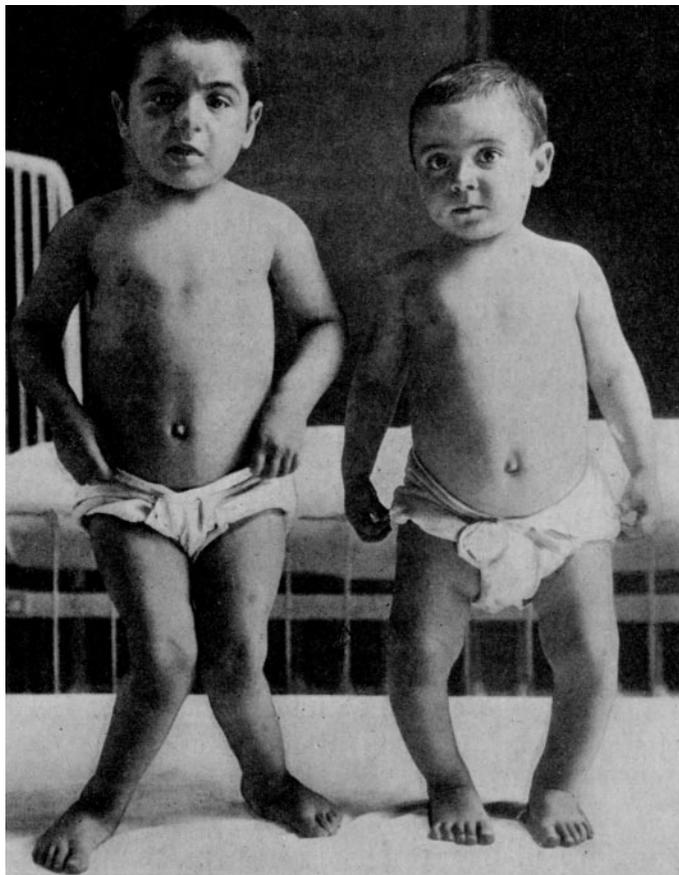
Here we see the beginning of the scheme for identifying vitamins by letters before their chemistry had been worked out. They then reported that leaves showed "Factor A" activity even though their ether extracts and also the plant oils that they had tested did not do so. It was also noticed that the deficiency of "Factor A" resulted in severe ophthalmia. This of course linked up with the clinical work previously described dealing with night blindness leading to xerophthalmia and its prevention with cod liver oil (2). We will return to the slow and complex progress in understanding "Factor A" in a later section.

### Rickets and vitamin D

In 1917 McCollum moved to the newly established and well-funded School of Public Health at Johns Hopkins University in Baltimore. There, in further rat experiments, he discovered that he had produced an experimental model of another human disease, rickets.

As previously described, rickets had become a serious problem among young children in the large industrial cities of Western Europe and the northern United States (Fig. 1) (2). It was particularly serious in Glasgow, Scotland's largest city, whose medical school had an active group concerned with the problem. They reported in 1908 that puppies fed bread or oatmeal with whole milk would develop rickets if kept indoors, but not if taken for outdoor walks (29). From a study conducted in the city's slums, it was concluded that inadequate fresh air and exercise were "potent factors in determining the onset of rickets," and in a further trial with puppies it appeared that having an outside run was more important than the intake of milk fat (30).

In contrast Edward Mellanby in England, who had worked under Gowland Hopkins and was familiar with the subject of 'accessory food factors,' reported in 1921 that he had produced rickets in puppies kept indoors by limiting their milk intake to 200 mL/d. However, he could prevent its occurrence with supplements of a variety of foods including butter and cod liver oil without allowing the dogs outdoors or giving them exercise (Fig. 2) (31). He and the Scots were therefore understandably skeptical of each other's conclusions, and it seemed to be an additional insult when Mellanby reported at a meeting held in



**FIGURE 1** A sister and brother (aged 5 and 3 y, respectively) showing the effects of earlier rickets, Rachford, 1912 (126).



**FIGURE 2** A puppy with rickets as a result of being reared indoors on a rachitic diet, Mellanby, 1921 (31).

Glasgow that oatmeal had a rachitic effect on his puppies. This was the traditional cereal food of the Highlanders who prided themselves on their physique. In reaction a Scottish newspaper published a cartoon of an addle-brained professor saying that porridge was useless because it did not agree with his dog, and then being offered some bones to see how he liked them (32,33).

