

A Short History of Nutritional Science: Part 2 (1885–1912)¹

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Before 1885, nearly all of the nutritional studies had been carried out in Western Europe and most were concerned with the need for either protein or energy. These lines of work continued in the 1880s but in the next 25 y, and in many more parts of the world, important new lines of work were being developed that would, in the long run, greatly broaden our understanding of nutritional requirements.

Protein research continued

Until this time, there had been little significant work in nutritional science in the United States, but Wilbur Atwater, born in 1844 in New England and by 1885, a professor of chemistry at Wesleyan University, was determined to change that. He had already spent several months in Munich studying the nitrogen balance procedures in use at the laboratory of Carl Voit, who had been Liebig's protégé. Voit believed that people with sufficient income to choose the diet that they preferred would instinctively select a diet containing the amount of protein that they needed to remain healthy and productive. His estimate was that the average German workman doing moderate physical work chose to eat 118 g protein/d, and this became his standard (1). Atwater found that American workmen were generally better off and ate more. They also, he thought, worked harder and he set his standard at 125 g/d (2).

With hindsight, it seems ironic that he should not have been more questioning concerning whether they really needed so much of this relatively expensive ingredient. Apparently he looked to the German school of nutritionists as the authorities in a field in which he was only a newcomer. Voit accepted that vegetarians who lived on a much lower protein intake could remain in nitrogen balance, but he remained convinced that such people "exposed themselves to disadvantages" (3). The American group suggested that even if protein was not directly used as the fuel for muscular contraction, it provided the nervous energy required to "wish to make the effort" (4).

The main thrust of Atwater's work in this period was to analyze foods by the proximate system (nitrogen, fiber, ash, ether extract, moisture and "carbohydrate by difference") and to use these values to teach the poor how they could obtain their requirement for protein, the most expensive of their needs, more economically (Table 1). An unfortunate effect of

recommending diets only on the basis of the economic provision of protein and energy was that fruits and green vegetables became dispensable luxuries. At this period, the purchase of food typically took ~50% of a working family's income.

The challenge to high protein standards came finally from Russell Chittenden, Yale University's Professor of Physiological Chemistry. He had found some relief from what may have been a rheumatic condition after he had deliberately reduced his general intake of food, and particularly that of meat, and was greatly impressed by having fully maintained both his physical and mental activity, although his intake of protein had not been >40 g/d (equivalent to 48 g for someone of the "standard" weight of 150 lb).

Chittenden then organized three controlled trials using low protein diets. In the first, Chittenden and three scientific colleagues remained healthy and in nitrogen balance for 6 mo on daily diets containing 62 g protein on average, after adjustment to "standard" body weight. The second trial used 11 corpsmen from the U.S. army who also remained in good health and physical condition with a standardized daily intake of 61 g protein (Fig. 1). In the final trial, a group of 7 Yale student athletes consumed ~64 g protein (standardized) per day, maintained their levels of athletic performance and said that they felt better for it (5).

Others were reluctant to accept Chittenden's recommendation of such diets as representing "physiological economy," and argued that the almost universal consumption of high protein diets in prosperous countries showed an important relationship that might not become apparent in short-term trials. He replied that his critics were reversing cause and effect; people did not become rich because they ate more protein, but ate meat and other more expensive high protein foods because they had already attained an income sufficient to afford them (6). Later studies have only confirmed Chittenden's findings.

Protein digestion and interconversion

Throughout the writings of Voit, Atwater and Chittenden, there was the unstated assumption that all proteins were of equal quality. Thus, Atwater had no doubt that meat protein in the diet could safely be replaced by the same quantity of protein from beans. With hindsight, this is surprising because Mulder's hypothesis that all proteins contained the same radical had collapsed, and even the ratio of carbon:nitrogen had been reported to differ between "legumin" extracted from beans and some animal proteins (7).

For most of the 19th century, even after the breakdown of Mulder's theory, it had been assumed by workers in nutrition

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TABLE 1

Examples of Atwater's calculations of the relative economy of different foods in supplying protein and energy¹

Food item	25 cents will pay for:		
	Total weight	Protein	Energy
	lb	g	kcal
Animal			
Oysters	1.21	41	285
Beef sirloin	1.27	86	1120
Cheese	1.67	213	3420
Beef liver	3.13	286	2095
Vegetable			
Potatoes	20	163	5900
Wheat bread	4.3	170	5500
Wheat flour	7.1	360	11750
Dried beans	5.0	520	8070
Fruit			
Oranges	2.5	7	375

¹ Source: (2).

that proteins ingested in foods were absorbed almost intact and then modified in some slight ways, if necessary, to convert them from "fibrin" to "albumin," for example. However, other workers studying the physiology of digestion first showed the existence of a substance (pepsin), secreted by the stomach wall, that converted proteins into more soluble derivatives. Liebig regarded this as being no more than breaking up aggregations of molecules, allowing them to pass through the gut more easily. A few years later, the pancreas was found to secrete another substance (trypsin) that further broke down the products of treating proteins with pepsin to produce materials that were noncoagulable, diffusible through parchment and included the chemicals tyrosine and leucine. This subject has been thoroughly reviewed, with full references, by Greenstein and Winitz in an easily available volume (8).

Now, tyrosine and leucine were already known as two of the compounds, first called "amino-bodies" and then "amino acids," that chemists had obtained by boiling proteins in strong acids. These breakdown products had not been considered of interest to nutritionists because the kind of destruction effected by strong, boiling acids had been assumed to be quite different from what happened under the mild conditions in the gut. However, the discovery of amino acids as products in a biological system was obviously highly relevant, especially because analysts had already reported that proteins appeared to differ in the relative quantities of different amino acids that they yielded on treatment with acids.

