
E. V. McCollum
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PREFATORY CHAPTER

MY EARLY EXPERIENCES IN THE STUDY OF FOODS AND NUTRITION

By E. V. McCollum

The Johns Hopkins University, Baltimore, Maryland

This chapter was written at the request of the Editorial Committee. Among the suggestions which the Editor offered as to the kind of chapter which would be acceptable was one to the effect that the writer might prepare ". . . an autobiographical sketch in which he describes his own experiences as a student, a teacher, and an investigator." It is this suggestion that has been followed. These reminiscences will be limited to my educational experiences and to the decade of 1907 to 1917, the period of my earliest experiences as a nutrition investigator. This was a decade of special importance for clarifying the ideas of workers in this field as to how to use animals effectively for discovering the existence of hitherto unsuspected nutrients, a task for which chemical procedures alone were inadequate. It was the decade when the initial successes were achieved in determining, in individual naturally occurring food substances, and in some mixtures of foods, the nature of the chemical deficiencies which limited their ability to support physiological well-being in an animal.

As a youth working on a Kansas farm it appeared to me that among all the people I saw, the life of the country doctor, with his many human contacts and general esteem, was the most desirable. I early resolved to study medicine. This resolution I kept through my high school years and my first year at the University of Kansas, in 1900. In my second year I listened with delight to the lectures of Dr. Edward Bartow on elementary organic chemistry and soon made up my mind that the chemistry of organic substances was the field in which I wanted to become proficient and to devote himself.

On completing the first course I proceeded at once to synthesize organic compounds, using the German edition of Gatterman's *Praxis;* I prepared in succession the forty-eight kinds of substances there described, and purified them as directed. Dr. Bartow taught me to use his copy of *Beilstein.* When I reached the synthesis of quinoline, by the method of Skraup, I had my first inspiration in research. As Dr. Bartow's lecture table assistant I had access to the store rooms, where I had seen eight bottles of substituted anilines. It occurred to me that if each of these substances were heated with sulfuric acid and glycerol, there would be obtained instead of quinoline, which resulted from the use of aniline, a series of derivatives of quinoline. I made a list of the names and formulas of the chemicals in these bottles, and then looked in *Beilstein* to see if any of them were described. I found none and told Dr. Bartow about what I had done and would like to do. He said the derivatives of aniline belonged to Professor E. C. Franklin, and that I should not use them without his permission. I took up this matter with Dr. Franklin, who taught me my first course in quantitative analysis, and my first course in physical chemistry, and he said he was done with them and that I was welcome to them. So I synthesized the new quinoline derivatives, and purified and analyzed them according to the directions of Dr. Bartow. We published the results under joint authorship.

In 1903 I received my A.B. and in 1904 I was awarded the M.A. Degree at Kansas University. For my thesis I presented the data which the late J. Arthur Harris and I secured in a study which we made of the composition of the gas mixture present in the hollow stems of the large water lily *Nelunbo lutea*, which was then very abundant in a lake not far from Lawrence. Harris was a botanist of note and the closest personal friend of my undergraduate years. We camped for a week and collected samples of the gas morning, noon, evening, midnight, and before sunrise, and secured samples on cloudy days as well as sunshiny ones. We never published our data.

While at Kansas University I gained credit in sixteen courses listed by the department of chemistry, and in September, 1904, I began to study under Professor Treat B. Johnson in the Sheffield Scientific School of Yale University. He was then just outgrowing his master, Professor Henry Lord Wheeler, who was still in charge of organic chemistry there. I devoted two years to syntheses in the pyrimidine series.

During these years I shared an apartment with Philip H. Mitchell, a student in physiological chemistry, who became head of physiology at Brown University. From him I heard much of the activities in the laboratory of Professor Mendel. A highly important personal relation was soon established between Samuel H. Clapp and myself. He was working in organic chemical research under Dr. Wheeler but soon became associated with Dr. Thomas B. Osborne, the eminent student of proteins of vegetable origin, at the Connecticut Agricultural Experiment Station in New Haven. Sam was employed by Osborne to apply the newly described "ester method" of E. Fischer, to the determination of the amounts of different amino acids yielded by different proteins on acid hydrolysis. He was a young man of unusual ability and at at once undertook to learn all he could about the chemistry of amino acids. We frequently dined together, and he often spent an evening with me. On these occasions he gave me a course of instruction on what he had learned about these substances. To my great interest he introduced me to the investigations of Miescher, Ritthausen, Kossel, E. Fischer, Abderhalden, and Osborne.

