Chapter 30

Historical aspects of the major neurological vitamin deficiency disorders: the water-soluble B vitamins

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INTRODUCTION

This chapter will review the major neurological disorders associated with deficiencies of the water-soluble B vitamins, including particularly beriberi, Wernicke-Korsakoff disease, pellagra, neural tube defects, and subacute combined degeneration of the spinal cord.

THIAMIN (VITAMIN \( \text{B}_1 \)) DEFICIENCY: BERIBERI AND WERNICKE–KORSAKOFF DISEASE

Peripheral nervous system manifestations of thiamin deficiency have been recognized for millennia in Asia in the form of a sensorimotor polyneuropathy called beriberi. People affected by beriberi first develop nonspecific constitutional symptoms including weakness, fatigue, irritability, anorexia, and abdominal discomfort. As the disease progresses, patients develop symptoms of peripheral polyneuropathy with paresthesias, neuropathic pain, and numbness (referred to as “dry beriberi”), often accompanied by congestive heart failure with pedal edema, pleural effusions, and pulmonary edema (“wet beriberi”).

The prevalence of beriberi increased greatly in Asia with a change in the milling process for rice in the late 19th century, around the time that the central nervous system manifestations of thiamin deficiency—Wernicke’s encephalopathy and Korsakoff’s psychosis—were recognized in Europe. Only in the 20th century were these disorders all clearly linked to a deficiency of a specific dietary factor, which was ultimately determined to be the vitamin now called thiamin. The isolation and synthesis of thiamin in the 1930s greatly improved the acute treatment of these disorders, but more importantly made possible the prevention of large outbreaks of beriberi, as well as the prevention of many sporadic cases of all neurological forms of thiamin deficiency, through food fortification.

Brontius’s description of the sensorimotor neuropathy of beriberi

Dutch physician Jacobus Brontius (1592–1631), frustrated by the meager earnings from his practice in Leyden, accepted a job in 1627 as physician for the Dutch East India Company in Batavia (now Jakarta), on the island of Java (in what is now southern Indonesia). In Batavia, Brontius observed and studied a wide range of novel tropical diseases, and gave the first European description of the sensorimotor polyneuropathy of beriberi in a book, De Medicina Indorum Libri IV, which was first published posthumously in 1642 (Brontius, 1745/1945). Brontius noted that the word beri-beri, meaning sheep, was applied because afflicted individuals had a steppage gait that resembled the gait of sheep. Among the clinical features recognized by Brontius were generalized weakness, tremulousness, and paresthesias.

Takaki and the dietary prevention of kakke (beriberi) in the Japanese navy

Although beriberi had been recognized in Asia for several thousand years, its incidence increased dramatically in the 1870s, when it became one of the most common diseases in Asia as an unrecognized consequence of a change in diet of the population. By this time, steam-driven mills had been introduced to Asia from Europe and were replacing the previous milling process (Verhoeft et al., 1999). The new steam-powered mills efficiently removed the so-called “polishing” and

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with these went essential nutrients, including thiamin. The new polished rice was considered to be superior in taste and quality and became a dietary staple throughout Asia.

From 1878 to 1882, approximately one third of enlisted Japanese sailors reported ill with kakké (i.e., beriberi) annually (Takaki, 1906a, b, c; Itokawa, 1976; Hawk, 2006). Japanese military physician Kanehiro Takaki (1849–1915) noted that the diets of Japanese sailors were relatively deficient in nitrogen content (i.e., protein) compared with the diets of British and German sailors, who were not susceptible to beriberi. As a result, Takaki incorrectly attributed the beriberi among Japanese sailors to a dietary deficiency of protein.

On a training cruise in 1883, 161 of the 278 Japanese sailors (58%) developed beriberi and 25 died (9%), prompting Takaki to push for dietary reforms. After receiving permission for a trial of a modified diet, Takaki arranged for a repetition of the training cruise the following year, with all factors held constant except for the diet, which was modified by increased amounts of meat, barley, and fruit (thus increasing the presumptively deficient nitrogen content). In contrast to the heavy toll the previous year, there were no deaths and only 14 cases of beriberi, all among sailors who refused to eat the full rations of meat and milk. With these dramatic results, the diets of all Japanese sailors were similarly modified, so that, by 1887, Takaki reported that there were only three cases with no deaths over the previous year, compared to more than 1000 cases annually prior to 1884.

**Eijkman and the polyneuritic chickens of Java**

In 1886, the Dutch government, hoping to find the cause of beriberi that had become a tremendous problem for its colonies in the East Indies, sent a commission to investigate under the direction of Cornelius Pekelharing, Professor of Pathology at the University of Utrecht, with Cornelius Winkler, a neurologist (Eijkman, 1929/1965; Carpenter, 2000). When Pekelharing went to Berlin to learn from Robert Koch (1843–1910) the latest microbiological techniques for use in his investigations of beriberi, he met Dutch military physician Christiana Eijkman (1858–1930), who had been studying with Koch since 1885 (only 3 years after Koch’s revolutionary discovery that tuberculosis is caused by a specific bacterium). Impressed with Eijkman, Pekelharing asked that he be assigned as an assistant to the commission.

From late 1886 through the summer of 1887, the commission focused on possible infectious causes of beriberi at a laboratory established in the Military Hospital in Batavia, Java (now Jakarta, Indonesia). In late 1887, Pekelharing and Winkler were recalled to Holland (where Winkler was appointed as the first professor of neurology in the Netherlands) and Eijkman was appointed director of the laboratory (Verhoef et al., 1999; Carpenter, 2000). Eijkman subsequently tried unsuccessfully to infect rabbits and monkeys with the microorganisms that his colleagues had isolated from people who had died of beriberi. Undaunted, Eijkman concluded that the responsible infection must be slowly progressive, with considerable time needed for clinically evident manifestations. To make sure that extraneous factors were not responsible for the observed results over a long time interval of disease development, many control animals were needed. By late 1889, he had begun using chickens for these injection studies, presumably because the chickens were cheaper and easier to maintain.

At that point, Eijkman was fortunate to observe a serendipitous event, astute enough to understand its possible significance, and diligent enough to pursue the necessary studies to evaluate the possibilities: a “polyneuritis” broke out among the laboratory’s chickens, which was characterized by an unsteady gait with frequent falls and difficulty in perching, later an inability to stand or fly attributed to ascending weakness, and finally slowed respiration, cyanosis, hypothermia, progressive lethargy, and neck extension preceding death (Eijkman, 1929/1965, 1990). Histological examination of peripheral nerves stained by the Marchi method demonstrated axonal degeneration, most pronounced in the legs, which was thought to resemble the changes seen in the peripheral nerves of people who had died of beriberi (Eijkman, 1990; Carpenter, 2000). Curiously, both injected and control chickens were affected, but, because they had been kept together in large cages, Eijkman suspected that the chickens he had injected with microorganisms had somehow contaminated the control chickens. However, in additional experiments he found that keeping the chickens in separate cages made no difference, causing him to wonder whether the entire institute had become infected. Further studies to elucidate the putative infection were unrevealing, but a new possibility presented itself when Eijkman learned that the onset of polyneuritis in chickens coincided with a change to polished rice, which only resolved when they were serendipitously switched back to brown rice (Eijkman, 1929/1965, 1990).

Eijkman then began “deliberate feeding experiments” with chickens. In 1889, Eijkman found that chickens fed on a diet restricted to cooked polished rice developed polyneuritis generally after 3 or 4 weeks, but recovered if returned to feed-grade unpolished rice (Eijkman, 1990). Despite his dietary experiments, Eijkman had trouble abandoning his initial microbiological framework: in keeping with the late-19th-century concept of “ptomaines,” Eijkman
suggested that “cooked rice favored conditions for the development of micro-organisms of a still unknown nature in the intestinal tract, and hence for the formation of a poison causing nerve degeneration” (Eijkman, quoted by Carpenter, 2000, p. 39).

Later experiments, from 1891 to 1895, demonstrated that the difference between polished and whole rice could not be attributed to inadequate preservation or contamination of the polished rice (e.g., by a microbial toxin), because: (1) freshly prepared polished rice could also cause beriberi; and (2) beriberi did not develop from brown or “rough rice” (i.e., with only the coarse husk removed but still containing the “silver skin” or pericarpium and the germ largely intact), even though this form of rice deteriorates much more quickly (Eijkman, 1929/1965, 1990). Disease development also did not depend on whether the polished rice was cooked or raw, on the water used for cooking (as it even developed with artesian or distilled water), or on the presence of coarse rice husks as a source of dietary fiber. Importantly Eijkman found that the polyneuritis could be cured or prevented by feeding the chickens either unpolished rice or the discarded rice polishings.

Feeding the chickens other starchy plant foods (e.g., sago and tapioca) produced identical results to those with cooked rice, making Eijkman wonder whether beriberi is at least in part due to lack of adequate food, as some of the birds were considerably emaciated—a suspicion seemingly confirmed when the birds recovered when fed only meat. However, birds fed on a combined diet of starch and meat ultimately developed beriberi, even though they did not become emaciated. Furthermore, simple starvation did not produce beriberi. Eijkman (1929/1965) concluded that “inanition in itself could not be the main cause of the disease (any more than ‘protein’ or ‘salt’ deficiency), even though it promoted it.”

Vorderman’s observational studies of beriberi in Java prisons

In late 1895, Eijkman discussed the etiology of beriberi with his friend Adolphe Vorderman (1844–1902), Inspector-General of Public Health, who was the government physician responsible for the medical inspection of prisons across Java. Because it was known that different prisons had different frequencies of beriberi, the two considered the possibility that the different prisons were using rice processed in different ways. Rations were highly standardized in Java prisons (e.g., 750 gm rice, 1 chili pepper, 150 gm of other mixed vegetables, etc.), but the type of rice was not specified and therefore open to the discretion of the prison governors, local market availability, prices, etc. (Carpenter, 2000). Vorderman wrote a form letter to each prison governor asking for the incidence of beriberi and the type of rice in use (Carpenter, 2000; Vandenbrouke, 2003). By 1896, preliminary results from available replies suggested that beriberi was almost exclusively confined to prisons using polished white rice. Based on these results, the government approved a larger and more in-depth study. Unfortunately, Eijkman was ill with malaria and had to return to Holland.

Vorderman spent the next 5 months visiting all 101 prisons scattered across the large island (some 54 000 square miles), taking samples of the rice used, recording the frequency of beriberi over the previous 18 months, and recording environmental information about each prison. When the rice samples were examined at the laboratory in Batavia, there was considerable variation in the completeness of deskinning, so that the rice had to be categorized as mostly polished (= 75% of grains were deskinning), mostly unpolished (< 25% of grains were deskinning), or intermediate. Of the 96 000 people imprisoned at institutions using unpolished rice, less than 1 in 10,000 developed beriberi, whereas of the 150 000 people at institutions using polished rice, 1 in 39 (2.8%) developed beriberi (Verhoef et al., 1999; Carpenter, 2000). Although beriberi prevalence varied dramatically with the type of rice used, it did not vary with the source of the rice (imported or locally produced), the age of the buildings, floor permeability, adequacy of ventilation, or degree of overcrowding. On the basis of these data, Vorderman persuaded governmental authorities to modify the prison diets to include more unpolished rice, and also more beans and other vegetables. Any subsequent change in incidence was apparently never formally studied (Carpenter, 2000), although it was reported that this public health measure rapidly eliminated beriberi from the prison populations (Verhoef et al., 1999; Vandenbrouke, 2003).

Vorderman’s study was a valuable early effort at observational epidemiology, but not without limitations. For example, Vorderman relied on retrospective clinical diagnoses (of variable validity), did not assess each prisoner’s duration of exposure to polished rice (especially when some sentences were as short as a few days and others were much longer), and did not address potential confounding factors (e.g., a difference in disease frequency for coastal and central prisons) (Carpenter, 2000).