There always seems to be a way around unwelcome findings and in 1895 Chittenden wrote: "We may well consider the formation of these amino acids in pancreatic proteolysis as a means of quickly ridding the body of any excess of ingested protein food, with the least possible expenditure of energy on the part of the system" (9). Thus, he was suggesting that the proteins that the body needed were still being absorbed pretty well intact, and it was just the unwanted surplus that was being broken down before its disposal. Even in 1902, a German textbook was saying essentially the same thing: "such a profound decomposition would be a waste of chemical potential energy, and a reunion of such products is highly improbable" (10).

However, other workers in Germany and Denmark were studying whether animals could use mixtures of amino acids as

substitutes for dietary protein. Most found that meat proteins treated with pepsin and trypsin for long periods, and apparently free of intact protein, did serve as nutritional substitutes, when fed to adult dogs, but that acid hydrolysates of protein, even after neutralization and removal of excess salts, did not (11).

It had been suspected that strong acid treatment was destroying some component of the protein because proteins, and even enzymic digests, gave a color reaction suggesting the presence of an indole derivative, but acid hydrolysates did not. Finally, in 1902, F. G. Hopkins and S. W. Cole, working in Cambridge, isolated the amino acid tryptophan, which contains an indole ring, from an enzymic digest and showed that it was destroyed by conditions of acid hydrolysis (12). Then in 1906, Hopkins and another colleague reported that mice receiving zein (which contains no tryptophan) as their sole protein source, lived longer if they also received a supplement of tryptophan (13). And in 1909, Abderhalden found that adult dogs could remain in nitrogen balance if the acid-hydrolysates of protein that they were receiving were supplemented with this amino acid (14). These results did not yet prove that tryptophan was utilized for protein synthesis because there was no growth, but they did show that this organic compound had some essential function.

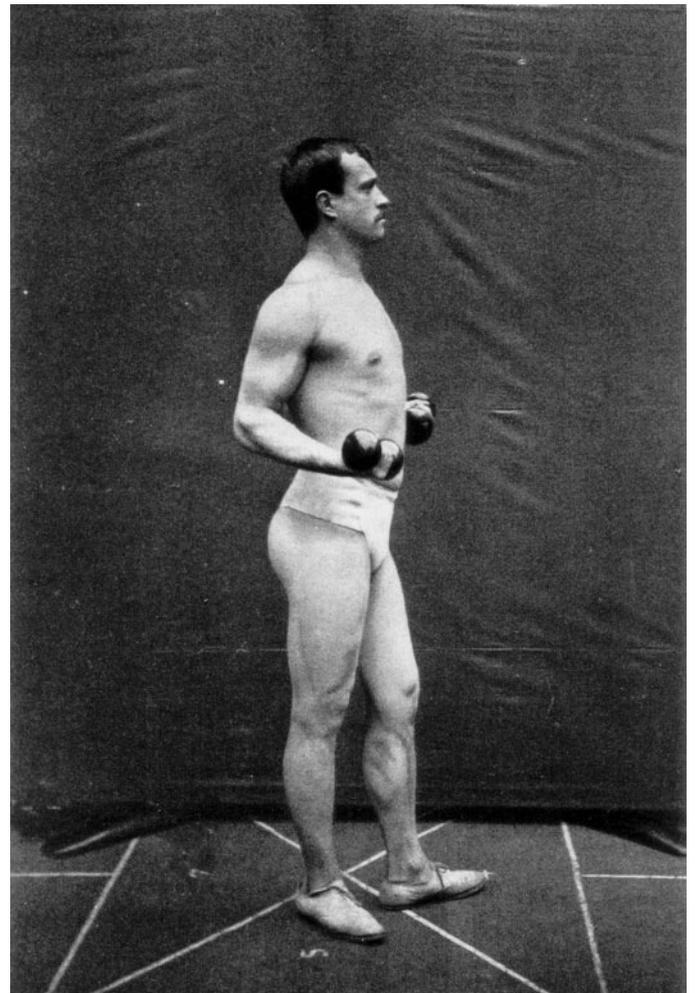


FIGURE 1 A photograph used by Chittenden to demonstrate that his subjects remained in good physical condition while consuming a relatively low protein diet. Reproduced from (5).

Calorimetry

After the work of Lavoisier and Seguin at the end of the 18th century, several workers in France and Germany gradually improved the equipment for measuring the respiratory exchange of animals and their heat output under different conditions (15,16). Finally in 1894, Max Rubner was able to demonstrate with a dog that its heat output did exactly match the heat of combustion of the foods that it was metabolizing, as measured by urea output and gas exchange measured at the same time (17).

We return now to the work of Wilbur Atwater, who also had an interest in the energy value of foods; his group established estimates for the metabolizable energy of the carbohydrates, protein and fat in mixed diets as 4, 4 and 9 kcal/g, respectively. These “Atwater factors” were slightly different from those proposed by Rubner, but have stood the test of time (18).

However, Atwater’s real ambition was to make a fundamental contribution to nutritional science by building and conducting research with a respiration calorimeter that would hold a human subject over long periods and also measure their heat output directly. **Figure 2** illustrates the kind of equipment needed for a human respiration calorimeter, but to measure the heat output at the same time is much more complex. This was an ambitious and expensive machine that took Atwater’s group five years to build and test. The first aim was to confirm that the heat produced by the human body was the same as that produced outside the body (i.e., *in vitro*) by the combustion of the same quantity of nutrients. This they were able to do with considerable precision (19).

They then made the controversial finding that ethanol, given to a subject in a series of small doses, could also serve as a source of useful energy (20). This was controversial because the liquor trade made use of it in its advertising, whereas their university (Wesleyan) was supported by the Methodist church, which recommended total abstinence from alcohol, and its members circulated pamphlets that described it as nothing but poison. Atwater responded that the Almighty would not wish moral teaching to be based on untruths (21).

After Atwater suffered a disabling stroke in 1904, his colleagues prepared for publication the further work demonstrating that the energy from combustion of either fats or carbohydrates could be used for mechanical work with at least similar efficiency (22). The equipment was then moved away from Atwater’s laboratory, and there is a sad story of his

daughter having to make up accounts of what it was still being used for to her bedridden father, who had been kept unaware of his group’s dispersal.