The controversy was soon resolved. Exposing an infant to sunlight had already been one of the traditional folk treatments for rickets in Northern Europe, and in 1919 exposure to ultraviolet lamps had also been reported to be effective. At this time, in the aftermath of World War I, there had been great food shortages in Central Europe, and a group of scientists financed by the Society of Friends and led by Harriette Chick of London's Lister Institute went to Vienna where the well-equipped Childrens Hospital had many cases of rickets. There they were able to demonstrate, with the aid of X-ray photographs, that either the use of cod liver oil or irradiation with ultraviolet light would cure the condition, which otherwise developed even under the most hygienic conditions (34).

By this time, McCollum and his medical colleagues at Johns Hopkins had, as already mentioned, found that the more convenient rat could also be used to provide a model for rickets if its diet were severely imbalanced in ratio of calcium to phosphorus. They then found that cod liver oil would prevent the disease even after it had been aerated in a way that destroyed its antiophthalmic (factor A) value (35).

The next extraordinary finding, back in Wisconsin in 1924, was that not only did irradiation of rachitic rats with ultraviolet light have a curative effect, but so did irradiation of the diet from which they had developed the disease (36). Many groups tried to determine what factor might be activated in this way. It was quickly traced to "lipid," then to the sterol fraction and finally to ergosterol and, in 1931, the activated material itself now named "vitamin D" was crystallized (37).

(The letter "C" had already been allocated to the antiscorbutic vitamin.)

With this new knowledge rickets ceased to be an intractable public health problem. It was later realized that there was more than one form of active vitamin, and that their relative activities depended on the species under consideration.

### Experimental scurvy

Surprisingly, after the discovery in 1907 that guinea pigs could provide an animal model for scurvy, very little use was made of them for several years (2). McCollum became interested because his rats clearly did not need antiscorbutics to survive, and if species could differ drastically in their requirements it would be a challenge to the general significance of results obtained with rats. He and a colleague fed guinea pigs a combination of "oats + milk." Some survived, but others died and were found to have "the cecum distended with putrefying feces" that they assumed to be causing autointoxication. In 1917 on the basis of this one finding, they wrote, "The significance of this interpretation is far reaching: it removes from the list one of the syndromes [scurvy] which has been generally accepted as due to dietary deficiency" (38).

Harriette Chick and E. M. Hume, the first independent women scientists to be mentioned in this history, were soon able to demonstrate that cow's milk had only a low antiscorbutic activity; guinea pigs receiving an "oats + milk" diet needed some 50 mL per day to remain healthy and if the milk went at all sour they would not touch it. It was essential therefore to monitor individual consumption in order to interpret the results. Scurvy consistently appeared with autoclaved milk that had apparently lost most of its vitamin C activity (39).

Because of suspicions that commercial lime juice was not an effective antiscorbutic, the same group now used guinea pigs to test this in what may have been the first bioassay for vitamin activity. They found that commercial lime juices had less than one tenth the activity of freshly squeezed lemon juice (Table 1) (40). The processing almost certainly included pumping through copper pipes and probably some form of sterilization. This of course confirmed that the Victorians had been correct to doubt that lime juice as they knew it was effective in preventing outbreaks of scurvy on long expeditions.

Other workers now attempted to isolate vitamin C from lemons, which proved difficult because of its instability. Strangely, it was first achieved in 1928 by Albert Szent-Györgi, who was not seeking a vitamin but a factor involved in

the catalysis of oxidation-reduction reactions in mammalian metabolism. He used adrenal cortex tissues from slaughterhouses and rapid *in vitro* assays (41). Only four years later after its isolation from lemon juice by another group was it realized that Szent-Györgi had already isolated the vitamin (42). Then, in a remarkably short time, chemists were able to determine its molecular structure, synthesize it and confirm the product's biological activity (43). Vitamins could no longer be described by skeptics as "hypothetical entities."

In 1939 a surgeon at Harvard Medical School fed himself a diet containing no vitamin C, but supplements of all other vitamins. After 26 weeks he developed hemorrhages on his legs, a wound inflicted on his back failed to heal and he rapidly became exhausted. Upon dosing with ascorbic acid these problems quickly disappeared (44).