Grijns’ dietary deficiency explanation of beriberi presaged the “vitamin doctrine”

Dutch physician Gerrit Grijns (1865–1944) from the University of Utrecht was assigned to continue the studies of beriberi in Java after Eijkman returned to
Holland in 1896. Having no preconceived ideas concerning etiology, Grijns considered additional possibilities that had not been addressed by Eijkman. Eijkman’s work had already indicated that it was not the protein in the silverskin of the rice that was important for preventing beriberi, so Grijns systematically excluded potential deficiencies of minerals or fats in the polished rice associated with beriberi (Carpenter, 2000). Grijns tested other foods and discovered that both mung beans and “pigeon peas” had antineuritic properties (Eijkman, 1929/1965; Carpenter, 2000). Grijns also excluded a toxic effect specific to rice starch by demonstrating that polyneuritis also developed in chickens fed only on autoclaved meat or on potato flour plus a protein supplement (i.e., mung beans in which the antineuritic properties were destroyed by autoclaving) (Eijkman, 1929/1965; Carpenter, 2000).

What dietary factor was left to consider, Grijns wondered, since the various components of a physiologically complete diet as then understood (i.e., sufficient proteins, carbohydrates, fats, inorganic salts, and water) had all apparently been excluded as possibilities? Grijns noted two well known but insufficiently appreciated facts that suggested foods might contain previously unidentified nutrients: (1) sailors suffering from scurvy could be cured by fresh meat and fresh green vegetables (or by citrus fruits or their juices, as was well known from James Lind’s experiments in 1747); and (2) infant formulas were not a satisfactory substitute for breast milk even with the same concentrations of protein, sugar, fat, and salts. Grijns’ subsequent attempts at extracting the antineuritic factor from rice bran were unsuccessful, but he discovered that the antineuritic factor had been destroyed by the processing method he used.

In 1901, Grijns considered two possibilities to explain the known facts concerning the etiology of beriberi: first, a “deficiency or partial starvation” of a substance that was necessary in small amounts for maintaining metabolic functions of the peripheral nervous system and the muscles; or second, the lack of a protective dietary factor that normally acts to maintain resistance of the peripheral nervous system to an environmental agent (e.g., a microorganism) that otherwise causes neural degeneration. In either case, Grijns supposed, beriberi was actually caused by a dietary deficiency of a specific natural substance found in certain foods (Carpenter, 2000).

Unfortunately, Grijns’ important work presaging the “vitamin doctrine” was published in Dutch and not widely recognized at the time. In 1929, Eijkman shared the Nobel Prize in Physiology or Medicine with Frederick Hopkins “for their discovery of the growth stimulating vitamins,” but Grijns’ important work was overlooked by the Nobel Prize Committee and Eijkman failed to give Grijns appropriate recognition in his Nobel Prize Lecture. Grijns research achieved wider recognition only after colleagues had his work translated and published in English in 1935 (Grijns, 1935).

Grijns’ work did lead to a clinical trial after Vorderman visited a mental hospital in Buitenzorg and discussed the treatment of beriberi with Hulshoff Pol, the physician in charge (Carpenter, 2000). Pol set up a controlled trial of 300 patients to compare the value of mung beans, green vegetables (suggested by Vorderman, because these were a dietary staple in the surrounding villages), regular disinfection (to assess the proposal of another physician that beriberi was spread by cockroaches), and a control with no specific treatment. As the patients were housed in 12 separate pavilions, the different treatments were allocated to four different groups of three pavilions each. After 9 months, none of the patients in the mung bean group had developed beriberi, compared with 19% in the green vegetable group, 42% in the regular disinfection group, and 33% in the control group. The three pavilions receiving beans were protected from developing beriberi, and further tests demonstrated that beans could reverse the pedal edema and congestive heart failure, though they did not restore the function of severely damaged nerves.

**Wernicke’s clinicopathological description of Wernicke’s encephalopathy**

Over a 1-year period in the late 1870s, German neuropathologist Carl Wernicke (1848–1905), working at the Charité in Berlin, treated three patients with an unusual constellation of neurological findings including mental disturbances, ophthalmoparesis, nystagmus, and ataxia (Wernicke, 1881/1977). The first was a 20-year-old woman Wernicke treated in early 1877 after she had swallowed sulfuric acid and developed protracted vomiting. After some degree of recovery, she developed photophobia, impaired vision, drowsiness, and gait ataxia. Examination disclosed somnolence, disorientation, apathy, and later extreme anxiety, nocturnal agitation, convergent strabismus with asymmetric lateral rectus weakness, vertical nystagmus, impaired convergence, optic disc swelling, and multiple flame-shaped retinal hemorrhages. The symptoms progressively worsened and she died within 2 weeks of onset. Wernicke subsequently observed similar symptoms in two alcoholic men, both of whom were hospitalized with agitated delirium.

Wernicke summarized the clinical features of his three cases, noting particularly the progressive opthalmoparesis, ataxia, and encephalopathy with either agitation or somnolence. In all three cases, pathological examination of the brain at autopsy demonstrated...
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numerous punctate hemorrhages symmetrically arranged in the grey matter around the third and fourth ventricles and the aqueduct of Sylvius. Wernicke considered these pathological changes to have resulted from an acute, inflammatory disease of the rostral brainstem involving cranial motor nerve nuclei of the extracranial muscles, analogous to poliomyelitis and its involvement of the grey matter of the anterior horns of the spinal cord—hence his term, “acute hemorrhagic polioencephalitis superior.”

Korsakoff’s clinical description of Korsakoff’s psychosis

From 1887 to 1889, in a series of three articles, Russian psychiatrist Sergei Sergeievich Korsakoff (sometimes spelled Korsakov, 1853–1900) gave a comprehensive description of a cognitive disorder now known as Korsakoff psychosis, occurring in conjunction with peripheral polyneuropathy (Korsakoff, 1889/1955; Victor and Yakovlev, 1955). As early as 1887, Korsakoff felt that the cognitive disorder and the polyneuropathy represented “two facets of the same disease . . . The pathologic cause provoking multiple neuritis may affect several parts of the nervous system, central as well as peripheral, and according to where this cause is localized there will be symptoms either of neuritis or of the brain” (quoted by Victor and Yakovlev, 1955, p. 395); hence his initial terms, “psychosis associated with polyneuritis” and “polyneuritic psychosis.”

By 1889, Korsakoff recognized that, “At times . . . the symptoms may be so slight that the whole disease manifests itself exclusively by psychic symptoms” (quoted by Victor and Yakovlev, 1955, p. 395); therefore, as Korsakoff stated in his final publication, “One might also call it psychosis polyneuratica, but using this designation one must remember that an idiopathic psychic disturbance may occur also in cases in which the symptoms of multiple degenerative neuritis may be very slight or even entirely wanting” (Korsakoff, 1899/1955, p. 402). The other terms suggested by Korsakoff—“toxemic cerebropathy (cerebropathia psychica toxemica)—were based on his concept that the diverse conditions associated with the disorder could all be “reduced to an incorrect constitution of the blood, developing under their influence and leading to an accumulation in the blood of toxic substances,” which could poison the central nervous system, the nerves, or both (Korsakoff, 1889/1955, p. 402).

Although many modern authors consider only a restricted form of cognitive disorder under the eponym of Korsakoff’s psychosis—i.e., the combination of anterograde amnesia and confabulation—Korsakoff originally described a much wider range of mental states, often occurring sequentially, including an agitated delirium, an apathetic acute confusional state, and a confabulatory anterograde amnestic state. It was, however, the confabulatory amnestic state, typically following an agitated delirium, that most intrigued Korsakoff:

Korsakoff based his conclusions on at least 46 patients—approximately two thirds of whom were alcoholics, with the remainder having a wide variety of conditions often associated with protracted vomiting, including postpartum infections, intestinal obstruction, abdominal tumor, typhoid fever, and jaundice. Korsakoff’s writings do not include a pathologic description of the disease. Also, he was apparently unaware of the important association of the cognitive and neuropathic features with the oculomotor findings and ataxia described by Wernicke in 1881. Korsakoff did mention that “sometimes there are ophthalmoplegia externa, nystagmus, and like manifestations,” but he attached no great significance to this, considered these symptoms among a range of other manifestations that indicated “a disturbance of the entire organism,” and did not pursue this further (Korsakoff, 1889/1955, p. 399).

Relationship between Wernicke’s encephalopathy and Korsakoff’s psychosis

Neither Wernicke nor Korsakoff appreciated the close relationship between the disorders the two of them described. It was not until the early years of the 20th century that Bonhöffer recognized the close relationship between Korsakoff’s psychosis, delirium tremens, and Wernicke’s encephalopathy (Bonhöffer, 1901, 1904). Bonhöffer also recognized that the lesions in Wernicke’s encephalopathy are not inflammatory. By 1904, Bonhöffer concluded that neuritis (neuropathy) and a memory disorder can be found in all patients with Wernicke’s encephalopathy. During the subsequent decade, several authors noted the frequent co-occurrence of Wernicke’s encephalopathy and Korsakoff’s psychosis.

Because Wernicke’s encephalopathy was often fulminant, autopsies were done on many of the cases, including Wernicke’s first three cases (Wernicke, 1881). In contrast, Korsakoff’s psychosis often required survival from Wernicke’s encephalopathy to be manifest, so that pathologic material was less available. Consequently Korsakoff was unable to describe the pathology, despite having evaluated at least 46 patients. The clinico-pathologic overlap was nevertheless ultimately recognized between Wernicke’s encephalopathy and Korsakoff’s psychosis (Gamper, 1928; Kant, 1932–1933; Campbell and Biggart, 1939).
For example, Kant (1932–1933) noted an amnestic disorder in all patients presenting with Wernicke’s encephalopathy, and also found the characteristic brain stem pathology of Wernicke’s encephalopathy in all fatal cases of Korsakoff psychosis.

In the 1920s and 1930s, careful pathological studies noted the selective distribution of symmetric lesions affecting the mamillary bodies, the grey matter immediately surrounding the third ventricle and involving the hypothalamus and the medial portion of the thalamus, the periaqueductal grey matter (including the oculomotor nuclei), the posterior colliculi, and less frequently the floor of the fourth ventricle (involving the dorsal vagal nuclei and the median eminence) (Gamper, 1928; Campbell and Biggart, 1939). Specific histological changes in affected areas included hyperemia and sometimes small hemorrhages, vascular irregularities and proliferation of small blood vessels, absence of inflammatory infiltration. Although predominantly a polioencephalopathy, white matter was sometimes affected, including the columns of the fornix adjacent to the mamillary bodies and the optic nerves.

Subsequent pathological studies have repeatedly documented a high frequency of cases of Wernicke-Korsakoff disease that went unrecognized during life, for example 86% in Harper’s (1979) series of 51 cases. Characteristic clinical findings of Wernicke’s encephalopathy (e.g., a triad of organic mental syndrome, ophthalmoparesis, and ataxia) were typically reported in clinical studies, but in only a minority of cases in pathological studies, suggesting various selection and reporting biases in both types of study, but also that cases with atypical clinical features were seldom being recognized during life (Cravioto et al., 1961).

Isolation and synthesis of thiamin

In the late 1800s and early 1900s, several investigators tried unsuccessfully to isolate the antineuritic substance (Jansen, 1956; Williams, 1961; Carpenter, 2000). In 1926, Jansen and Donath, working in Batavia (where Eijkman had worked), finally crystallized the substance from rice polishings (Jansen and Donath, 1926; Jansen, 1956; Williams, 1961; Carpenter, 2000). Jansen and Donath had known that the protective factor was a relatively small molecule that was dialyzable and probably an organic base (Jansen, 1956). Work was frustratingly slow, however, until they were able to identify a small tropical bird (i.e., the bonbol) that was more susceptible than chickens and therefore developed manifestations of deficiency more quickly and more reliably. Jansen and Donath ultimately isolated about 100 mg of a chemically pure substance that was extraordinarily potent in the prevention of polyneuritis in bonbols and also effective in the treatment of affected pigeons. The investigators sent 40 mg of these crystals to Eijkman in Utrecht where he was able to demonstrate their prophylactic and curative properties in the pigeon polyneuropathy model (Jansen, 1956; Carpenter, 2000).

In 1931, A. Windaus and colleagues in Gottingen isolated the pure, crystalline vitamin from yeast and demonstrated the presence of a sulfur atom in the molecule that had previously been overlooked by Jansen and Donath (Jansen, 1956; Williams, 1961; Carpenter, 2000). With knowledge of the empirical chemical formula, Robert Williams and colleagues proceeded to elaborate the chemical structure, and in 1936 Williams and J. K. Cline completed the chemical synthesis (Williams and Cline, 1936; Williams, 1961), followed nearly simultaneously by the same feat in two other laboratories (Jansen, 1956; Carpenter, 2000). The structure proved to include a pyrimidine ring linked by a methyl group to a thiazole ring. As a result of the chemical synthesis of thiamin, dietary supplementation became feasible, and by the 1950s synthetic forms of the vitamin were produced cheaply and used to enrich polished rice (Jansen, 1956).