Wilbur Atwater is remembered as the “father of nutritional science” in the United States. This is justified not solely by his own research, but at least equally by his administrative work at the U.S. Department of Agriculture where he organized food consumption studies in many parts of the country. He also set in place a policy of encouraging long-term basic work in nutrition at agricultural experiment stations. The fruit of this policy will be seen, for example, in part 3 of this sequence, in which we will review Thomas Osborne’s long-term work at the Connecticut experiment station on the amino acid composition of proteins, and his collaboration with Mendel on their nutritional significance.

Anemia

Anemia, or chlorosis (literally “the green disease”), had long been a common problem among young women in their teens. By 1885, it was agreed that the blood of an anemic patient would show a decreased blood cell count and proportionally an even greater fall in hemoglobin level. It was also generally agreed that the condition would respond to the administration of pills containing ferrous sulfate. However, it was not generally accepted that this response was the straightforward result of the iron being absorbed through the wall of the gut and incorporated into molecules of hemoglobin. There was first the theoretical objection that: “it was just as unlikely that the animal kingdom could make hemoglobin from inorganic iron as that it could make protein from potassium nitrate and starch” (23,24). Of course we have already seen the 19th century belief that only the plant kingdom was capable of synthesizing protein.

Second, German workers had found that when they added a supplement of ferrous sulfate to a dog’s diet of meat, the additional iron recovered in the feces was at least nearly equal to that ingested and, given the inherent variability in such determinations, they concluded that inorganic iron was essentially “indigestible.” Typical results from one study are summarized in **Table 2** (25).

At this time, investigators had the most experience in studying the digestibility of proteins, which had been found to be equally well digested at any level of intake. There was no reason therefore to suspect that the digestibility of another

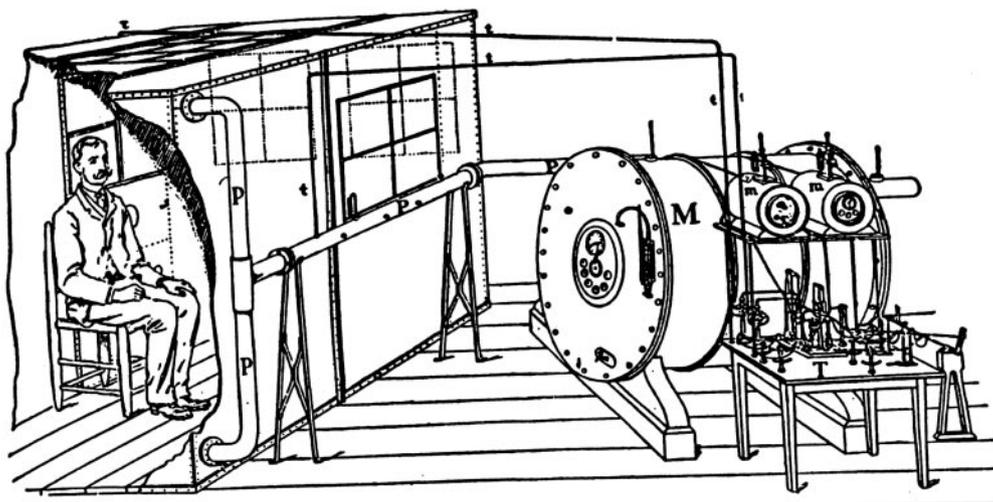


FIGURE 2 A diagram used by Atwater to illustrate the kind of equipment needed for a human respiration calorimeter. Reproduced from (15).

TABLE 2

Iron balance in a dog, used as evidence for the unavailability of inorganic iron¹

Period	Diet	Fe intake	Fecal Fe	Urine Fe	(Net balance)
<i>d</i>		mg/d			
0-6	Meat alone	25.0	24.5	3.2	(-2.7)
7-21	Meat + FeSO ₄	57.0	51.3	3.9	(+1.8)
Difference between treatments		+32.0	+26.8	+0.7	(+4.5)

¹ The calculated "net balance" was not included in the original paper (25).

nutrient would depend on the level ingested or on the prior status of the test animal.

Looking at the data in Table 2 through modern eyes, one wonders why the authors in the earlier period missed the equally obvious point that the values obtained with meat alone indicated that organic iron was also "indigestible." It illustrates how easy it is to see what one wants to see in a set of data, and to be blind to anything that does not fit in with one's preconceptions.

Human nature, as we have said before, seems to allow us to explain our way out of almost anything, and this was no exception. Here the argument was that there is never really a shortage of organic iron in the diet, but that some people unfortunately have a dyspepsia accompanied by the production of hydrogen sulfide in their small intestine, and that this compound has such a strong affinity for iron (and some other metals) that it takes even organic iron into an indigestible complex and makes it nutritionally unavailable. The value of ferrous sulfate is explained by the hydrogen sulfide reacting with it still more readily, and leaving no excess to react with the organic iron that therefore remains available for use. It was also believed that nontoxic compounds of other metals, such as bismuth and manganese that also formed insoluble sulfides, should be equally effective (24).

In the 1890s, these ideas were challenged by Ralph Stockman, a physician at Edinburgh University. He first tested the usefulness of subcutaneous injections of small quantities of ferrous citrate into anemic patients and found good responses in both red cell counts and hemoglobin levels in the blood. He then gave other patients keratin-coated capsules containing ferrous sulfide. According to the prevailing theory, these should have been inactive when released by digestion of the coating in the small intestine but, in fact, they too resulted in an improved blood picture. On the other hand, giving either bismuth oxide or manganese dioxide (both supposedly able to bind and neutralize hydrogen sulfide as well as ferrous salts) proved ineffective with anemia patients (26). All of these results were contrary to what would be expected in terms of the "dyspepsia" theory.