### Beriberi and vitamin B

We have seen already that McCollum and others had produced signs of polyneuritis in rats using purified diets with a source of vitamin A and had characterized the "antiberiberi factor" as vitamin B. It was then realized that autoclaved yeast, although it had lost its "anti-neuritis" activity, still promoted growth in rats so that it must contain a second factor; these were then called B<sub>1</sub> and B<sub>2</sub>, respectively.

The isolation of B<sub>1</sub> was achieved in 1926 by Dutch scientists in Java using small "rice birds" fed on washed white rice supplemented with cod liver oil for their assays. Starting with nearly 700 pounds of rice polishings, they obtained 100 mg of crystals so potent that only 10 µg was needed to cure a deficient pigeon (45). The next problem was to determine the structure of the crystals of the chloride salt that had an empirical formula of C<sub>12</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>4</sub>OS. This was finally achieved and a biologically active compound synthesized in 1936 (46,47). It was named Thiamin(e) as "the vitamin containing sulfur (*thios* in, Greek)".

Meanwhile Rudolph Peters at Oxford was investigating the function of the vitamin. It was known that deficient subjects maintained unusually high levels of pyruvic or lactic acid in their blood after exercise, and his group obtained evidence that thiamin pyrophosphate served as a cofactor for the enzyme pyruvate decarboxylase. He introduced the term "biochemical lesion" to describe the effect of its deficiency (48). This was the first of a long series of findings that B-vitamins generally served as part of coenzymes concerned with different aspects of metabolism.

### Pellagra in the United States

This is a disease characterized by dermatitis of areas exposed to sunshine, and gastrointestinal and mental disturbances (Fig. 3). There probably were a few earlier cases of pellagra in the Southern States, but from 1905 it became common. At one mental hospital in 1906 there were 88 sufferers of whom 57 died (49). By 1909 pellagra had spread to many parts of the South, and nearly 350 physicians attended the first national conference held in South Carolina. There were many ideas as to the cause of the disease. One from Italy, where pellagra had long been a problem, was that corn that had been damaged or inadequately dried became moldy with pathogenic fungi (50). Another, because it occurred mostly in the poorer areas, was that flies made their way into inadequately screened outdoor privies and then carried infected excreta onto food being prepared in kitchens (51).

Joseph Goldberger, put in charge of the Public Health Service's pellagra program in 1914, began systematic investi-

TABLE 1

A selection of the results obtained from feeding guinea pigs a scorbutic diet supplemented with lemon juice or lime juice from different sources (38)

Material tested	Daily dose	Degree of protection <sup>1</sup>
	<i>ml</i>	
Lemon juice, fresh	1.5	++
Lemon juice, fresh	2.5	+++
Lime juice, fresh	2.5	+
Lime juice, fresh	5	++
Lime juice, navy issue (stored)	5	0
Lime juice, navy issue (stored)	10	0+
Lime juice, 6 commercial samples	5	0-+

<sup>1</sup> Full protection, +++; partial, ++; slight, +; none, 0.



**FIGURE 3** A patient with pellagra showing dermatitis on the parts of her body that had been exposed to strong sunlight, H.F. Harris, 1916 (127).

gations. Because nurses or physicians treating the sick never developed the condition, he believed that pellagra was not infectious and was willing to put his life on the line for it. He received subcutaneous injections of blood from patients, then had skin eruptions rubbed into his nasal mucosa, and finally ate some of their excreta; then some of his fellow physicians followed (52). It was a tradition of the Service that officers would expose themselves to the same degree of risk as those in the Armed Services. In this case none was made ill.

Goldberger thought that an unbalanced diet was responsible and persuaded authorities in Mississippi to allow 12 prisoners to volunteer to eat for six months an experimental diet that might induce pellagra. In return the prisoners would be released at the end of the period—if still alive! The diet had abundant corn and other cereals but no meat or dairy products. After five months, six of the men had developed dermatitis on the scrotum and in a few cases, on the back of their hands (53). Goldberger was satisfied that this was pellagra, but the volunteers immediately fled after obtaining their release and he could not demonstrate their condition to physicians who doubted whether he truly had produced the disease (54).

In another study in South Carolina mill villages, Goldberger's group found that pellagrous families had purchased a very similar pattern of foods to that of healthy families, but the latter nearly all kept a cow and were obtaining abundant milk (55).