Vitamin $B_1$ was initially named aneurin (for anti-neuritic vitamin), but was subsequently named thiamine (for thio = sulfur-containing vitamin), or more recently thiamin (Carpenter, 2000).

The metabolic role of thiamin

In the 1930s, Rudolph Peters and colleagues in Oxford developed a biological assay of thiamin deficiency using acute opisthotonous in pigeons as the biomarker. They discovered that: (1) lactic acid was elevated in the brain of thiamin-deficient pigeons, particularly in the brainstem, even before clinical signs were evident (whereas exercise increased brain lactate levels fairly evenly across different brain areas); (2) elevated brain lactate levels were associated with decreased oxygen uptake, especially in the brainstem when pyruvate was the substrate; (3) elevated brain lactate was associated with a decreased rate of oxidation of pyruvate; (4) thiamin increased the respiration of brain tissue, with the essential biochemical step being the addition of thiamin pyrophosphate (coenzyme) as a cofactor for pyruvate dehydrogenase; and (5) ingested non-phosphorylated thiamin (i.e., from plant rather than animal sources) must be phosphorylated to be active as a cofactor (Knippersley and Peters, 1930; Meiklejohn et al., 1932; Peters, 1936; Ochoa and Peters, 1938; Banga et al., 1939; Ochoa, 1939; Victor et al., 1989).
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Subsequent studies demonstrated the range of activity of thiamin in intermediary carbohydrate metabolism, including acting as a coenzyme for pyruvate dehydrogenase (in the decarboxylation of pyruvate to acetyl-CoA), a-keto-glutarate dehydrogenase (in the decarboxylation of a-keto-glutarate in the Krebs cycle), and transketolase (in the pentose phosphate pathway) (Platt and Lu, 1939; Horecker and Smyrniotis, 1953; Racker et al., 1953). Although many of the biochemical pathways in which thiamin is utilized have been well studied, how thiamin deficiency actually produces the clinical and pathological manifestations of beriberi and Wernicke-Korsakoff disease is less well understood, with conflicting evidence available concerning the roles of the different thiamin-dependent enzymes (Victor et al., 1989).

Etiology of Wernicke–Korsakoff disease

After the reports of Wernicke and Korsakoff in the 1880s, alcoholism was recognized as the most frequently associated underlying cause of the disorder. Still, a wide range of other associated conditions was also recognized in which thiamin deficiency could be attributed to inadequate intake, impaired absorption (e.g., protracted vomiting and gastrointestinal disturbances), increased requirements (e.g., fever, carbohydrate loading), or some combination of these. Wernicke’s encephalopathy was often associated with protracted vomiting, particularly in pregnancy (hyperemesis gravidarum) (Wernicke, 1881; Henderson, 1914).

In the 1930s and early 1940s, a deficiency of B vitamins was proposed as the cause of Wernicke-Korsakoff disease, and shortly thereafter the therapeutic effects of thiamin were demonstrated in this condition. In 1933, Bender and Schilder suggested that Wernicke’s encephalopathy was due to a vitamin deficiency rather than to alcohol toxicity. In 1937, Wagoner and Weir reported clinical improvement with B vitamins in Wernicke’s encephalopathy following protracted vomiting during pregnancy. In 1939, Bowman and colleagues reported the therapeutic effects of thiamin in patients with Korsakoff’s psychosis. Campbell and Biggart (1939) implicated thiamin deficiency as the common etiologic factor resulting from various conditions associated with Wernicke’s encephalopathy, including alcoholism, hyperemesis gravidarum, and carcinoma.

In 1941, Jollife and colleagues at the Psychiatric Institute of Bellevue Hospital in New York documented the rapid resolution of oculomotor palsies, and the slower improvement in ataxia and peripheral neuropathy with thiamin treatment, whereas the cognitive changes—especially Korsakoff’s psychosis—proved to be intractable. They concluded that the ophthalmoplegias of Wernicke’s encephalopathy responded to thiamin, but the entire syndrome was probably due to a “combination of several nutritional deficiencies” (Jolliffe et al., 1941).

In the late 1930s and early 1940s, similar pathologic changes were produced in animal models by maintaining the animals on thiamin-deficient diets (e.g., rats, foxes, fish, and pigeons) (Prickett, 1934; Alexander et al., 1938; Alexander, 1941). For example, in 1934, C. O. Prickett of the Alabama Polytechnic Institute demonstrated neuropathological changes in the brainstem of rats maintained on a diet deficient in thiamin but supplemented with other vitamins: after 40 days the animals became ataxic and soon died with bilateral petechial hemorrhages in the floor of the fourth ventricle and involving the vestibular nuclei and the nucleus solitarius (Prickett, 1934). Similarly, in 1938, Leo Alexander of Boston and colleagues demonstrated that experimental beriberi in pigeons produced lesions which appeared similar to those described by Wernicke in 1881.

Even into the 1950s, the details of the clinical features associated with isolated thiamin deficiency were contested. In 1952, Phillips and colleagues reported detailed studies of nine patients with classic Wernicke’s encephalopathy (i.e., with ophthalmoplegias, nystagmus, ataxia, and mental disturbances) who were given a diet composed solely of glucose and minerals, with specific vitamins added after periods of observation (Phillips et al., 1952). None of the clinical features improved before administration of thiamin, despite alcohol withdrawal, bed rest, and addition of other vitamins (i.e., niacin, calcium pantothenate, pyridoxine, folie acid, ascorbic acid, riboflavin, or cyanocobalamin). Instead, the ophthalmoplegias progressed and the nystagmus decreased only in association with the increasing oculomotor paresis. With administration of thiamin, the ophthalmoplegias improved markedly in from 1 to 6 h, confirming that ophthalmoplegias is due to a specific lack of thiamin. The nystagmus and ataxia improved more slowly and less completely, while the mental changes improved only minimally with improved attention but with some greater confusion. The authors felt that the evidence for a causal association between thiamin deficiency and the nystagmus and ataxia was “less conclusive,” and that no definite conclusions were possible concerning the relationship between mental disturbances and vitamin deficiency.

Studies from the 1930s and thereafter tried to utilize assays of blood or urine thiamin, or assays of blood pyruvate and lactate, for clinico-pathological correlation, clinical diagnosis, and treatment monitoring. However, neither blood nor urinary thiamin levels are sensitive indicators of tissue stores of thiamin, and elevated blood pyruvate and lactate levels are not suffi-
ciently specific (Platt and Lu, 1939; Wortis et al., 1942; Sauberlich, 1967; Victor et al., 1989).

In the early 1960s, the etiological relationship between thiamin deficiency and Wernicke’s encephalopathy was further supported by chemical analyses that demonstrated elevation of erythrocyte transketolase levels in the blood of patients with the disorder, consistent with thiamin deficiency (Brin et al., 1956, 1958; Dreyfus, 1962; Victor et al., 1989). Subsequently transketolase levels have been developed into a useful clinical test for Wernicke’s encephalopathy (Dreyfus, 1962; Dreyfus and Hauser, 1965; Sauberlich, 1984; Victor et al., 1989).

**NIACIN DEFICIENCY: PELLAGRA**

Delirium, dementia, psychosis, and depression were common neuropsychiatric features of pellagra as it was seen in the 18th and 19th centuries in Europe and in the early portion of the 20th century in the United States (Lombroso, 1892, quoted by Marie, 1910; Lanska, 1996, 2004).

**Recognition of pellagra**

Pellagra was apparently unknown prior to the introduction of maize into Europe from the New World. Gaspar Casal (1691–1759), physician to King Ferdinand of Spain, described the signs and symptoms of pellagra in 1735, noted that the condition was known locally as mal de la rosa (disease of the rose), because of the erythematosus rash on sun-exposed areas of the body, and linked it with poverty and a diet with little milk, meat, or other foods of animal origin (Marie, 1910; Etheridge, 1972; Bollet, 1992; Rajakumar, 2000). In 1771, the Italian Francesco Frapolli noted that, in Italy, the disease was associated with poverty and a diet largely restricted to maize-based polenta, exacerbated by sun exposure, and known locally as pellagra (pelle, skin, and agrafa, rough) (Frapolli, 1771/1945; Marie, 1910; Niles, 1916). In addition to the dermatitis recognized by Cašal and Frapolli, other clinical manifestations included dementia (or depression), diarrhea, and death—the “4 D’S.”

From the time of Casal’s description, endemic pellagra was recognized across large areas of Europe, particularly Spain and Italy, where peasants subsisted on nutritionally marginal corn-based diets, but also in France, Romania, Bulgaria, Yugoslavia, Austria, Hungary, Russia, as well as Egypt and North Africa. In the United States, endemic pellagra arose much later as a result of dietary deficiencies arising from the cotton monoculture of the South following the Civil War (Etheridge, 1972; Bollet, 1992; Lanska, 1996). A number of scattered cases of pellagra were reported from the time of the Civil War up into the early-20th century, although not all reported cases were recognized as such at the time of the reports, and the diagnoses in the others were doubted. Beginning in 1907, outbreaks of pellagra were reported in various asylums, and by 1910 the disease was recognized throughout most of the southern states and in several other states (Searcy, 1907; Etheridge, 1972; Bollet, 1992; Lanska, 1996).

Even if there were occasional (at that time unrecognized) cases in the United States prior to 1900, it was only after 1900 that pellagra became a significant public health problem, particularly in the South. As was the case with beriberi in Asia in the 19th century, the epidemic of pellagra in the South followed the introduction of a new grain processing method that effectively removed much of the vitamins from the processed grain. Specifically, in the case of pellagra there was a shift from use of coarsely ground corn meal produced in local, water-driven, grist mills before 1900 to use of finely bolted meal produced by large milling companies, which was degenerated to prevent development of rancidity during storage and shipment (Sydenstricker, 1958).

**Pellagrous dementia**

From the earliest descriptions of pellagra in the United States around 1907, several investigators noted prominent neuropsychiatric manifestations including depression, delirium, and dementia. In 1907, Ray et al. (1907–1908) from the State Hospital for the Insane, in Columbia, South Carolina, reported to the South Carolina State Board of Health similarities between the neuropsychiatric features of pellagra and those of syphilitic general paresis and acute delirium. Similarly, in 1909, in a report for the 35th annual meeting of the American Neurological Association, neurologist Eugene Bondurant (?)–1950) from Mobile, Alabama, noted that pellagra may begin with lassitude and dysthymia, with subsequent development of emotional lability, psychosis, depression, delirium, and dementia, typically with depression predominating (Bondurant, 1910).

In 1915, psychiatrist H. Douglas Singer of the Illinois State Psychopathic Institute in Kankakee argued that previous statistics concerning the frequency of neuropsychiatric disturbance in pellagra were biased, among other things having been ascertained in psychiatric facilities without careful clinical evaluation and without reference to a population base in the community. Using data from Spartanburg County, South Carolina over the period January 1912 to June 1913, obtained from community surveys conducted by the Thompson-McFaddin Pellagra Commission and from persons adjudged insane with and without pellagra in...
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the county, Singer provided data supporting a incidence rate of insanity of approximately 520 cases per 10,000 pellagrins per year, 75-times the rate of diagnosed insanity in the general population of that county (calculated from Singer, 1915, p. 149). Singer also noted that there was an “extraordinary frequency of pellagra arising [de novo] in hospitals for the care of the insane” and that patients with neuropsychiatric disorders were also somehow predisposed to develop pellagra (Singer, 1915, p. 150).

By the 1920s and 1930s, the triad of neuropsychiatric dysfunction with skin and gastrointestinal manifestations was well known and thought to be specific, but insensitive, for the diagnosis of pellagra, particularly in the early stages (Stevens, 1922; Meakins, 1936). In 1943, Virgil Sydenstricker (1889–1964), Chairman of the Department of Medicine at the University of Georgia School of Medicine (now the Medical College of Georgia), again noted that the neuropsychiatric manifestations of pellagra were variable, not specific, and could be the presenting manifestation of pellagra (Sydenstricker, 1943).