Stockman went on to investigate the assumption that there was always plenty of iron in ordinary human diets and found that there was a source of interference in the standard method of iron analysis in use in that period (27). When a sample contained a large proportion of carbohydrates, some survived the initial ashing step, and in the final stage, in which the extract was titrated with potassium permanganate that oxidized ferrous to ferric ions, an additional quantity of permanganate was reacting with carbohydrate breakdown products.

Because of this, bread had been considered to be as rich in iron as meat. With Stockman's revised procedure, bread was found to have only ~5 mg iron/kg, whereas meat had ~40 mg/kg (28).

Analyses of five actual daily diets, consisting mainly of tea, milk, bread and butter, which were being consumed in surprisingly small quantities by anemic young women, showed an average intake of only 3 mg iron. In contrast, the more varied and abundant diets of healthy nurses were all found to contain 9-10 mg iron. He concluded that the combination of low iron intake and menstrual losses was sufficient to explain the occurrence of chlorotic anemia in young women, and also that when red blood corpuscles break down, the great bulk of the released iron is retained and reutilized so that both the excretion and the requirement for the element are quite small. Stockman's work illustrates the importance of analytical procedures being specific and accurate, and how an inefficient procedure may seriously mislead investigators concerning the cause of a clinical problem.

Beriberi

By the 1880s, Japan had developed a navy using warships built in Europe and employing the general practices of Western navies. However, an alarming proportion of the sailors was succumbing to a disease known in Japan as *kakké*, but soon recognized to be the same as that called *beriberi* elsewhere in Asia and classified as a "polyneuritis" (Fig. 3). It was characterized by initial weakness and loss of feeling in the legs, and then the development of heart failure and breathlessness, together with edema in some cases (29). Kanehiro Takaki, a naval surgeon who had received postgraduate training in England, was made responsible for eliminating the problem. The only difference that he could find in the conditions of the Japanese sailors compared with those in European navies in which the problem did not exist, was that their diet was considerably lower in protein content and well below the "Voit" standards accepted then. (This was before the work of Chittenden, discussed above.)

One voyage of a cadet training ship, to New Zealand and back, had been particularly stricken, with over one half sick with beriberi and 25 dead. Takaki persuaded the authorities to repeat the voyage with the rations modified to include more meat, condensed milk, bread and vegetables at the expense of rice, so as to increase the proportion of protein. This time there were no deaths and only 14 cases of the disease, all among men who had not been willing to eat the full extra rations. Takaki believed that he had confirmed that the disease resulted from a deficiency of protein and, indeed, the problem virtually disappeared from the navy after the rations were changed throughout the fleet (30,31).

In the same period, there was a similar problem of beriberi in the native army, recruited by the Dutch in their East Indian colony (now Indonesia), which had been sent into the field to suppress a local uprising. In view of recent successes by Robert Koch and others in identifying the microorganisms responsible for several diseases, the Dutch government dispatched a small team led by Professor Pekelharing, who was trained in bacteriology, to spend 8 mo investigating the beriberi outbreak. He had no knowledge of Takaki's work at that time.

Their examinations of autopsy material showed evidence of neural degeneration. No bacteria were found in the blood of beriberi patients in a base hospital, but they were found in both sick and healthy soldiers in Atjeh where the fighting was in progress. It was also of interest that soldiers posted there would develop the disease only after ~60 d in the area. There, Pekelharing also found that a single injection of blood from a



FIGURE 3 Victims of beriberi, with difficulty in walking, in one of the Javanese prisons surveyed by Vorderman. Reproduced from (38).

beriberi patient into a dog had no bad effect, but that some dogs that had received as many as 20 repeated injections over a period of 6 wk, did become sick and gave indications of nervous degeneration (32).

In his final report, Pekelharing wrote that he believed the disease to be the result of an unusual type of bacterial infection, but that more work was required to confirm this, and he recommended that this be carried out by Christian Eijkman, a young army physician who had been assisting him, and was permanently stationed in the colony. Eijkman was therefore relieved of military duties and put in charge of a modest research unit under civilian control, but adjacent to an army hospital on the outskirts of Batavia (now Djakarta) that contained many beriberi patients.

Chicken polyneuritis

Eijkman decided, in view of the variability of individual animals, that he would need to use large numbers, and he chose chickens because they were readily available and cheap to buy, as well as to house and feed. This remains a surprising choice for an animal model considering that they are not even mammals, but it turned out to be a lucky one. He began by trying to infect birds by injecting them with blood from hospital patients and, after a few months, he did begin to see birds with an unsteady gait, somewhat reminiscent of beriberi cases. However, the same condition was seen in uninjected control animals kept in the same compound. This could, of course, have resulted from the infection jumping from bird to bird. Examination of autopsied birds showed the presence of degenerated nerves and this encouraged him to hope that he had indeed induced a condition comparable to the human disease. He therefore started more trials with the controls further separated from those injected, but now he failed to see the problem developing in any of his chickens (33).

I believe that at this point most of us would have abandoned trying to use chickens that were proving so inconsistent, but Eijkman thought that there must be some explanation. From the local servant responsible for the maintenance of the birds he discovered that, at the time when the birds were showing leg weakness, the man had, for some months, been able to beg leftover cooked rice from the hospital kitchen and to use that to feed the birds. But then a new cook had

been appointed who had said that he was not going to give military rice to civilian chickens (34).

Eijkman at once began tests with the leftover cooked rice and found that its use did lead to leg weakness in his chickens after 3–8 wk, whereas, at the same time, chickens fed either uncooked hospital rice or rough, feed-grade rice, remained healthy for the 3 mo of the trial. He then looked into the technology of rice preparation and learned that the “white rice” used in the hospital had had the grains “polished” to remove the bran layer, after the usual preliminary removal of the husks. He also learned that the local peoples in their villages would pound rice grains each day, to remove just the husk by winnowing, and then they would cook and eat the “brown rice” with its bran still attached. This was no problem when it was consumed fresh but, for supplying an army for which foodstuffs often had to be shipped and stockpiled for a period, brown rice was unsuitable because it would become rancid under tropical conditions and hence unpalatable.