They now tried to find an animal model with which they could assay the antipellagrous activity of different foods and

extracts. Early work with monkeys and rats failed to elicit anything resembling the human disease. McCollum too had been finding that rats failed to show anything like pellagra when fed diets resembling those used in Goldberger's prison experiment and referring to the doubts as to whether pellagra had been produced there, he concluded that "probably . . . pellagra is caused by an infectious agent" (55). So the man whose name is linked to the discovery of vitamins wanted at one point to erase both pellagra and scurvy from the list of deficiency diseases.

Goldberger's group persevered with searching for an animal model. Dogs given mixtures with mostly cornmeal and no meat or milk powder developed a condition called 'black-tongue', showing red lips with patches of necrosis, drooling and loss of appetite. The group considered this to be a model for pellagra after the dogs responded rapidly to yeast, and this proved equally valuable for pellagrins (57).

Dogs were then used to assay fractions obtained from yeast and liver. Finally in 1937 after nicotinic acid had been found to be a bacterial growth factor, both it and nicotinic amide were found in Wisconsin to be extremely potent in curing blacktongue, and also pellagrous patients in Alabama (58,59). This was the only example of a vitamin, an already familiar chemical, now being given the blander name of "niacin." This is not the end of the story; it will be continued in Part 4 of this series.

### Riboflavin

It was soon realized that the "B<sub>2</sub>" in autoclaved yeast was a complex of factors. The first to be investigated, and originally called 'vitamin G,' was tentatively identified with the green-fluorescent pigment of whey (60). It was isolated and then synthesized in 1935, and named riboflavin (61). It was then discovered that some dogs on a "blacktongue" diet that had collapsed with a prior condition of "yellow liver," could be saved by giving them riboflavin. It was also found that pellagrins who still showed cheilosis (lesions about the mouth) after being treated with niacin responded rapidly to riboflavin (62). It appeared that most patients with signs of pellagra were also at least marginally deficient in riboflavin in addition to niacin.

### Folic acid

The trail to the discovery of this vitamin began in India. Lucy Wills went to Bombay in 1928 to investigate the macrocytic (large cell) anemia of pregnancy most commonly seen in Mohammedan women. After failing to associate the condition with infection or deficiency of vitamins A or C, she found that yeast and its proprietary extract "Marmite" were highly effective in curing the condition (63). Back in England she and colleagues reported in 1937 that feeding a poor Bombay diet to rhesus monkeys also induced macrocytic anemia and leucopenia; this responded to both Marmite and to crude liver extracts, but more refined liver extracts given parenterally, and effective in patients with pernicious anemia, had no effect (64). Others confirmed that the condition in monkeys did not respond to any of the known vitamins and called the deficient factor "vitamin M" (for monkey) (65).

In the same period workers interested in poultry nutrition had found that chicks fed purified diets containing all of the then known vitamins still grew slowly and developed a macrocytic anemia, and in 1944, that this could be prevented by giving them crystalline "vitamin B<sub>c</sub>" isolated as a growth factor for certain bacteria (66). The same or a closely related compound had also been obtained from spinach and named "folic acid" because it had come from "foliage." These materials were

also active in treating the experimental monkey anemia and it was hoped that they would be clinically effective in India when supplies became available (65).

The chemical identification and syntheses of these active compounds will be considered in Part 4.

### Other B vitamins

Once it was clear that “vitamin B-2” was a combination of different factors, the race was on to identify them. Space does not permit describing this demanding work but in principle, it was of the same type that had been used to separate and identify the first vitamins discovered using baby chicks, yeast and lactobacilli, as well as rats (68,69). By 1937 pantothenic acid, B<sub>6</sub> [the ‘pyridoxine (PN)’ group] and biotin were added to the list of water-soluble vitamins (Table 2). Choline also was recognized to be essential for poultry in addition to having a lipotropic value for mammals under some conditions (70). “The existence of other factors, including B<sub>4</sub>, B<sub>10</sub> and B<sub>11</sub> was claimed and then quietly abandoned” (71).