Etiologic theories of pellagra

By the early-20th century, toxic, infectious, nutritional, and hereditary theories of the etiology of pellagra found limited empiric support (Roberts, 1913a; Niles, 1916). Each new theory spawned various, often aggressive therapies, including cecostomy with colonic irrigation, arsenical administration, and dietary manipulation. But initial therapeutic results were misleading and ultimately disappointing. Identification of effective therapies was hampered by poorly controlled studies with small sample sizes, failure to consider placebo effects or natural history, and inclusion of cases with mistaken diagnoses or unrecognized concurrent conditions.

The major etiologic theory of pellagra at the time of initial recognition in the United States was the “zeist” theory (a term derived from *Ze a m ays*, the scientific name for maize or Indian corn), which attributed pellagra to the ingestion of corn. Although corn was widely held to be in some way responsible for pellagra, proposed mechanisms varied widely. Support for the corn theory came mainly from ecologic observations in which groups of individuals served as the unit of analysis: (1) The appearance and certainly the recognition of pellagra followed historically the introduction of corn as a staple food into Spain, France, Italy, and other countries of southern Europe; (2) Endemic pellagra occurred only in countries where corn was grown and used extensively among the rural poor; (3) Countries where corn was not grown or used as food were free of pellagra, even if contiguous to or surrounded by pellagrous countries; and (4) A change of food generally resulted in a diminution or disappearance of pellagra, especially if all corn or corn products were removed from the diet. Such evidence, while supportive, was not definitive and not universally accepted.

Opponents of the zeist theory (“anti-zeists”) countered that: (1) Although pellagra was first recognized as a specific disease in the early-18th century, this did not prove it was not present earlier; (2) Pellagra was endemic only over a small part of the extensive area where corn was cultivated, and, indeed, it was absent from many places where maize was a staple food; (3) Cases were reported among people who reportedly had not eaten corn or corn products, and from places where corn was not cultivated; (4) In many places the apparent frequency of pellagra increased or decreased without any apparent change in dietary habits.

One subset of zeist theories proposed that corn was associated with a pellagra-causing toxin: for example, toxins could be a component of natural corn, could be elaborated by microorganisms such as bacteria or fungi involved in corn spoilage (Reed, 1910; Bass, 1911), or might be produced in the alimentary canal (MacNeal, 1913). By the time pellagra was recognized as endemic in the United States, world opinion was strongly against corn containing a toxic substance, unless the corn had been modified in some way (e.g., by microorganisms), because corn was consumed in many places with apparent impunity.

Some authorities, particularly the Italian physician Cesare Lombroso (1836–1909), suggested that a toxin was produced in spoiled corn, which was not present in good corn, and many investigators subsequently considered pellagra to be analogous to ergotism resulting from ingestion of toxic products of a fungus growing on rye used as a foodstuff (Lombroso, 1892; Marie, 1910; Reed, 1910; Bass, 1911; Voeglin, 1914; Lanska, 2004); however, the distribution of pellagra did not coincide well with the very irregular and variable distribution of spoiled corn.

Another subset of the zeist theories considered corn to have inadequate nutritional value. The initial formulation of this theory was that a corn-based diet provides insufficient protein, but chemical analyses of corn did not substantiate this and many recognized authorities argued persuasively against this possibility (Lavinder, 1909; Niles, 1916). For example, in 1916, Niles stated “this explanation is inadequate. If corn is lacking in certain nutritive qualities—in gluten, in nitrogenous matter—so is rice, which, nevertheless does not produce pellagra” (Niles, 1916, p. 62). More sophisticated analyses later showed that corn is deficient in certain amino acids, and this imbalanced protein became viewed as the responsible factor.

A number of infectious theories were also proposed, and various investigators and authoritative
groups (e.g., Sambon, 1905; Marie, 1910; Siler et al., 1914a, b, 1917) supported an infectious etiology, although no consistent organism was implicated (Sambon, 1905), affected individuals were not febrile, there was no evidence of inflammation, and attempts at transmitting the condition with body secretions or skin scrapings failed (McCafferty, 1909; Anderson, 1911; Lavinder, 1911; Singer et al., 1912; Lavinder et al., 1914). Some considered that a microorganism was eaten with corn and subsequently set up an intestinal infection (which could of course explain some of the gastrointestinal manifestations of pellagra), while others suggested that a filterable virus is responsible (Harris, 1913), and still others considered that a vector, such as a mosquito, common sand fly, or gnat is responsible for transmitting a parasitic condition similar to malaria, filariasis, or trypanosomiasis (Sambon, 1905; Lavinder, 1910; Marie, 1910; Roberts, 1913a, b).

Based on human-to-monkey transmission experiments using a combination of subcutaneous, intravenous, and intracerebral injections of large quantities of material originating from two fatal human cases of pellagra, Harris (1913) claimed to have produced pellagra in two of three injected monkeys and therefore concluded that pellagra was caused by a filterable virus (Harris, 1913, p. 1950). However, these results were not replicated, and other studies attempting to transmit the disease from human cases to monkeys were “uniformly negative” (Singer et al., 1912, p. 175; Anderson, 1911; Lavinder, 1911; Lavinder et al., 1914). Around 1909, at the Mt. Vernon Hospital “for the colored insane” in Tuscaloosa, Alabama, Assistant Superintendent E. L. McCafferty, MD, also performed unethical human experiments in unsuccessful attempts to transmit pellagra to unaffected institutionalized patients: “We tried to infect some patients by swabbing out the sick one’s mouth and rubbing it into the well one’s mouth; also swabbed the sores on the hands and feet, and scarified well ones’ hands, but failed to get any results,” he wrote (McCafferty, 1909, p. 229). Unfortunately, misuse of vulnerable human subjects for experimental research studies in attempts at transmitting what would later be found to be deficiency diseases was not isolated to this report. For example, as noted by Vedder (1913, p. 144):

Fraser and Stanton, in the course of their work on beriberi [in Malaya beginning in 1907], performed a large number of human experiments, in which they tried by every conceivable method, including insect transmission, to infect healthy individuals from beriberi patients. These experiments were all negative, but were unfortunately suppressed by the [British] Government for political reasons.

Indeed, these experiments are not reported in a volume of Fraser and Stanton’s collected papers on beriberi (Fraser and Stanton, 1924).

Joseph Goldberger and the “P-P factor”

In 1914, with pellagra morbidity and mortality expanding rapidly in the South, the US Public Health Service commissioned Dr Joseph Goldberger (1874–1929) to study the disease. From 1914–1929, Goldberger completed well-designed epidemiologic investigations, tested theories with human experiments, and developed an animal model (Parsons, 1943; Terris, 1964; Kraut, 2003).

Goldberger’s first paper was published in June 1914. He emphasized three epidemiologic facts that had been repeatedly observed: (1) In various medical institutions, the inmates developed pellagra after varying periods of residence, but ward personnel, attendants, and nurses were uniformly exempt despite prolonged and close contact with affected patients; (2) Pellagra preferably affected rural areas; and (3) Pellagra is associated with poverty (Goldberger, 1914a). Based on these considerations, Goldberger boldly proposed a plan of prevention that emphasized dietary changes with a reduction in cereals, vegetables, and canned foods, and an increase in fresh meats, eggs, and milk (Goldberger, 1914a; Terris, 1964).

Goldberger initiated a series of observational studies to clarify factors that might have etiologic significance, and that could be tested with more formal experiments or applied with presumptive preventive approaches. In 1914, surveys of state institutions showed that those developing pellagra ate an unbalanced diet low in protein. In 1920, community surveys showed that pellagrous households had diets deficient in animal protein (lean meat, milk, butter, cheese, eggs), recognized vitamins (particularly the “fat soluble A” factor), and minerals (Goldberger et al., 1920a,b,c,d; Sydenstricker, 1933; Kasius, 1974).

No differences were observed between pellagrous and nonpellagrous households in terms of cereal supplies (particularly maize), caloric content of the diets, or the proportion of calories derived from carbohydrate and fat combined (Goldberger et al., 1920a,b,c,d; Sydenstricker, 1933; Kasius, 1974). The incidence of pellagra varied by age, gender, family income, and season, but there was no clear relation to sanitary conditions (Goldberger et al., 1920a,b,c,d; Goldberger and Sydenstricker, 1927; Sydenstricker, 1933; Kasius, 1974).

Goldberger proceeded to test his theory and exclude alternatives with careful human experiments. From 1915 to 1923, he and his colleagues showed that initiation of a well-balanced diet at two Mississippi orphanages and the Georgia State Asylum eliminated...
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pellagra from these institutions, places where it had previously been prevalent (Goldberger et al., 1915a, b, 1923). Those fed a diet of fresh meat, milk and vegetables, instead of a corn-based diet, did not develop pellagra, and those already affected rapidly recovered (Goldberger, 1914a, b; Goldberger et al., 1915a, b, 1923). A subsequent return to the institutional diet resulted in a return of pellagra, which again disappeared with resumption of the well-balanced diet (Goldberger et al., 1923).

In 1915, with the cooperation of Governor Earl Brewer, Goldberger experimented on 11 healthy volunteer prisoners at Rankin State Prison Farm near Jackson, Mississippi, with the prisoners offered pardons in return for their participation (Goldberger and Wheeler, 1915, 1920a, b; Bollet, 1992; Harkness, 1996; Kraut, 2003). The prisoners were fed a milk- and meat-free cornmeal diet, and six were felt to have developed cutaneous manifestations of pellagra within 6 months, although later studies suggested that some of the cutaneous manifestations (particularly the scrotal lesions) were those of riboflavin deficiency (Sebrell and Butler, 1938; Oden et al., 1939; Horwitt et al., 1949, 1956; Carpenter and Lewin, 1985).

Proponents of competing theories, particularly those championing an infectious etiology, challenged Goldberger’s results (e.g., MacNeal, 1916). Intending to convert his critics, Goldberger performed a remarkable series of demonstrations. In 1916, he showed that pellagra could not be transmitted by injection of blood from pellagrous patients, by swabs of nasal and pharyngeal secretions swabbed onto healthy volunteers, by ingestion of capsules containing scabs of pellagrins’ rashes, or by ingestion of capsules containing pellagrins’ fecal material (Goldberger, 1916; Kraut, 2003). Goldberger used himself, his wife, and his colleagues as the subjects of these “filth parties.” None of them contracted pellagra, but they did not convert all of their critics either (MacNeal, 1916; Kraut, 2003).

In the 1920s, Goldberger and colleagues determined that pellagra developed despite supplementation with minerals, known vitamins, and a liberal supply of protein of presumably good biological quality (Goldberger and Tanner, 1922, 1925; Goldberger et al., 1926; Goldberger, 1927). Goldberger and colleagues demonstrated that yeast extract, milk, and fresh lean beef are capable of preventing pellagra, whereas soy beans, cowpeas, butter, cod-liver oil, and canned tomatoes are not (Goldberger and Tanner, 1925; Terris, 1964). After raising the possibility that pellagra resulted from a deficiency or imbalance in dietary amino acids (Goldberger and Tanner, 1922), Goldberger ultimately concluded that pellagra is a dietary deficiency disease that could be cured by a pellagra preventive factor or “P-P factor” (later, “vitamin P-P”) that was lacking in corn, but that could be found in meat and milk.

Goldberger and colleagues also began experiments with dogs, after learning in 1922 that the dog disease named “black tongue” (sometimes spelled blacktongue) was the canine equivalent of human pellagra (Chittenden and Underhill, 1917; Goldberger et al., 1922, 1926, 1928; Goldberger and Wheeler, 1928). From 1922 to 1928, Goldberger and colleagues demonstrated that black tongue could be produced experimentally with a diet containing mainly corn meal (Goldberger et al., 1926, 1928; Goldberger and Wheeler, 1928). Further studies evaluated the black tongue-preventive properties of a wide variety of foods and correlated these results with the pellagra-preventive properties of the same foods.

By 1926 Goldberger and associated established that a small amount of dried brewer’s yeast could cure or prevent pellagra less expensively than fresh meat, milk, and vegetables (Goldberger and Tanner, 1925). A heat-stable component of yeast was shown to prevent the development of black tongue (Goldberger et al., 1928). Entering yeast manufacturers, in need of business during Prohibition, seized the opportunity and filled newspapers with propaganda: “only brewer’s yeast will cure and prevent pellagra.” A gullible populace of worried well “pellagra-phobics” responded by buying more and more yeast. After 1928, yeast was provided free in endemic areas by state and county health departments and the American Red Cross (Davies, 1964).