He considered the disease in the birds to be a variety of peripheral polyneuritis, on the basis of his finding damaged peripheral nerves on autopsy examination. From reading the literature, he learned that in humans it appeared usually to be caused by some kind of poisoning, perhaps only indirectly through the production of toxins by bacteria. His first thought therefore was that: “the cooked hospital rice favored conditions for the development of micro-organisms of an unknown nature in the intestinal tract, and hence for the formation a poison that caused nerve degeneration.” One aspect of the disease in chickens that differed from human beriberi was that the birds uniformly lost weight, but giving birds a reduced amount of brown rice so that they too lost weight did not result in leg weakness.

Eijkman now began a long series of feeding trials, partly interrupted by his suffering bouts of malaria, and it was to be six years before he made a progress report on his work. One of his first findings had been that the disease would, after all, appear in birds receiving uncooked white rice, although usually only after a longer period than with the cooked material. He therefore had to abandon his first idea that the disease was caused by pathogenic microorganisms that had thrived in the cooked rice during its overnight storage. He also discovered that sick birds could be cured by switching them to a diet of

brown rice. He therefore concentrated on what factor in the bran coat, still present in brown rice, could be responsible for its protective effect. He wondered if it could be the fiber content of the bran, but he found that giving ground-up bran was still effective, whereas giving ground-up husks, as an alternative source of fiber, was not.

In parallel experiments, he had found that feeding birds either sago or tapioca starch also resulted in both their losing weight and developing the characteristic leg weakness, so that the effect was not peculiar to rice. Then he tried feeding a bird each day 500 g tapioca and 25 g raw meat, which contained at least as much protein as did a brown rice diet. The bird gained weight but, after 4 wk, developed the usual leg weakness. This impressed Eijkman as showing that chicken polyneuritis was not inevitably accompanied by emaciation. When the bird was changed to a diet of meat alone it gradually recovered, and other birds fed meat alone from the beginning also remained healthy. He concluded that the only common factor in diets that produced this condition was starch, and that starch was probably subject to fermentation in the intestines by microorganisms that produce a toxin and further, that the bran surrounding the rice grain provided an antidote to the toxin (35).

Even this account of his work has omitted many experiments, some of them with other species that yielded inconclusive results. It is no wonder that he used as the text for a lecture given after his return to the Netherlands: "Simplicity is not characteristic of truth" (36). One must feel for this man. His wife had died on his first tour in Indonesia. He himself was still suffering from bouts of malaria and would soon have to leave the tropics for good, and he knew that others were skeptical whether the disease in chickens, and the new kind of phenomenon into which he had stumbled, had any real relation to beriberi. Indeed one critic, after reading his report, was to write that: "It must be considered the most inadequate product which can be found in the literature from the Director of a scientific institute" (37).

One finding was to console him, however, after his return to the Netherlands in 1896. Before leaving Batavia he had talked about his work with Adolphe Vorderman, the medical inspector of prisons in Java. It occurred to them that there might already be a natural experiment in progress with different prisons using different kinds of rice. Surprisingly, there were 101 prisons and 250,000 prisoners in this island, roughly the size of Greece. Vorderman made a thorough investigation and found that, indeed (as seen in Fig. 4), the incidence of beriberi was vastly greater in the prisons in which mostly white rice was in use (38,39). It appeared that less than one prisoner in 10,000 developed beriberi in the "brown rice" prisons, and one in 39 in the prisons using mostly white rice. Most of the prisoners had very short sentences, but among the long-term prisoners receiving white rice, ~1 in 4 had developed beriberi. Moreover, other factors that had been suggested as being conducive to the disease, such as overcrowding and poor ventilation, showed no evidence of having any adverse effect. This was a strong indication therefore that Eijkman's work did have relevance to the human disease.

The man who succeeded Eijkman in Batavia was Gerrit Grijns, whose background was similar to that of his predecessor. His first work was to try to fractionate rice bran to discover the character of the active material that it contained; at first, he was disappointed to find that his manipulations seemed to destroy the activity. Then he realized that this gave him the opportunity to test Eijkman's idea that the appearance of the chicken disease depended on the presence of starch. He autoclaved meat and fed it as the sole food for 8 cockerels and they all died, with all but one showing characteristic paralysis. The

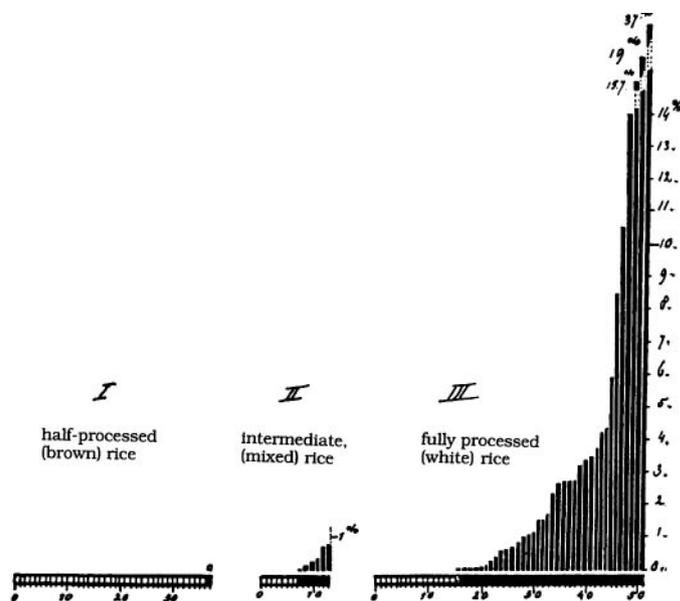


FIGURE 4 Vorderman's figure illustrating the contrasting incidence of beriberi in Javanese prisons according to the type of rice in use. Reproduced from (38).

evidence for starch being responsible for the condition, perhaps through its stimulating a toxic fermentation, was therefore discredited. Grijns also showed that several varieties of beans were even more effective than rice bran in supplementing a chicken's diet of white rice, and he ended his 1901 paper with these historic sentences:

"There occur in natural foods, substances, which cannot be absent without serious injury to the peripheral nervous system. The distribution of these substances in different food-stuffs is very unequal. . . The separation of these substances meets with the difficulty that they are so easily disintegrated. . . They cannot be replaced by simple compounds" (40).