### The fat-soluble vitamins

**Vitamin A and carotene.** In the first section of this history Part 3, we reviewed early work that led to the concept of “vitamin A” as a fat-soluble vitamin needed by young rats to support growth and to prevent the development of xerophthalmia. In Denmark during World War I, when fats were in short supply, an unintended experiment occurred in a children’s home. In one group of 16 children, 8 developed xerophthalmia, while no cases developed in a second group. The only difference in the diets of the two groups was that the second had received whole cream milk in the previous six months. Carl Bloch, the pediatrician in charge, then began to give cod liver oil to the affected group; their eye problems cleared up in eight days and they began to grow faster (72,73). Clearly, the work with rats had some practical relevance.

The problem now was to identify the vitamin, which appeared to exist in at least two forms: a highly colored form in leaves and carrots, and a colorless form in animal fat. Crystals of  $\beta$ -carotene, a polyunsaturated hydrocarbon, were obtained from carrots and found to be active. The colorless factor was more difficult to obtain but the activity of extracts correlated with a characteristic color formation with antimony trichlo-

ride that could be differentiated from the color obtained with carotene. It was then found that giving carotene to rats depleted of vitamin A resulted in the reappearance of the color reaction of the “animal” factor in extracts from their livers (74). Thus carotene appeared to be a precursor of the final vitamin, and this was confirmed when the actual vitamin was finally isolated from fish liver oils in 1939 using centrifugal molecular stills, and its structure identified (75,76).

Synthesis proved particularly difficult. A major contributor wrote, “After so many years, victory has come and the romance of high hopes and bitter disappointment will in a few years simply be recorded in textbooks of organic chemistry in a few terse sentences” (76). Sad but realistic. The vitamin, now named “retinol,” was an alcohol attached to a long unsaturated carbon chain linked in turn to a  $\beta$ -ionone ring, and  $\beta$ -carotene could be considered as two retinol molecules condensed through their alcohol groups.

**Vitamins E and K.** In 1922 H. M. Evans and Katharine Bishop working at Berkeley found that a purified diet with vitamin supplements that supported good growth in female rats nevertheless failed to support normal reproduction; the embryos were being resorbed before the end of pregnancy (77). Lettuce was the first food found to prevent this problem, but then wheat and in particular, wheat germ oil. Cod liver oil seemed unexpectedly to increase the problem. The active factor was named “vitamin E” and following further investigations by many groups, it was isolated in 1935 and named “tocopherol” (from Greek terms signifying “the childbirth-producing alcohol”). Three years later the Swiss chemist Paul Karrer synthesized it by condensing phytyl bromide with trimethyl hydroquinone (78). He too received a Nobel Prize for his work on the chemistry of several vitamins.

During this period there was a change in thinking about the effects of a deficiency of vitamin E. In rats, males showed testicular degeneration, but it was realized that in pregnancy it was the fetus that failed to develop rather than the dam being at fault. It was also found that vitamin E deficiency in lambs and rabbits resulted in muscular degeneration rather than infertility; and in chicks it resulted in exudative diathesis and/or encephalomalacia, both related to disturbances in the vascular system (79).

Vitamin E became a popular treatment, backed by reports of early successes, for a number of clinical conditions including abortions, impotence and various forms of muscular dystrophy, but with more controlled testing, few if any of these claims could be confirmed (80).

Hemorrhaging in chicks, which responded to dosing with cabbage, was another disease that at one time was thought to possibly be caused by a deficiency of vitamin E. The Danish worker Henrik Dam reported in 1935 that it was the deficiency of a new fat-soluble vitamin, which he named “vitamin K” in recognition of its essential role in blood coagulation (“Koagulation” in Danish and German) (81). It was discovered to occur naturally in modified forms in many plants and to be produced by bacterial growth in stored animal products (82,83). The vitamin also cured the hemorrhaging of patients with obstructive jaundice who lacked bile to aid absorption of the vitamin and of cattle that had been eating sweet clover hay that contained an anti-vitamin (84).

Herman Almquist and a colleague at Berkeley could have published the discovery of vitamin K before Dam, but had to delay publication until a controversy on campus about the cause of the chick disease had been resolved and so missed a Nobel prize. By this period there were so many people at work on nutritional problems that such things were almost inevitable. Many years later Almquist was to write philosophically,

TABLE 2

*Timelines for the discovery of the vitamins up to 1944.  
Modified, with permission, from Combs, 1992 (128)*

Vitamin	Year		Structure determined	Synthesis achieved
	proposed	Isolated		
Thiamin	1901	1926	1936	1936
Vitamin C	1907	1926	1932	1933
Vitamin A	1915	1939	1942	—
Vitamin D	1919	1931	1932	1932
Vitamin E	1922	1936	1938	1938
Niacin	1926	1937	1937	1867 <sup>1</sup>
Biotin	1926	1939	1942	1943
Vitamin K	1929	1939	1939	1940
Pantothenic acid	1931	1939	1939	1940
Folate	1931	1939	—	—
Riboflavin	1933	1933	1934	1935
Vitamin B <sub>6</sub>	1934	1936	1938	1939

<sup>1</sup> This compound, nicotinic acid, was known long before its vitamin activity had been discovered.