After Minot and Murphy showed that liver and liver extracts could cure pernicious anemia (1926–1928), Goldberger and Sebrell showed that liver extract could also prevent black tongue (Goldberger and Sebrell, 1930). In the early 1930s, many physicians tried liver extracts in the treatment of pellagra with equally positive results, but although liver extracts were more potent than yeast, they were prohibitive expensive and often ineffective parenterally in the dosages used (Sydenstricker, 1958; Davies, 1964).

Goldberger never identified the elusive P-P factor, because his research was cut short by his death in 1929, but the year he died the Committee on Vitamin B Nomenclature of the American Society of Biological Chemists recommended naming the P-P factor vitamin G in his honor (Seidell et al., 1929).

Endemic pellagra was a manifestation of complex social issues

Once the relationship between poverty, diet, and pellagra was established—primarily by Edgar Sydenstricker (1881–1936) and Goldberger—it became clear that pellagra was a manifestation of complex social issues
and could not be easily eradicated even with the availability of a medicine that was curative (Sydenstricker, 1915, 1933; Goldberger et al., 1918, 1920a, b, c, d; Goldberger and Sydenstricker, 1927; Kasius, 1974). Indeed, elimination of endemic pellagra would require improving the diet of a large portion of the rural Southern population, a behavioral and social change of tremendous magnitude and complexity. Nevertheless, with the impetus of further economic collapse of the Southern cotton monoculture and the manpower requirements of World War II, the needed political resolve developed and sufficient social reform occurred, so that eradication of endemic pellagra in the United States was accomplished by the 1950s.

The antebellum cotton- and tobacco-based agriculture of the South had initially been compromised by the lack of slaves after the Civil War, but soon produced further economic servitude in the form of sharecropping, in which the landowners got a quarter to a half of the crop. Financially-strapped sharecroppers devoted all available land to the cash crops (i.e., cotton and tobacco), and lived on inadequate unbalanced diets consisting largely of cheap corn meal. This entrenched sharecropping system would not be abandoned voluntarily by the powerful landowners or by some indebted sharecroppers, who had no ready alternatives.

Unlike the elimination of slavery, which required political resolve and a civil war, the social change needed to eliminate sharecropping was initiated in large part by a tiny beetle. The boll weevil crossed the Rio Grande River from Mexico in the early 1890s and spread east and north to affect all the cotton growing regions of the United States by 1920. The boll weevil contributed greatly to the increasing economic woes of the Southern farmers during the 1920s, and consequently to a marked rise in pellagra incidence and mortality in the late 1920s. Boll weevil devastation of the cotton crops was a major reason for the subsequent development of crop diversification and crop rotation principles, including those developed and promoted by agricultural chemist George Washington Carver (1864–1943).

During the depression, cotton was no longer an economically viable crop. The agricultural extension services encouraged farmers to reduce the acreage under cotton, keep livestock for personal food use, and diversify cultivated crops. Southern farmers learned to alternate soil-depleting cotton or tobacco crops with soil-enriching crops, such as peanuts, peas, soybeans, sweet potatoes, and pecans. From 1928 to 1933, total acreage under cotton or tobacco declined markedly and the production of vegetables and farm products for home use increased equally dramatically (Davies, 1964). With these changes, pellagra mortality declined precipitously in the early 1930s, and subsequently plateaued until the advent of an effective treatment (i.e., niacin) was discovered in 1937.

**Niacin**

In 1937, Conrad Arnold Elvehjem (1901–1962), an agricultural biochemist at the University of Wisconsin, finally isolated the P-P factor from active liver extracts, showed that the P-P factor is nicotinic acid (subsequently named *niacin* for *nicotinic acid vitamin*), and demonstrated that nicotinic acid and nicotinic acid amide cure black tongue in dogs (Elvehjem et al., 1937, p. 938).

Soon after Elvehjem’s initial report in 1937, further animal trials demonstrated that niacin cured pellagra in pigs and monkeys (Harris, 1938), and human clinical trials confirmed that niacin had dramatic therapeutic effects and rapidly cured pellagra in people, including the cutaneous and cognitive manifestations (Fouts et al., 1937; Smith et al., 1937; Elvehjem et al., 1938; Matthews, 1938; Schmidt and Sydenstricker, 1938; Spies, 1938; Sydenstricker et al., 1938; Spies et al., 1938a,b,c,d, 1939). In 1937, Paul Fouts of the Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indiana University, along with colleagues, noted that, “All patients showed distinct improvement in general condition and mental attitude within 48 h of onset of therapy” (Fouts et al., 1937, p. 406). Also in 1937, David Smith and colleagues from Duke University noted a dramatic recovery in a 42-year-old pellagrin treated with intravenous nicotinic acid (Smith et al., 1937). In 1938, Tom Spies of the University of Cincinnati College of Medicine and the Cincinnati General Hospital, along with colleagues, reported that treatment with nicotinic acid had dramatic results in 60 pellagrins with acute or subacute psychosis (Spies et al., 1938d). Spies subsequently lauded the beneficial effects of nicotinic acid in the treatment of pellagra, but nevertheless recognized that simply replacing the niacin deficiency was not a long-term solution; instead, maintenance of an adequate diet was necessary (Spies et al., 1939).

**Dietary modification and food fortification**

Dietary modification was the first truly effective approach for the prevention of pellagra, but on a wide scale social reform was needed to ensure implementation. Initially dietary modification proved impractical because of economic conditions and dietary habits. Even with free distribution of yeast by the American Red Cross and by state and county health agencies, or with availability of inexpensive nicotinic acid, pellagra persisted in endemic areas, because of ignorance,
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The vast majority of sporadic cases in the United States and other developed countries are now seen in alcoholics, although very rarely other patients can develop the disease, because of malabsorption, iatrogenic situations (e.g., total parenteral nutrition with inadequate supplemental niacin), or when they subsist on bizarre diets owing to mental illness or extraordinary circumstances (Sydenstricker, 1958; Spivak and Jackson, 1977).

The niacin-tryptophan connection and niacin neurochemistry

By the 1940s, it became clear that the total niacin content of foods was not the only factor in the development of pellagra. Diets in some areas where pellagra was rare were found to contain less niacin that did
neutral amino acids (e.g., leucine, isoleucine, valine, methionine, and phenylalanine) that compete for blood–brain barrier uptake through a single amino acid carrier mechanism. Low platelet levels of serotonin and low urinary excretion of 5-hydroxyindolacetic acid (i.e., the main metabolite of serotonin) have been documented in pellagrous dementia (Krishnaswamy and Ramanamurthy, 1970).

**FOLATE DEFICIENCY: NEURAL TUBE DEFECTS**

Folate deficiency was initially recognized clinically as a macrocytic anemia in the 1920s, which was only clearly separated from pernicious anemia by the mid-20th century. When folate was finally isolated in the mid-1940s, it was shown to correct the macrocytic anemia associated with pernicious anemia, while the neurological manifestations progressed. Beginning in the 1960s, folate deficiency was increasingly recognized as the major cause of preventable neural tube defects. In the early 1990s well-designed randomized trials established that folate supplementation could prevent neural tube defects. Subsequent studies have established genetic predispositions for neural tube defects in offspring in the form of gene polymorphisms for enzymes involved in folate-dependent homocysteine metabolism. These latter findings help to explain how the genotype of the mother, the genotype of the unborn child, and environmental factors (e.g., folate intake) can all impact on the risk of neural tube defects.

**Isolation and synthesis of folic acid**

Folate was identified as the active substance in brewer’s yeast in the late 1930s. In 1941, Mitchell and colleagues isolated this factor from spinach leaves and named it folic acid, a derivative of *folium*, the Latin word for leaf (Mitchell et al., 1941; Hoffbrand and Weir, 2001). In 1945, Angier and colleagues reported the successful synthesis of folic acid (Angier et al., 1945), several years before the synthesis of vitamin B12 (in 1948). Angier and colleagues showed that folic acid is composed of a pteridine ring, paramino-benzoic acid, and glutamic acid, and they called it pteroylglutamic acid (Angier et al., 1945; Stokstad, 1979). Soon after the synthesis of folic acid was achieved in 1945, it was demonstrated to be effective in the treatment of macrocytic anemias that are generally refractive to treatment with refined liver preparations, including the macrocytic anemias of malnutrition, pregnancy, sprue, and celiac disease.

Naturally occurring folates were subsequently found to vary in composition from the synthetic pteroylglutamic acid, with, for example, multiple polyglutamate residues, additional single-carbon units attached to the nitrogen atoms, etc. (Hoffbrand and Weir, 2001). The term “folic acid” now refers to the synthetic compound, pteroylglutamic acid, which is not present in natural foods, while the term *folate* refers to the large family of natural or synthetic compounds with similar vitamin activity, including synthetic folic acid and the natural folates (Hoffbrand and Weir, 2001; Wald, 2001).

**Risks of administering folic acid to patients with pernicious anemia**

In 1939, M. M. Wintrobe had reported that yeast or yeast extracts could produce a hematological response in patients with pernicious anemia (Wintrobe, 1939), a finding confirmed by others in the early 1940s (Vilter et al., 1945). Although administration of folic acid was subsequently found to be safe in normal people and those with various neurological disorders, and to be temporarily effective in correcting the anemia of Addisonian pernicious anemia (Moore et al., 1945; Vilter et al., 1945; Harvey et al., 1950; Weissberg et al., 1950), beginning around 1946 a number of reports appeared indicating that folic acid would not prevent the progression of the central nervous system dysfunction in patients with pernicious anemia, and several anecdotal reports suggested that administration of folic acid might accelerate neurological dysfunction or even precipitate abrupt neurological worsening in some cases (Vilter et al., 1946; Heine and Welch, 1947; Vilter and Spies, 1947; Berk et al., 1948a; Bethel and Sturgis, 1948; Haden, 1948; Wagley, 1948; Wills, 1948; Dickinson, 1995).

Despite the concern at the time (and since), available data from historical cases and case series (although methodologically limited) do not support a difference in the rates of progression of neurological deterioration in patients with untreated pernicious anemia (i.e., without administration of vitamin B12) with and without administration of folic acid (Dickinson, 1995). The rate of progression of neurological manifestations in patients with untreated pernicious anemia is widely variable, and in some cases marked deterioration develops over periods of a few weeks (Dana, 1899a,b; Duckworth, 1900; Richmond and Williamson, 1905; Kennedy, 1913; Globus and Strauss, 1922; Dickinson, 1995).

**Folate metabolism and the expanding role of folates in pathogenesis of various diseases**

In the 1950s and 1960s, the biochemical reactions involving folates were elucidated, and folates were found to be essential for transfer of single carbon units in the conversion of the amino acid homocysteine to methionine
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(by the B_{12}-dependent enzyme methionine synthase), in purine (adenine, guanine) and pyrimidine (thymine) biosynthesis (and thus in the biosynthesis of DNA and RNA), in DNA methylation, and in numerous other cellular reactions. The need for folate was found to increase with rapid tissue growth and cell division (at least in part because of the need for folate in DNA and RNA biosynthesis), as in hematopoiesis, epithelial growth and differentiation, spermatogenesis, pregnancy, and fetal development. Among the earliest clinical manifestations of folate deficiency are hematological changes, including hypersegmentation of neutrophils, production of megaloblasts in the bone marrow, and eventually development of macrocytic anemia. The increased rate of folate-dependent tissue growth and differentiation during pregnancy (McPartlin et al., 1993) increases dietary folate needs by about 0.2–0.3 mg per day (Czeizel, 1995): as a result, pregnant women with marginal folate levels, as in the cases studied by Wills in India in the 1930s (Wills and Mehta, 1930, 1931; Wills, 1931), were found to be susceptible to potentially fatal macrocytic anemias and to fetal malformations, particularly neural tube defects (Hibbard, 1964).

From the 1950s to the 1990s, the range of folate-responsive disorders has expanded. Recognition that folic acid therapy enhances tumor growth led to development of folate antagonists for anticancer therapy, including development of methotrexate. Studies of children with inborn errors of metabolism identified forms of homocysteinuria with methylmalonic aciduria, impaired methionine synthase, and resulting defective remethylation of homocysteine; pathologic studies in such cases demonstrated marked vascular pathology, suggesting an association between vascular disease and hyperhomocysteinemia. Subsequent studies demonstrated abnormal methionine metabolism in significant proportions of patients with unexplained atherosclerotic cardiovascular disease, and later studies demonstrated elevations of serum homocysteine in patients with cerebrovascular and peripheral vascular disease.