Grijns, too, had to return to the Netherlands in 1902 to spend two years recovering from another tropical disease, but his work was immediately pursued by others. Hulshoff Pol, the physician in charge of a mental hospital in Indonesia in which there had been a serious problem of beriberi, heard of his experiments with beans and decided to test their value for his subjects. He had the men who had already developed the disease transferred to a hospital unit. For the trial, the subjects, all initially healthy, were housed in six separate buildings. His control groups, housed in three buildings, consumed their standard rations, and some in each building developed the disease over the next 9 mo (altogether 19 out of a total of 58). Those in three other houses, chosen at random, received the standard daily ration supplemented with 150 g mung beans, and none of the 78 men in these houses developed the disease. Those who had developed the disease with consumption of the standard diet were then given the same supplement of beans and they were cured (41). This result was a further confirmation of the relevance of work with a small animal model for the cure and prevention of a human disease.

By 1905, the Dutch workers in Indonesia had demonstrated fairly convincingly that this disease was the results of white rice lacking some unknown, heat-labile component. However, this was not yet accepted in other parts of Asia in which the disease was a problem. During the Russo-Japanese war of 1904–1905, many Japanese soldiers serving in Manchuria suffered from beriberi (the estimates ranged from 90,000 to

200,000) and many were brought back to temporary hospitals in Japan and treated on the basis that it was an infection (42).

It was a chronic problem in Malaysia also, and Leonard Braddon, who had served there as a British colonial medical officer became convinced, without knowing of the recent Dutch research, that white rice was the culprit. But he believed that the “polishing” of the grain left it with a porous surface that allowed it to pick up pathogenic fungi that had accumulated in the milling machinery; in addition, during storage, the fungi continued to proliferate in the white rice and were responsible for the signs of beriberi (43).

Workers at the Malaysian Institute for Medical Research, who had learned of the Dutch work and begun their own studies with chickens, found that adding alcoholic extracts from brown rice to a diet of white rice prevented the appearance of polyneuritis in their birds, whereas feeding the brown rice from which the alcoholic extract had been taken now induced the disease (44). These findings could be explained only in terms of the white rice being deficient, rather than toxic.

The United States, which had become the occupying power in the Philippine Islands after the Spanish-American war of 1898, was also faced with the problem of beriberi among its native troops, and the authorities there called an international meeting in 1910 to discuss the problem. This was well attended, with delegates from Japan, Java, Malaysia, Thailand, Sri Lanka and French delegates from the area that is now Vietnam. It was fairly generally agreed that the disease was restricted to those who had white rice as their staple food, and the American delegation suggested that the “public health” approach should be to make its production illegal, or to tax it so highly that it could be afforded only by those better off who would, in any case, eat a more varied diet containing other items that would make up for the deficiency of the rice. Other delegates thought this to be impracticable because of the problem of brown rice going rancid during storage in the tropics (45).

In Japan it had been noticed some years earlier that breast-fed infants were subject to a disease called “*taon*” that was characterized by vomiting, edema and stoppage of urine secretion. The death rate was very high, and it seemed as if their mother’s milk was poisonous to them because they could recover if given cow’s milk. Physicians working with the U.S. Army Medical Corps in the Philippines made similar observations; they suspected that the condition was analogous to adult beriberi, and found that infants would also recover if breast-feeding was supplemented with extracts made from rice bran, using alcohol, which was then evaporated (46).

By this time, workers in Java and elsewhere were already trying to concentrate and isolate the active factor from rice bran, in the distant hope of being able, eventually, to identify and even synthesize it. It was discovered in Germany that pigeons, which were easier to house, could be used instead of chickens and showed a characteristic head retraction when deficient in the antiberiberi factor (**Fig. 5**). It now became fairly simple for this problem to be pursued in established laboratories in the West just as easily as in countries in which beriberi existed. For example, because rice polishings had a relatively high concentration of phosphorus, a worker in Germany tested phytin, phosphonucleic acids and other phosphorus-containing compounds for their potency. These were found to be inactive, but dried yeast was discovered to be highly active (47).

In 1911, Casimir Funk, a Polish chemist working at the Lister Institute in London, was the first to claim to have isolated active crystals from rice polishings: they consisted of

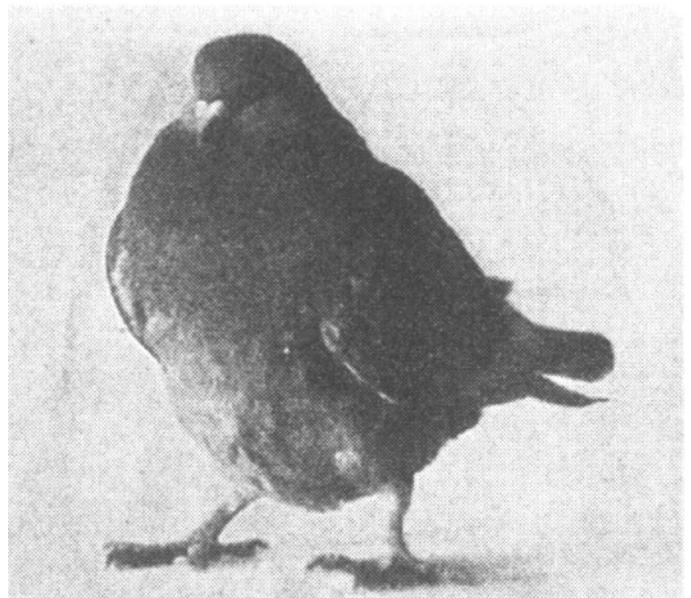
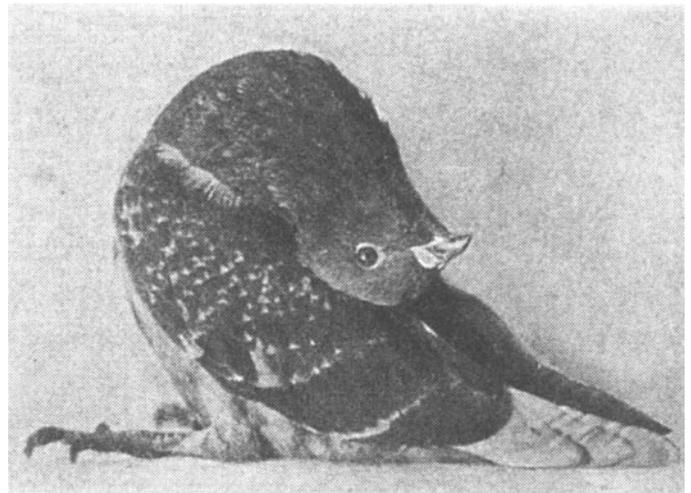