“More often than not, a discovery is really born of a converging culmination of scientific leads . . . to all of which many have contributed . . . a stage has been set . . . there are many who can do the job if they happen to be on the scene” (85).

### Essential fatty acids

In 1929 George and Mildred Burr, who had moved from working with H. M. Evans and were now at the University of Minnesota, went to great trouble to prepare diets that were completely free from fat using sucrose instead of corn starch (because the latter contains 0.7% lipid unextractable with ether) and supplying vitamins A and D by saponifying cod liver oil and adding only the nonsaponifiable fraction. They found that rats fed such diets for many months failed to reach their normal mature weight; they also lost fur and their tails became inflamed and scaly. In the following year they found that giving the animals small quantities of methyl linoleate, but not butterfat, prevented the condition (86). Much more would be discovered later about the functions of the polyunsaturated acids.

### Proteins and amino acids

We now return to the beginning of the period. One aim of the early work with rats was to investigate the relative nutritional value of different proteins and this became possible with diets that included protein-free milk (skimmed milk acidified, heated and filtered). Osborne and Mendel had already found that gliadin, isolated from wheat, supported far slower growth than was obtainable with casein (Fig. 4). Now they found that with 18% zein, the corn protein that lacked tryptophan and lysine, rats failed to grow unless the diet was supplemented with both these amino acids (87,88). It appeared therefore that certain amino acids were “essential,” meaning that they could not be synthesized by the body and needed to be provided in the diet.

Further work showed that mixing proteins deficient in different amino acids could result in mutual complementation (89). However, proving that the value of proteins was deter-

mined only by their digestibility and amino acid balance took considerably longer.

Chemical methods of amino acid analysis were slow and subject to interference (90). However, microbiological methods taking advantage of the complex requirements of lactobacilli were developed in the 1940s and allowed many more assays to be completed (91). From these it was possible to determine the “chemical score” of food proteins calculated as the lowest percentage value when the level of each essential amino acid was compared with that found in whole egg (taken as a provisional standard). These “scores” were then found in most cases to correlate well with the relative values of the same foods as determined in protein quality tests with young rats, based either on weight gain or nitrogen balance (92).

In 1930 William Rose, who had earlier been a graduate student with Mendel and attended Chittenden’s lectures, was a professor at the University of Illinois, Urbana campus. He and his group set out to develop amino acid mixtures that would support good growth in rats. They began with 19 known amino acids, but the animals failed to grow. With hindsight it is surprising that their list did not include methionine, discovered at Harvard in the 1920s as being required for the growth of certain bacteria (93). However, it was largely ignored by nutritionists for another decade, perhaps because they knew that rats receiving casein as their sole protein would grow faster if cystine were added to the diet, so that cystine appeared to be the sulfur-containing essential amino acid. Even with the inclusion of methionine, rats still failed to grow at Urbana unless they received in addition the mix of amino acids obtained from an acid-hydrolysate of casein.

Attempts to isolate an active factor from the hydrolysate proved very frustrating until it was realized that it was supplying two additional materials. With further careful fractionation, Madelyn Womack working with Rose, discovered that one was isoleucine (94). At that time there was no method for determining isoleucine separately from leucine. It was included in the original mix, but at a level well below what turned out to be its requirement.

The second factor was a previously unknown amino acid, which they identified as 2-amino-3-hydroxybutanoic acid and named threonine (95). When it was included in the amino acid mix the rats grew well so that this was a most important finding. Reviewers have referred to “the very high level of chemical competence and skill” with which these studies were conducted and, for example, to “fivefold crystallizations of tryptophan and histidine,” even after analyses had shown them to be essentially pure (96).

Starting in 1942, Rose set out to extend the study to human adults. This involved preparing much larger quantities of amino acids, and then careful control of the energy intakes of volunteers and measurement of their nitrogen balance. All of this took time and the findings will be considered in Part 4.