Prevention of neural tube defects with folic acid

In approximately one in 500 to one in 1000 pregnancies, the neural tube fails to close properly 28 days after conception, producing a neural tube defect—either with failure of closure of the cranial end producing anencephaly or encephalocele, or with failure of closure of the caudal end producing spina bifida or myelomeningocele. Neural tube defects are the most common congenital malformations and are thought to have multifactorial causes, including a combination of both genetic and environmental factors.

In 1964, British obstetrician Brian Hibbard suggested an association between fetal neural tube defects and maternal deficiency or defective metabolism of folates (Hibbard, 1964). In 1976, Richard Smithells at the University of Leeds and colleagues demonstrated that women with megaloblastic anemia during pregnancy have a high frequency of neural tube defects in their offspring (Smithells et al., 1976). In 1980, Smithells and colleagues reported a non-randomized trial of multivitamin supplementation among women who had previously given birth to one or more infants affected with neural tube defects (Smithells et al., 1980): there was a 5% recurrence rate for the non-supplemented group compared with a 0.6% recurrence rate for the supplemented group.

Additional observational studies and non-randomized clinical trials were published during the 1980s and 1990s that documented protective effects of higher folic acid intake or of vitamin supplements containing folic acid during the periconceptional period (i.e., from 1 month before pregnancy through the first trimester) among women who had not previously had a pregnancy affected by a neural tube defect (occurrence studies) and among women who had a previous pregnancy affected by a neural tube defect (recurrence studies). These studies showed a wide range of estimated efficacy in the occurrence of neural tube defects with folic acid supplementation, but the summary efficacy estimate across the various studies indicated an overall 50% reduction in risk of neural tube defects (Wald, 1993).

The strongest evidence in support of periconceptional folic acid supplementation came from two large randomized trials published in the early 1990s (MRC Vitamin Study Research Group, 1991; Czeizel and Dudas, 1992; Wald, 1993; Czeizel et al., 1994; Czeizel, 1993a, b, 1995). The Medical Research Council study under the direction of Nicholas Wald at St. Bartholomew’s Hospital Medical College in London was a multi-center, multinational, randomized, double-blind, controlled, recurrence prevention trial conducted in 33 centers in seven countries (MRC Vitamin Study Research Group, 1991; Wald, 1993). The MRC study found a 72% reduction in recurrence of neural tube defects with 4 mg of folic acid daily over the period from before conception and during the first trimester among women with a previous neural tube defect-associated pregnancy (MRC Vitamin Study Research Group, 1991; Wald, 1993). The Hungarian study, conducted by Andrew Czeizel of the National Institute of Hygiene in Budapest, was a randomized, double-blind, controlled, occurrence prevention study with periconceptional supplementation with multivitamins,
should consume 0.4 mg (4000 μg) of folate daily (Centers for Disease Control and Prevention, 1992; Czeizel et al., 1993a, b; Czeizel and Duda’s, 1995). Among approximately 5000 women with confirmed pregnancy and an “informative offspring,” maternal periconceptional folate supplementation produced a significant decrease in the first occurrence of neural tube defects compared to a placebo-like (i.e., trace element) control group.

A meta-analysis of data from these trials and a previous small (underpowered and not statistically significant) trial by Laurence and colleagues collectively indicated that periconceptional folate administration reduces both the occurrence and recurrence risks of neural tube defects by at least 70% (Laurence et al., 1981; MRC Vitamin Study Research Group, 1991; Wald, 1993). Subsequent studies have generally supported these findings and suggest that periconceptional multivitamin supplementation can significantly reduce the occurrence of other congenital abnormalities in addition to neural tube defects (Kirke et al., 1992; Czeizel, 1993a, b; Czeizel et al., 2004).

Data from the randomized controlled trials have been used to establish governmental recommendations concerning folic acid intake, and have also been used to establish health policy concerning vitamin fortification of foodstuffs (Honein et al., 2001). In 1991, the US Centers for Disease Control published a review of the evidence for the prevention of recurrent neural tube defects and recommended 4 mg of folic acid for women who had previously had an infant or fetus with a neural tube defect (Centers for Disease Control, 1991). In 1992, the US Public Health Service recommended that all women capable of becoming pregnant should consume 0.4 mg (4000 μg) of folic acid daily (Centers for Disease Control and Prevention, 1992; Cornel and Erickson, 1997). Because naturally occurring folate is less readily absorbed than synthetic folic acid, in 1998 the Institute of Medicine recommended that women of childbearing age consume 0.4 mg daily from dietary supplements or fortified foods for the primary prevention of neural tube defects (Institute of Medicine, 1998).

Potential strategies for increasing folate levels among women are dietary modification, folic acid supplementation, and food fortification (Centers for Disease Control and Prevention, 1992; Wald and Bower, 1995; Czeizel, 2000; McNulty et al., 2000). Despite various education campaigns, the estimated dietary folate intake for US women averages only 0.2 mg daily and it was considered impractical to have women systematically increase their intake of folate-rich foods (e.g., fruits, leafy green vegetables, and grains) sufficiently to raise daily folate intake to 0.4 mg daily (Centers for Disease Control and Prevention, 1999). Folic acid supplementation can also be effective, but vitamins are used consistently by less than a third of women of childbearing age, and the remainder do not consider taking vitamin supplements until after they discover that they are pregnant (McNulty et al., 2000). Unfortunately, neural tube defects develop in the fourth week post-conception, i.e., before a pregnancy is confirmed. Furthermore, about half of pregnancies are unplanned (Grimes, 1986; Forrest, 1994), but even women who plan their pregnancies are poorly compliant with folate supplementation (Clark and Fisk, 1994; Scott et al., 1994; Wild et al., 1997; McNulty et al., 2000). Therefore, an approach relying on supplementation will not prevent most of the folate-preventable cases of neural tube defects. Food fortification, in contrast, can cost-effectively increase folate levels across the population without requiring a change in behavior (Romano et al., 1995).

In 1996, the US Food and Drug Administration selected flour, corn meal, pasta, and rice for mandatory folic acid fortification beginning in January 1998 at a level of 140 μg per 100 g of cereal grain product (Food and Drug Administration, 1993a,b, 1996a,b). This was estimated to result in an average adult consumption of approximately 100 μg of folic acid daily from fortified cereal grain products, effectively ensuring that about half of reproductive-age women will receive the recommended 0.4 mg daily from all sources (Food and Drug Administration, 1993a,b; Romano et al., 1995). This level of fortification—deemed the best possible accommodation between concerns for the fortification of the target population of women of childbearing age and the safety of the much larger non-target population—was expected to prevent many but not all neural tube defects that might be prevented by sufficient maternal folic acid intake. Folic acid fortification was limited to relatively low levels because of a fear that folic acid would correct the hematological abnormality in patients with vitamin B12 deficiency, potentially delaying diagnosis, and allowing progression of central and peripheral nervous system manifestations of vitamin B12 deficiency (e.g., Reynolds, 2002). Many have challenged the logic and ethics of this rationale and the resulting national fortification decisions (e.g., Wald and Bower, 1994), but levels of fortification remain modest. As a result, dietary modification and folic acid supplementation continue to be necessary and appropriate modes of intervention.

Since 1996, folic acid fortification has produced a significant improvement in population folate status in the United States (Jacques et al., 1999; Lawrence et al., 1999; Honein et al., 2001; Erickson, 2002; Mathews et al., 2002; Rader, 2002; Centers for Disease Control and Prevention, 2004; Pfeiffer et al., 2005), and
similar improvements have been observed in other countries that have adopted this strategy (Ray et al., 2000). In 1999, data from the Framingham Offspring Cohort showed that fortification of enriched grain products with folic acid was associated with a substantial improvement in folate status of the population (Jacques et al., 1999; Rader, 2002). Similar results were demonstrated in populations enrolled in large managed care plans (Lawrence et al., 1999), and in representative samples of women participating in the National Health and Nutrition Examination Survey (NHANES) (Centers for Disease Control and Prevention, 2000; Peiffer et al., 2005).


Gene polymorphisms for enzymes involved in folate-dependent homocysteine metabolism

In the 1990s, several studies demonstrated hyperhomocysteinemia in mothers of children with neural tube defects, despite the absence of folate or vitamin B12 deficiency in these mothers, while other studies identified hyperhomocysteinemia in children with spina bifida, despite the absence of folate or vitamin B12 deficiency in these children. Because of this, several groups examined enzymes involved in homocysteine metabolism for potential mutations or polymorphisms linked to an increased risk of neural tube defects, suspecting that disturbed homocysteine metabolism in either mothers or their offspring could cause neural tube defects.

In 1995, Nathalie Van der Put and colleagues reported that a common thermolabile variant (i.e., 677C→T) in the 5,10-methylenetetrahydrofolate reductase gene among mothers is associated with decreased function of the enzyme and with increased risk of neural tube defects in their offspring (Van der Put et al., 1995, 1996, 1997, 2001). Methylene tetrahydrofolate reductase catalyzes the formation of 5-methyltetrahydrofolate, the biologically active form of folate needed for the remethylation of homocysteine to methionine, so either inadequate folate, a defective methylenetetrahydrofolate reductase enzyme, or a combination of these increases plasma homocysteine concentrations. Among folate-deficient people, homozygotes for the 677C→T mutation (i.e., the TT genotype with two mutant T alleles) have higher plasma homocysteine concentrations than those with the CC genotype.

Other studies have noted the same polymorphism in children with hyperhomocysteinemia and spina bifida (Bjerke-Monsen et al., 1997; Shaw et al., 1998; Shields et al., 1999), and Denis Shields and colleagues found a much stronger relationship between the genotype and phenotype of the child than between the genotype of the mother and the phenotype of the child (Shields et al., 1999). When the genotypes of both the mother and the child were considered, the 677C→T mutation accounted for at most 25% of the observed protective effect of folate (Van der Put et al., 1995), suggesting that other defective enzymes either in folate metabolism or folate transport may also be involved (Van der Put et al., 1998; Brody et al., 2002). Subsequent studies have demonstrated that elevated maternal homocysteine concentrations in women with such polymorphisms can be lowered by supplemental folate intake, even in women with normal folate levels to begin with (Kang et al., 1988; Nelen et al., 1998; Brouwer et al., 1999; Fohr et al., 2002).

Although methylenetetrahydrofolate reductase gene polymorphisms are only moderate risk factors for neural tube defects (Van der Put et al., 1997), at a population level they contribute to a significant portion of the observed burden of neural tube defects. They also help to explain how the genotype of the mother, the genotype of the unborn child, and environmental factors (e.g., folate intake) can all impact on the risk of neural tube defects.

**CYANOCOBALAMIN DEFICIENCY: SUBACUTE COMBINED DEGENERATION OF THE SPINAL CORD**

Pernicious anemia was recognized clinically in the mid-19th century, but the associated neurological manifestations—particularly the myelopathy now known as subacute combined degeneration of the spinal cord—was not recognized clinically and linked with pernicious anemia until the end the 19th century. In the 1920s, Minot and Murphy showed that large quantities of ingested liver could be used to effectively treat pernicious anemia. Cyanocobalamin (vitamin B12) was finally isolated by the mid-20th century, and this greatly improved the treatment of pernicious anemia and the associated neurological manifestations.

**Pernicious anemia**

Originally at a meeting of the South London Medical Society in 1849, and subsequently in a monograph in 1855, Thomas Addison (1793–1860) at Guy’s Hospital in London described several cases with “idiopathic”
anemia characterized by pallor, weakness, and progressively worsening health leading to death (Addison, 1855/1942). Later this condition was called Addisonian anemia, at least until Biermer of Zurich named it *perniciöse Anämie* (i.e., pernicious or fatal anemia) when describing 15 cases of severe anemia (of mixed etiologies) in 1872 (Biermer, 1872; Haden, 1948).