FIGURE 5 A pigeon showing characteristic head retraction after it has been fed white rice for 2–3 wk (*upper panel*), and the same bird 3 h after receiving a yeast extract (*lower panel*). Reproduced from (65).

an organic base, and 50 mg was sufficient to cure a deficient pigeon (48). In the following year, a group of Japanese workers obtained even more active material (49). It was later realized that these were both still mixtures and highly competitive attempts at purification were to continue for many more years.

Rickets

Rickets in young children, characterized by inadequate calcification of the bones with bowed legs on walking and deformities of the ribs, was becoming a more-and-more common problem in large cities in Western Europe and the United States in this period. It could not be the direct result of an inadequate intake of calcium salts because it was as rife in areas in which the water had a high concentration of “lime” as elsewhere. It also occurred in well-to-do families in which the child was plump and well fed. Walter Cheadle, in his review of the problem, concluded that the common factor was the child not being breast-fed, but receiving either skim milk or newly patented “artificial foods” that were high in starch, but con-

tained much less fat than breast milk. He referred also to the problem in the London Zoo, where the lion cubs were ignored by their dams and had been dying of rickets until their diet of horse meat was supplemented with cod liver oil and ground bones (50).

Theobald Palm, who had been a medical missionary in Japan, had been surprised at the complete absence of rickets there. He organized a world-wide survey by fellow missionaries of the incidence of the disease and concluded that it was always absent where people were in long hours of good sunlight, not blocked by industrial smoke (51). Putting the work of the two authors together it appeared that the disease appeared only when two conditions were met: sunlight was limited **and** the child was fed an inadequate substitute for breast milk. In the following period, these ideas were to be subjected to further tests with animal models, but there was already sufficient evidence concerning how the disease could be avoided.

Infantile scurvy

By 1885, a London pediatrician, Thomas Barlow, had observed in some children with rickets an additional problem reminiscent of adult scurvy (52). Postmortem examinations had shown effusion of blood around the ends of the long bones and the separation of the rib bones from their connecting cartilage. These were characteristic effects of scurvy in adults as seen by Lind and other early investigators, but not at all characteristic of rickets. In France and Germany the same condition was recognized as becoming common and was called "Barlow's disease."

It was being seen increasingly in the United States also, and by 1897, over fifty papers had been published on "Barlow's disease." In the following year the American Pediatric Society had completed an enquiry into 356 cases in which the method of feeding was known. Of these, only 12 had been receiving breast milk, and the great majority had been receiving either cow's milk that had been sterilized or "condensed," or proprietary powders reconstituted with water. It had also been found that giving children orange juice together with raw cow's milk, or even raw cow's milk alone would result in their recovery (53).

A major cause of death in young children before this time had been "summer diarrhea," which was believed to be caused in large part by infection due to gross bacterial contamination of the milk brought into large cities. There had therefore been an active and successful program in many cities to make sterilized milk available for feeding to young children. Pediatricians were therefore understandably reluctant to incriminate this product, which was so beneficial in other respects.

The problem was also debated in Paris and Berlin. One idea was that the heat process resulted in changes to some of the milk proteins that made them less easily digestible, so that indigestible residues putrefied in the large intestine and caused autointoxication. This, of course, was an analogous idea to Eijkman's explanation for rice starch producing beriberi in chickens. But the idea was not supported by the condition being cured, even when sterilized milk continued to be given, by supplementing it with either potato gruel or orange juice. No animal model of the disease was available as had been the case with beriberi.

Adult scurvy

During this period, examples continued to occur of people on some expeditions in the Arctic succumbing to scurvy even

though they regularly took one ounce of lime juice each day, whereas on other expeditions in which people were stranded on land, they kept healthy without lime juice, but did eat raw or lightly cooked meat and blood (54). Frederick Jackson, the leader of one successful expedition, concluded: "The use of lime juice neither prevents nor cures scurvy . . . [it] is a disease developed through eating tainted food. . . . No scientific study of scurvy has been prosecuted since the discoveries of Pasteur have shown us the havoc produced by bacteria as a cause of disease." He put the blame specifically on canned meat, which had replaced the traditional salt meat that had been taken on earlier expeditions. He suspected that, before the canning process, the meat had deteriorated and the bacteria multiplying in it had produced ptomaines and other toxic materials that survived autoclaving, even though the bacteria themselves were killed by the procedure. He quoted his own experience of surviving by eating fresh game and also the experience of the Hudson's Bay Company who had found that when scurvy had broken out in one of their depots, it was enough to send them a good huntsman so that their diet could be supplemented with fresh meat (55).