Meanwhile, the availability of isotopes had allowed a new approach to studying the fate and distribution of nutrients in the body. In 1939 Rudolf Schoenheimer and his colleagues at Columbia University reported results from feeding rats for three days a physiological dose of *l*-leucine, doubly labeled with N<sup>15</sup> and deuterium (replacing hydrogen in the side chain). They found that less than one third of the N<sup>15</sup> had appeared in the urine, but that 57% was incorporated into body proteins, much of it in other amino acids with the exception of lysine (97). It was assumed that this was the consequence of transamination reactions.

Schoenheimer thought at this time of protein molecules opening to release one amino acid molecule at a time into the blood stream before reattaching a replacement. In any case, it



**FIGURE 4** Two female rats from the same litter at 140 d of age after receiving: (A) 18% casein as the sole protein source, and (B) gliadin (from wheat); other diet components being identical, Osborne & Mendel, 1911 (23, Plate 1).

appeared that most synthesis of body proteins must be coming from recycled amino acids rather than from newly digested dietary protein. As he was to write, "If the starting materials are available, all chemical reactions which the animal is capable of are carried out continually" and "The synthesis of amino acids, like that of fatty acids . . . proceeds even when there is no obvious need for it" (98).

### Mineral elements

The first workers with purified diets felt that they could meet their animals' mineral requirements by giving them the residual ash from combustion of an apparently satisfactory food such as milk or dog biscuits. Then when the problems of supplying vitamins and amino acids had been solved they could begin to study individual mineral requirements. One early finding, already quoted, was that rats would become rachitic if either the calcium or phosphorus content of the diet was greatly increased in the absence of vitamin D or irradiation.

The development by 1929 of analytical procedures using emission spectroscopy allowed the detection of trace elements in foods. Thus, it was found that cow's milk contained strontium and vanadium in addition to the previously detected iron, copper, zinc and manganese, as well as larger quantities of calcium, magnesium, potassium, sodium and phosphorus (97). Chlorine and iodine had been found using other procedures. There was no doubt in the minds of workers that the more abundant elements were essential, and studies now began on possible requirements for the 'trace elements' (100).

At Wisconsin copper had already been found to be required for the production of hemoglobin in rats fed purified diets (101). Then in 1931 a deficiency of copper was found to be responsible for a characteristic sickness occurring in cattle in parts of Florida (102). In the same year McCollum's group reported results with young rats fed a purified diet designed to be as low as possible in manganese content. They grew normally, but the males were sterile with testicular degeneration, and the females would breed when mated with normal males, but were unable to suckle their young (103). Again, a few years later manganese deficiency was recognized at Cornell as being a practical problem, this time in intensive poultry production and responsible for "perosis" (a crippling deformity of the leg bones) in young birds (104).

Magnesium was known to be present in both bones and soft tissues of animals but it proved difficult to obtain a deficiency condition in rats. Finally, by feeding rats a diet that after careful purification contained only 1.8  $\mu\text{g/g}$  of the element, McCollum's group produced a characteristic condition of tetany (105). In contrast, grass-fed cattle would sometimes develop tetany that responded to dosing with magnesium salts even when the level in their blood appeared normal (106).

It also proved possible to produce zinc deficiency with a highly purified diet on which rats showed slow growth and loss of hair (107). However, no evidence of zinc deficiency being a practical problem was seen in the period under review. In contrast, serious problems affecting cattle and sheep in parts of Australia were recognized in 1937 as being due to cobalt deficiency without its having first been produced experimentally in laboratory animals (108). The special function of cobalt in ruminant nutrition would only be worked out in a later period.

The importance of iron in the prevention of microcytic anemia in human subjects was previously discussed (2). Interestingly, iron deficiency had been much less of a problem in traditional animal husbandry, in large part because animals had access to soil which is rich in iron. However, when sows

began to be brought indoors in the 1920s for farrowing, the piglets had a higher death rate. This was found to be the result of anemia and workers in Scotland prevented it by adding iron salts to the sows' feed (109). It was thought that the piglets obtained additional iron from contamination with her feed and feces rather than from any additional iron appearing in the sow's milk and this was confirmed in Wisconsin (110).