In 1870, Fenwick in London associated stomach atrophy with this form of anemia and demonstrated that stomach mucosa from an affected fresh cadaver could not digest boiled egg white with prolonged incubation, whereas mucosa from a control stomach could (Fenwick, 1870; Mackay and Whittingham, 1968; Florkin, 1973; Okuda, 1999). Subsequently, Cahn and Mering showed that a patient with pernicious anemia had no hydrochloric acid in the stomach contents, a finding later demonstrated to be pervasive in this disorder (Cahn and Mering, 1886; Faber and Block, 1900; Hurst and Bell, 1922; Levine and Ladd, 1924). Soon it was recognized that achlorhydria precedes the development of anemia (Hurst and Bell, 1922).

It was not until the 1850s – after Addison’s original communication – that the first red cell counts were done by Vierordt, and that hemoglobin was discovered by Funke (Funke, 1851; Vierordt, 1851; Haden, 1948). In 1875, William Pepper (1843–1898) of Philadelphia noted the extreme hyperplasia of the bone marrow in patients with pernicious anemia (Pepper, 1875). In 1880, Paul Ehrlich (1854–1915), using aniline dyes developed by his cousin Carl Weigert (1845–1904), identified large erythroid precursor cells that he called “megaloblasts” in stained blood smears of patients with pernicious anemia (Ehrlich, 1880). Subsequent hematologists noted characteristics of megaloblastic anemia in the peripheral blood (i.e., macrocytes, poikilocytes, and hypersegmented neutrophils) and bone marrow (e.g., megaloblasts, metamyelocytes, and megakaryocytes) (Billings, 1902). Later, Francis W. Peabody of the Thorndike Memorial Laboratory in Boston hypothesized that this macrocytic anemia was due to maturation arrest of erythroblasts in the bone marrow (Peabody, 1927; Florkin, 1973; Kass, 1978).

**Subacute combined degeneration of the spinal cord**

In 1884, Lichtenstein described cases of pernicious anemia with neurologic manifestations felt to be suggestive of tabes dorsalis (Lichtenstein, 1884; Haden, 1948). Following Lichtenstein’s report in 1887 of progressive myelopathy associated with pernicious anemia in three cases, two with autopsy, similar cases were reported by a number of authors over the next several decades (Dana, 1891a, b; 1899a, b; 1908; Putnam, 1891; Minnich, 1893; Taylor, 1895; Lloyd, 1896; Russell, 1898; Russell et al., 1900; Putnam and Taylor, 1901; Billings, 1902; Bramwell, 1915; Wolman, 1918; Hurst and Bell, 1922; Weil and Davison, 1929; Greenfield and O’Flynn, 1933).

Clinicians of the period struggled to distinguish the clinical and pathologic features of this condition from those of other recognized causes of progressive myelopathy (Dana, 1891a, b), particularly those that could affect multiple white matter tracts of the spinal cord, i.e., what New York neurologist Charles Loomis Dana (1852–1935) had referred to categorically as “combined sclerosis or mixed-system myelitis” (Dana, 1887, p. 1). In the absence of a valid diagnostic test (i.e., before recognition of vitamin B12 deficiency as the cause), early cases were particularly hard to distinguish from other conditions, and many of the early reports included cases with other etiologies (Pant et al., 1968). Nevertheless, subacute combined degeneration of the spinal cord was clearly linked with pernicious anemia by about 1900.

In 1891, Boston neurologist James J. Putnam (1846–1918) of Harvard Medical School and the Massachusetts General Hospital reported eight cases, four with autopsy. The neurologica presentation and course in these early cases typically included progressive paresthesias, incoordination, impaired sensation and position sense in the arms and legs, preserved and even exaggerated muscle stretch reflexes and ankle clonus, and weakness progressing to terminal paraplegia (Putnam, 1891).

Early neuropathological studies by Putnam and Dana, along with later neuropathological studies, demonstrated that the degeneration of white matter tracts was initially uneven and patchy, with small foci enlarging and coalescing to involve entire white matter columns and the most severe involvement generally affecting the posterior columns of the lower cervical and thoracic cord, extending in some cases to the medulla (Putnam, 1891; Dana, 1891a, b; Pant et al., 1968). The degenerative process affects most dramatically the myelin sheaths (although axons are also damaged), with marked swelling of myelin sheaths giving a vacuolated “sieve-like” appearance to stained spinal cord sections, evident even in some of the earliest published pathological illustrations (Putnam, 1891; Dana, 1891a, b; Pant et al., 1968).

Sensory symptoms and signs are early and prominent features of the disease. Although Putnam initially remarked that “the nerve roots were more or less diseased” (Putnam, 1891, p. 72), by 1901 he and Taylor reported on a “common freedom from degeneration of nerve roots, both motor and sensory, and peripheral nerves” (Putnam and Taylor, 1901, p. 92). Even if in individual cases “somewhat imperfectly staining
bundled [within the nerve roots] may be made out . . . [the] dorsal roots at no point show the changes which are an essential part of the pathological process in tabes [dorsalis]” (Putnam and Taylor, 1901, p. 76). Severe cases showed some involvement of the posterior roots (Putnam, 1891), but not enough to explain the severe degeneration of the posterior columns as a secondary phenomenon (Pant et al., 1968).

Involvement of peripheral nerves was suggested clinically (Woltman, 1918), but initial pathological reports of peripheral nerve involvement with subacute combined degeneration of the cord were scanty and inconsistent (Putnam, 1891; Putnam and Taylor, 1901). Peripheral nerve involvement was recognized pathologically by the 1930s and 1940s (Greenfield and Carmichael, 1935; van der Scheer and Kock, 1938; Foster, 1945; Ungle, 1949), with later studies also demonstrating slowed peripheral nerve conduction velocities (Mayer, 1965).

Clinical manifestations of optic atrophy were elaborated since the 1930s by a number of authors (Courville and Nielsen, 1938; Kampmeier and Jones, 1938; Turner, 1940), with degeneration of the papillomacular bundle previously demonstrated pathologically by Bickel (1914) and more extensive degeneration of the optic nerves anterior to the optic chiasm demonstrated later by Adams and Kubic (1944).

The association of subacute combined degeneration of the spinal cord with anemia was recognized by many of the early authors describing this form of myelopathy, although only some emphasized a strong or universal relationship with pernicious anemia specifically (Lichtheim, 1887; Billings, 1902; Bramwell, 1915; Woltman, 1918; Hurst and Bell, 1922; Weil and Davison, 1929). By the 1920s, subacute combined degeneration of the spinal cord was clearly associated with both pernicious anemia and gastric achlorhydria (Hurst and Bell, 1922; Greenfield and O’Flynn, 1933).

In 1900, Russell and colleagues suggested the name “subacute combined degeneration of the spinal cord” (Russell et al., 1900, p. 40). Other names proposed included Putnam’s earlier “system sclerosis, associated with diffuse collateral degeneration” and “primary combined sclerosis” (Putnam, 1891), and later “diffuse degeneration of the spinal cord” (Putnam and Taylor, 1901); Dana’s earlier “sub-acute combined sclerosis of the spinal cord” (Dana, 1899a) and “subacute ataxic paralysis and combined sclerosis” (Dana, 1899b); “combined sclerosis of Lichtheim-Putnam-Dana type,” and “combined systems disease” (Pant et al., 1968). Although Putnam and Taylor (1901) objected to this terminology, after about 1910 the term most often used was “subacute combined degeneration of the spinal cord” (Bramwell, 1915; Hurst and Bell, 1922; Weil and Davison, 1929; Greenfield and O’Flynn, 1933; Weir and Gatenby, 1963; Robertson et al., 1971).

**Toxic and infectious theories for pernicious anemia and subacute combined degeneration**

At the beginning of the 20th century, pernicious anemia and the associated subacute combined degeneration of the spinal cord were considered by many investigators to result from infectious or toxic causes (Dana, 1899a, b; Russell et al., 1900; Hunter, 1901; Billings, 1902; Bramwell, 1915; Hurst and Bell, 1922; Weil and Davison, 1929; Weiss, 1991). As early as 1901, Hunter suggested that pernicious anemia is the result of infection and release of an exotoxin. He attributed the glossitis to a specific microorganism, which when swallowed produced gastritis and ultimately gastric atrophy, and he further attributed the anemia to the release of a toxin in the intestinal tract, which when absorbed into the portal circulation caused hemolysis (Hunter, 1901; Haden, 1948).

In 1922, Hurst and Bell similarly proposed that pernicious anemia resulted from “oral sepsis, absence of free hydrochloric acid from the stomach contents, and consequent intestinal infection and intoxication” (Hurst and Bell, 1922, p. 266). In support of this theory, they noted the constant association of pernicious anemia with gastric achlorhydria, evidence that the gastric achlorhydria precedes the development of anemia, the frequent presence of gastrointestinal symptoms (e.g., diarrhea, “bilious attacks” with occasional vomiting, “flatulent dyspepsia,” etc.), the fact that gastrointestinal symptoms precede the development of neurological manifestations, the frequent finding of bacteria in the sockets of infected teeth, and the finding of identical bacteria colonizing the atrophic stomach. Hurst and Bell’s bacterial toxin theory led to specific (albeit ineffective) treatments, in an attempt to rid the body of the causative infection and the secondary generation of the putative toxins, including extraction of all teeth; tonsillectomy; ingestion of large doses of hydrochloric acid and milk soured by active lactic acid bacilli; administration of a vaccine prepared from streptococci isolated from extracted teeth, duodenal contents, or feces; administration of arsenic; blood transfusion, etc. (Hurst and Bell, 1922).

In 1926, Minot and Murphy noted—at the time of their initial presentation of the first truly effective therapy for pernicious anemia (i.e., oral liver)—that the bacterial toxin theory was widely held. As they predicted, proponents of toxic or infectious theories were not easily swayed by studies demonstrating improvement with certain diets, even if others at the time felt
that “the prompt and regular beneficial effect of liver feeding on erythropoiesis seemed unlikely to be the result of elimination of bacterial infection in the bowel” (Castle, 1974, p. 25), and even if intestinal flora were unchanged after such patients were returned to (relative) health after regularly consuming a diet with large amounts of liver (Castle, 1929). Many investigators nevertheless continued to consider the presence of pathologic bacterial colonization as persuasive evidence in support of a bacterial toxin theory, without adequately considering that such colonization could be a non-causal association.

Microbial exotoxin theories gained further support in the early 1930s with the discovery in Finland of a macrocytic anemia associated with infestation of the small bowel by a tapeworm, particularly when it was shown that the anemia promptly improved after elimination of the parasite (Birkeland, 1932; Castle, 1974). Even in the 1950s, after the identification of vitamin B₁₂, toxic and infectious theories of the pathogenesis of pernicious anemia were still debated (Crosby, 1983). However, the postulates of Robert Koch (1843–1910) for establishing an infectious cause for pernicious anemia were never fulfilled for any of the putative organisms, nor was a systematic experimental effort ever undertaken with that goal in mind: specifically, the investigators never showed that the putative organisms isolated from people would cause pernicious anemia in susceptible animals, nor did they demonstrate that the organisms could then be recovered from such animals and re-grown in pure culture.

**Minot and Murphy and the liver therapy for pernicious anemia**

During the first quarter of the 20th century, a wide variety of therapies were employed in the treatment of pernicious anemia – hydrochloric acid, iron, arsenic, removal of sources of infection including all teeth, drainage of the intestinal tract, splenectomy, blood transfusion, and special diets (Haden, 1948). With the exception of transfusion, which could prolong life somewhat, these therapies were largely ineffective and life expectancy was less than 2 years.

Beginning around 1917, George H. Whipple (1878–1976) and colleagues, first in San Francisco and later in Rochester, New York, established that certain supplemental foods, such as spinach, beef muscle, and particularly liver “had a powerful effect upon hemoglobin regeneration” (Whipple, 1934/1965, p. 347; Weiss, 1991). Although Whipple did not test or apply his ideas in people, he did suggest in 1922 that pernicious anemia is a disease “in which all pigment factors were present in the body in large excess but with a scarcity of stroma-building material or an abnormality of stroma-building cells” (Whipple, 1922, 1934/1965, p. 348). By 1925, Whipple with Freida Robscheit-Robbins (1893–1973) demonstrated that liver increased the amount of hemoglobin regenerated in dogs maintained on a basal diet and kept chronically anemic by weekly bleedings accomplished by aspiration from the jugular vein (Robscheit-Robbins and Whipple, 1925). Although not immediately recognized, the magnitude of assimilation of inorganic iron was the most important factor in hemoglobin production in this experimental paradigm.