On Jackson's return to London, he obtained the collaboration of the Professor of Physiological Chemistry at London University, and they fed monkeys meat from either freshly opened cans or from cans that had been left open for several days, so that the meat had become sour. Unfortunately, the monkeys had been newly imported and were not acclimatized to their conditions; all developed diarrhea and died within 8 wk. However, the observers believed that they had seen spongy gums in 5 of the 8 animals receiving soured meat, and in none of those eating from the freshly opened cans (56). Their work was presented to a prestigious audience at a meeting of the Royal Society in London and it was to have considerable influence.

The next British expedition, this time to the Antarctic, was provisioned in terms of the ptomaine theory. Before it sailed in 1901 the senior surgeon said: "the benefit of the so-called antiscorbutic is an illusion An animal food is scorbutic if bacteria have been able to produce ptomaines in it, . . . otherwise it is not" (57). After a winter during which they lived largely on canned meat that was inspected and approved by the surgeon, sledging began and, within a very few weeks, scurvy became a serious problem. The policy was now reversed: lime juice was placed on the tables at meal times, although still not made a standard issue, seals were killed to provide fresh meat and the surgeon began to grow mustard and cress. Gradually, most of the men recovered their health (58).

Guinea pig scurvy

In 1902, Axel Holst, a Norwegian professor of bacteriology and hygiene who had been concerned at the appearance of what had been diagnosed as beriberi in the crews of Norwegian sailing ships, seized an opportunity to visit Grijns in Batavia and to see his work on chicken polyneuritis. On his return to Oslo, he attempted to obtain a closer model of "ship-beriberi" by using a mammal as his experimental species, and chose guinea pigs. He fed them grains, either whole or milled, and found that they all died within ~30 d. When the carcasses were opened he saw "pronounced hemorrhages" and looseness of the molar teeth. Theodor Frölich, a pediatrician with experience of infantile scurvy, confirmed that the condition appeared to be scurvy with no evidence of any kind of polyneuritis. The two men then found that the condition was not produced by semistarvation, and that it was prevented by giving two traditional antiscorbutics, lemon juice and fresh

cabbage (59). They also confirmed that cow's milk lost most of its antiscorbutic activity when it had been autoclaved to sterilize it.

This was important work, providing an animal model for scurvy, analogous to that of chicken polyneuritis for beriberi, and supplying supplementary evidence that the disease was a deficiency, rather than the result of some kind of intoxication.

Night blindness and xerophthalmia

The most important work related to these conditions that was published in this period was a study by Masamichi Mori in Japan of 1500 cases of the disease known locally as "hikan." He recognized it to be identical to the condition of night blindness and xerophthalmia seen in the West, where some cases progressed to keratomalacia and even blindness, as did some of his cases. He described the diets of the weaned young children as lacking in fat. He found that cod liver oil was the most effective treatment, olive oil inactive and sea-lamprey oil of intermediate activity. He surmised that the cod liver oil was probably the best absorbed of the three. He also considered animal milk to be protective, but that breast-fed infants could develop the condition if the mother was also showing signs of the disease (60). Despite the preexisting knowledge of the value of animal liver and cod liver oil in the treatment of night blindness, some textbooks were still failing to recommend them (61).

Goiter

During this period, there was little advance in the understanding of this condition. In 1895, a leading German surgeon reported successes from feeding animal thyroid glands to patients, and suggested that the hypertrophy of their own glands might be their body's response to receiving too little of their secretions. However, this treatment fell out of favor again, perhaps due to its being given in too large quantities, which had unpleasant side effects (62). The true nature of the problem was not to be established for another 20 years.

Cattle fed single-grain diets

Stephen Babcock, the agricultural scientist famous for developing in 1890 a convenient apparatus for determining the fat content of milk, was skeptical concerning the usefulness of the "proximate analysis" determinations (nitrogen, ether extract, crude fiber, moisture and ash) for the nutritional evaluation of foods and feedstuffs. He is supposed to have irritated Atwater by telling him that if he really believed in the system, he should be willing to recommend using cow dung, with its favorable proximate analysis, as feed for cows. At Wisconsin, on his official retirement, he was succeeded as Professor of Agricultural Chemistry by Edwin Hart, and Babcock challenged him to feed breeding heifers ingredients all from a single cereal grain, and to compare the result with a diet made up from mixed cereals.

Hart agreed and, with a group of colleagues, used sixteen 6-mo-old heifers and constructed three rations each based entirely on either corn, oat or wheat products, and balancing the proportions of ground grain, gluten and straw to obtain the same energy value and proximate analysis. A fourth ration was a mix of the other three. The trial was begun in 1906 and continued for two full reproductive periods; the results are summarized in Table 3. The heifers receiving the all-wheat ration quickly lost condition and performed extremely badly, with none of their calves surviving and two of the cows also

TABLE 3

Summarized results from the Wisconsin single grain experiment, with 4 heifers per group¹

Grain used	Mean weight gain of heifers in 1 y	Total calves in 2 y		Mean milk yield
		Born	Healthy	
	lb	n		lb/d
Wheat	353	5	0	12.1
Mixed	410	6	3	20.6
Oats	408	8	6	24.7
Corn	471	8	8	26.0

¹ Sources: (63,64).

dying before the end of the trial. In contrast, the corn-fed heifers maintained their condition and had healthy, strong calves, with the results from the other treatments being intermediate (63,64). This was an expensive trial for the Experiment Station, and the authors, at the conclusion of the final report, wrote: "We have no adequate explanation of our results." However, in the long run it was to prove highly productive because it provided the launch point for the research programs that were to make the University of Wisconsin a leading international center for nutritional science.

One man recruited by Hart to work on the "single grain project" was a young chemist, E.V. McCollum, who had previously been working with Mendel at Yale. At the end of the trial, Hart assigned him the task of finding out what was wrong with (or perhaps deficient in) an all-wheat diet. McCollum felt that the quantities of feed eaten by cattle were too great for him to be able to control their quality, and that he must try to work with a smaller species that would also have a shorter life cycle. Despite his dean's displeasure, he started on work with rats, and that was the beginning of a considerable saga to be described in Part 3 of this history.

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