In 1937 Robert McCance and Elsie Widdowson, working in London at that time, published a classic paper arguing that contrary to current opinion, humans had little or no ability to excrete iron, and that there must therefore be a mechanism that regulated its absorption according to need (111). This was confirmed by work with dogs using radioactive iron when it became available from the Berkeley cyclotron. It was interesting that absorption did not increase immediately after dogs had been bled to induce anemia, but only seven days later when body iron stores had been exhausted as a consequence of increased synthesis of red cells (112).

### Iodine

As previously stated, the old idea that goiter was caused by deficiency of iodine became discredited in the 1800's as a result of toxic overdosing (1). However, it gradually became recognized that iodine was concentrated in the thyroid gland and that the enlarged thyroid in goiter had a low iodine content (113). The first thyroid hormone, thyroxine was also found to contain iodine in its molecule (114).

Old observations that the incidence of goiter did not seem to parallel the deficiency of iodine in local water and food supplies had made many enquirers believe that other factors must also be at work. In 1928 workers at Johns Hopkins discovered that rabbits fed mainly cabbage and being used for a study of infections had developed goiters (115). Deliberate studies with rats then showed that most Brassica plants and also unprocessed soybeans had goitrogenic activity that responded to higher intakes of iodide (114,115). However, feeding Brassica seeds resulted in goiters that were reversed with thyroxine but not with iodine (116).

In 1917 David Marine organized a large-scale trial of iodine supplementation of schoolgirls in an area of Ohio where the disease was endemic (119). The results of reexamination of these subjects six months after their first treatment are summarized in Table 3. Longer periods of treatment produced more complete freedom from the problem with no evidence of harm. After this iodized salt began to be produced in many parts of the world with government encouragement.

### Fluorine

Fluoride ions were recognized in the 1930s as being toxic for livestock in areas where fluoride-rich ash was scattered as a

**TABLE 3**

*Results from the reexamination of the thyroids of schoolgirls in Akron, Ohio 6 mo after dosing with 2.0 g sodium iodide (119)*

	Control groups	Treated groups	
Number initially normal	1257	908	
Worsened after 6 months, %	27.6		0.2
Number initially enlarged	1048	1282	
Worsened after 6 mo, %	14.1		0.2
Unchanged after 6 mo, %	72.0		39.5
Improved after 6 mo, %	14.0		60.3

result of mining or smoke from the manufacture of aluminum, with animals becoming stiff and lame and without appetite (120). In the same period it was realized that mottling of the enamel of human teeth in particular areas was associated with a relatively high fluorine content of the local water supply (121). It was then appreciated that at the other extreme children growing up where the water was low in fluoride had more dental caries. This was accepted only with great reluctance, and the fluoridation of water supplies low in fluoride was delayed for several years (122).

### Diet restriction and life span

Much of the research in this period was concerned with discovering what was needed to obtain maximum growth rates in young animals, presumably with an underlying assumption that this was “a good thing.” However, in 1917 the Connecticut group reported that stunting female rats for their first year of life by restricting their food supply actually led to their living and remaining fertile, with vigorous young, to a greater age (123).

Clive McCay had learned of this work while with Lafayette Mendel on a postdoctoral fellowship. Mendel had told him that a younger man was needed to pursue such long-term work. McCay, who had moved to a faculty position at Cornell in 1927, decided to take it on. He and his colleagues confirmed that, indeed, the life of rats, particularly males, could be greatly extended by restricting their diet for an initial year or even two years, though he commented that, “it seems little short of heresy to present data [supporting] the ancient theory that slow growth favors longevity” (124,125). However, they also had to report that when the heating failed in the animal room it was only the skinny ones that succumbed!

In addition to the advances in scientific knowledge in this period, there were important advances in its practical application. Distributing iodized salt, as already mentioned, significantly reduced the incidence of goiter. But there can be difficulties; for example, fortifying foods with iron salts may result in rancidity and destruction of vitamin A. Solving such technical problems can be as demanding as the original discoveries of nutrients, but is outside the scope of these articles as is the work of developing standards for the level of individual nutrients in adequate diets and also the evaluation of nutritional status in different population groups.

Those who had begun their careers in the 1930s looked back on it as “the golden age of nutrition,” with its rapid discoveries of one nutrient after another. We are certainly indebted to them for their findings, but did they leave anything still to be discovered? That is what will have to be considered in Part 4.

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