Prior to 1925, dietary supplementation with small amounts of liver had been tried in a small number of cases of pernicious anemia without marked or consistent results. But these early investigators had neither systematically nor persistently fed these often anorexic patients large quantities of liver (e.g., a half pound or more daily), nor had they carefully quantified the amounts consumed or the results (Weiss, 1991). In 1925, George R. Minot (1885–1950), at Peter Bent Brigham Hospital in Boston, and William P. Murphy (1892–1987), at the Collis P. Huntington Memorial Hospital of Harvard University, decided to hospitalize a group of patients with pernicious anemia to systematically assess liver as a treatment.

By 1926, Minot and Murphy reported clinical and hematological improvement in 45 patients with pernicious anemia treated with a dietary regimen that incorporated large quantities of liver—“From 120 to 240 Gm., and even sometimes more” (Minot and Murphy, 1926, p. 472). Clinically the patients improved, often dramatically so, in conjunction with improvements in hematological indices. This clinical improvement could be sustained for many years, well beyond the previous life expectancy of such patients (Murphy, 1934/1965). A major component of the hematological response in such patients was, in retrospect, likely due to folate which the subjects could readily absorb, rather than to vitamin B₁₂, which the subjects with pernicious anemia could at best only marginally absorb (Chanarin, 1991). Importantly, patients with (relatively mild) neurological dysfunction also improved, for example with significant improvements in gait, suggesting in retrospect that sufficient vitamin B₁₂ was also absorbed with this regimen. However, patients with more severe neurological dysfunction showed at best slow and limited improvement (Minot and Murphy, 1926).

Using the reticulocyte response as an index of erythropoiesis, Minot and Murphy were able to recognize liver as the essential component of the regimen. With Edwin J. Cohn, a physical chemist in the Laboratories of Physiology at Harvard Medical School (later recognized for his fractionation of plasma proteins), they then tried unsuccessfully “to isolate the active principle” (Minot, 1934/1965). Nevertheless, they did demon-
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strate that potent extracts could be given parenterally in very small quantities (Minot, 1934/1965; Murphy, 1934/1965).

Whipple, Minot, and Murphy were subsequently recognized jointly with the 1934 Nobel Prize in Physiology or Medicine “for their discoveries concerning liver therapy in cases of anemia” (Minot, 1934/1965; Murphy, 1934/1965; Whipple, 1934/1965).

Castle’s intrinsic and extrinsic factors—linking gastric and hematological abnormalities

In 1926, after Minot and Murphy’s success with the liver therapy for pernicious anemia, William B. Castle (1897–1990)—then an assistant resident at the Thorndike Memorial Laboratory of Boston City Hospital, which had recently come under the direction of Minot as successor to Francis Peabody—decided to pursue his belief that gastric achlorhydria (“achyia gastric”) was etiologically linked to pernicious anemia (Castle, 1966, 1974; Kass, 1978; Crosby, 1983; Herbert, 1984; Jandl, 1995). Castle, in considering why normal individuals do not have to eat large amounts of liver every day to maintain a normal blood count, noted that: (1) gastric achlorhydria precedes the other clinical manifestations of pernicious anemia (including the hematological and neurological manifestations); and (2) even when the blood of a patient with pernicious anemia is returned to normal with liver feeding, there is “a total lack of any amelioration in the secretory incapacity of the stomach” (Castle, 1929, 1974, p. 5; Johansen, 1929). Based on these observations, Castle proposed that “a virtual dietary deficiency might be produced in the presence of a diet entirely adequate for a normal individual, by the notable defect in the process of gastric digestions necessarily imposed by the absence of functional gastric juice . . .” (Castle, 1929, 1974, p. 6). Castle suggested first that an essential step of gastric digestion was impaired, thereby disrupting absorption of an essential dietary factor, and second that this defective process might be circumvented by utilizing gastric juices or some other component of the gastric digestive process from individuals with normal stomachs.

To test this idea and its subsequent elaborations, Castle devised and implemented an ingenious series of experiments (Castle, 1929; Castle and Townsend, 1929; Castle et al., 1930; Castle and Ham, 1936). Castle’s first patient was a 60-year-old woman with untreated pernicious anemia who was first given two rare beef patties (200 gm) daily for 10 days, without a reticulocyte response (Castle, 1929). Castle then fed himself raw beef patties daily instead of breakfast and an hour later regurgitated his semi-liquid stomach contents using pharyngeal stimulation (Castle, 1929; Kass, 1978; Weiss, 1988). This mixture was treated with hydrochloric acid and incubated for 6 to 30 h, then filtered and neutralized, and immediately introduced into the stomach of the patient using a Rehfuss tube. Within 5 days the subject’s reticulocyte began to increase, peaking at 10 days, with a subsequent increase in the red blood cell count of over 1 million red cells per cubic millimeter within 30 days (Castle, 1929). Similar responses were observed in seven of nine additional patients, but two patients remained refractory (apparently because they lived in another city and the predigested material had to be transported to them, necessitating a prolonged delay after neutralization with sodium hydroxide). Although not stated in the original paper, Castle later acknowledged being the source of the normal human gastric juice (Weiss, 1988, p. 157).

Castle concluded “that in contrast to the conditions within the stomach of the pernicious anemia patient, there is found within the normal stomach during the digestion of beef muscle some substance capable of promptly and markedly relieving the anemia of these patients” (Castle, 1929, 1974, p.13). He and his coworkers further demonstrated that this response is not due to gastric juice alone (Castle and Townsend, 1929), and that contact between normal gastric intrinsic factor and dietary extrinsic factor is necessary for an erythropoietic response (Castle and Ham, 1936).

Castle subsequently labeled the essential substance secreted by a normal stomach as “intrinsic factor,” and the substance present in food as “extrinsic factor” (Castle et al., 1930). Castle and colleagues showed that intrinsic factor is a thermolabile substance present in gastric juice, but not present in saliva or duodenal contents free of gastric juice (Castle et al., 1930). Although Castle did not clearly identify the role of intrinsic factor as an intestinal transport vehicle for extrinsic factor (later identified as vitamin B₁₂), he did establish that: (1) the intrinsic and extrinsic factors have to interact for effective erythropoiesis in patients with pernicious anemia; and (2) nutritional deficiencies could result from malabsorption or impaired metabolism in addition to inadequate intake.

Around this time, desiccated, defatted whole hog stomach as a replacement source of intrinsic factor was shown to be modestly successful in clinical trials. Later studies demonstrated that intrinsic factor is a glycoprotein with a molecular weight of 60 kD secreted by gastric parietal cells (Hoedemacher et al., 1964).

Isolation, structure, synthesis, and biochemical reactions of Vitamin B₁₂

Over two decades, from the late 1920s until the late 1940s, increasingly potent liver extracts were manufactured that could be given either intramuscularly or
intravenously (Castle, 1966). Progress was slow in isolating the active substance in these factors, in part because of the initial need for bioassays using untreated cases of pernicious anemia, and in part because of inadequate separation methods (Castle, 1966; Okuda, 1999). In 1947, following the development of microbiological assay techniques (Shorb, 1947) and improved chromatographic techniques, vitamin B₁₂ was finally isolated as pink crystals of cyanocobalamin—containing cobalt, nitrogen, and phosphorus—by Karl Folkers and colleagues at Merck and Company in the United States, and nearly simultaneously by E. Lester Smith at Glaxo Laboratories in England (Rickes et al., 1948; Smith, 1948; Smith and Parker, 1948; Okuda, 1999). Shortly thereafter, Castle and colleagues identified vitamin B₁₂ as Castle’s extrinsic factor, but found that oral vitamin B₁₂ even with a source of intrinsic factor was still not as potent as parenteral vitamin B₁₂ (Berk et al., 1948b).

By 1955, Dorothy Crowfoot Hodgkin (1910–1994) of Cambridge University determined the molecular structure of cyanocobalamin using computer-assisted x-ray crystallography, work for which she received the 1964 Nobel Prize in Chemistry (Hodgkin et al., 1955; Hodgkin, 1964/1972). The complex structure of vitamin B₁₂ included a single cobalt atom at the center of a tetapyrrole or “corrin” macro-ring structure. A complete chemical synthesis of vitamin B₁₂ was finally achieved in 1960 by an international consortium of chemists. Subsequent biochemical work demonstrated that only two enzyme systems required forms of vitamin B₁₂ in man: adenosylcobalamin in the conversion of methionine to methionine by methionine synthase (Sakami and Welsh, 1950; Flavin and Ochoa, 1957).

Selected clinically important studies after isolation of vitamin B₁₂

Shortly after the isolation of vitamin B₁₂, Randolph West (1948) demonstrated the efficacy of injected vitamin B₁₂ in pernicious anemia, and West and Reisner (1949) were among the first to assess the response to parenteral vitamin B₁₂ of the neurologic manifestations of subacute combined degeneration of the cord in patients with pernicious anemia. In five patients with spinal cord lesions, West and Reisner observed “varying degrees of improvement” and noted that “none has become worse,” and that “All of these patients with spinal cord lesions are walking readily” after treatment. Changes noted included improvements in ambulation, improvements in position sense with improvement or normalization of a previously positive Romberg sign, and in some cases resolution of abnormal muscle stretch reflexes or cutaneous reflexes, but generally no evident improvement in vibratory sense abnormalities.

In 1957, Dorscherholmen and Hagen subsequently demonstrated that there are two mechanisms involved in B₁₂ absorption (also see Schilling, 1958). With physiologic (i.e., 1–2 μg) doses of oral radioactive vitamin B₁₂, radioactivity appeared in plasma within several hours and reached a peak at 8–12 h, a process dependent upon intrinsic factor, but if much larger oral doses were administered the radioactivity appeared in plasma much sooner as a result of passive diffusion, independent of the presence or absence of intrinsic factor. The passive diffusion mechanism has subsequently been utilized clinically for the treatment of pernicious anemia with large (1000 μg) daily oral doses of vitamin B₁₂.

In 1957, Booth and Mollin showed that patients in whom the ileum had been resected did not absorb vitamin B₁₂ well, and then that radioactive vitamin B₁₂ administered orally prior to laparotomy was localized to the terminal ileum several hours later using a Geiger counter during surgery (Booth and Mollin, 1957, 1959). Additional studies showed that when vitamin B₁₂ is released from foods by peptic digestion it is bound to intrinsic factor, affording partial protection against gut microorganisms and parasites during transport through the gut to the terminal ileum, where the complex binds to microvilli of the intestinal epithelial cells. The vitamin B₁₂ is released into the interior of these cells, and then enters the blood stream, where it is transported by specific serum proteins (particularly transcobalamin II) to target cells.

In 1957 and 1958, Michael Schwartz and colleagues in Copenhagen observed that the therapeutic efficacy of hog intrinsic factor preparations declined with use in pernicious anemia patients (Schwartz et al., 1957, 1958). Shortly thereafter, and through the 1960s, several lines of evidence converged in support of an autoimmune basis for pernicious anemia: (1) corticosteroids improved B₁₂ absorption and reduced anemia; (2) gastric and serum autoantibodies to intrinsic factor and gastric parietal cells are present in the majority of patients; and (3) other autoimmune diseases (e.g., Hashimoto’s thyroiditis, insulin-dependent diabetes mellitus, Addison’s disease, and vitiligo) are common in such patients (Mackay and Whittingham, 1968; Okuda, 1999; Whittingham and Mackay, 2005).

Pernicious anemia is now understood to begin with an autoimmune gastritis in which parietal cell antibodies produce atrophic gastritis with resultant decline in intrinsic factor production over decades. Although the recognition of antibodies to hog intrinsic factor
HISTORICAL ASPECTS OF THE MAJOR NEUROLOGICAL VITAMIN DEFICIENCY DISORDERS

led to discovery of the autoimmune nature of pernicious anemia, these antibodies apparently do not decrease the effectiveness of hog intrinsic factor in promoting the absorption of vitamin B₁₂, and the waning of efficacy of hog intrinsic factor was therefore attributed to “local effects in the intestinal tract” (Castle, 1966). In 1988, the principle target of these antibodies was identified by Karlsson and colleagues as the acid-producing H+/K+-adenosine triphosphatase (ATPase) in the cell membrane of gastric parietal cells (Karlsson et al., 1988).

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