Upon entering post-graduate study in organic chemistry at Yale I was given a key to the private library of Professor Wheeler and at once began a program of examining his scientific journals. I would take down in succession the volumes of a series of journals and turn every page, leisurely, until I came upon a title which interested me. Then I would read carefully the introduction, in which the writer gave some account of previous investigations bearing on his problem, and stated his objective and plan of experiment. I would then examine his experimental data and study the conclusions which he drew from them. Before proceeding further in the volume I would reflect on what I could do in order to shed further light on this problem. Of course these efforts were not very productive of new ideas, but I enjoyed acquiring detailed information about the properties of many substances and their chemical behavior. I formed reading habits which familiarized me with the manner in which mature men applied themselves to scientific investigations. In this way I saw all of the pages of Liebig's *Annalen*, the *Berichte*, and other chemical journals, and went afield to examine the volumes of Pflüger's *Archives*, the *Zeitschrift für physiologische Chemie*, *Biochemische Zeitschrift*, and *Comptes rendus*. This practice of examining old journals was for me an excellent one since it gave me a clearer perspective of the historical development of organic and biochemical investigations than I could have secured by any other course open to me at that time.

Early in 1906 Clapp gave notice to Dr. Osborne of his intention to give up his position on August 1st and go to Germany for study. Dr. Osborne discussed with Dr. Johnson the matter of finding a man to take Clapp's place. Since I had completed the work which was to be submitted for my dissertation for the Ph.D. degree and could soon finish writing it, it was arranged that I should begin work in Dr. Osborne's laboratory on April 1st and help Clapp, before he left, with the chemical work on hand from previous ester distillations and go through with him the preparation of the esters and a distillation operation. Accordingly I had the good fortune to spend four months with an expert in amino acid chemistry.

Throughout the spring and summer I was hopeful of finding a position in a university where I could teach organic chemistry and try my hand at independent research. No suitable opening came to our notice during that period, so on October 1st I entered the laboratory of Professor Mendel for a year of study of physiological chemistry. I had received the Ph.D. degree in June.

Throughout the academic year 1906–1907 I heard the lectures of Mendel and F. P. Underhill and spent the days in the laboratory gaining familiarity with the analytical methods applicable to biochemical work. I also attended the courses given by Dr. Chittenden on toxicology and on nutrition. The last was a subject in which he was deeply interested. He was a firm believer in the merits of abstemiousness in protein consumption, for which regimen he gave us what seemed to be convincing reasons. It is interesting to note that during this year, so far as I can recollect, there was no mention of beri-beri, scurvy, rickets, or pellagra, by three distinguished teachers of physiological chemistry. Mendel called our attention to the recent experiments of Willcock & Hopkins illustrating the supplementary value of tryptophan for zein, and differences in the amino acid content of proteins, so far as this was known, were discussed from the nutritional interest which they aroused. His instruction was based to a great extent on the researches of Voit, Pettenkofer, Rubner, Atwater, Lusk, and Pavlov, but everything known about metabolic processes was set before his students. At that time no one anywhere surpassed Professor Mendel in thoroughness of instruction in biochemistry. This is supported by the long list of his students who became professors in this field in universities and came to distinction as teachers and investigators.

In the spring of 1907 Dr. Mendel showed me a letter from Professor E. B. Hart, who, the year before, had succeeded Dr. S. M. Babcock as head of agricultural chemistry at the College of Agriculture of the University of Wisconsin. He inquired for a young man trained in biochemistry who was interested in the study of animal nutrition. Mendel thought the opportunity a good one for me, and since I still had no prospect of securing the kind of position I wanted in organic chemistry, I accepted, after corresponding further with Hart. I entered on my duties in midsummer 1907.

Professor Hart explained to me the plan of his famous experiment with cows restricted to rations derived from single plant sources: the wheat, corn (maize), and oat plants respectively. The experiment had been suggested by Dr. Babcock and was a distinct departure from animal feeding studies of the past. The rations included all parts of the plant except the root, and the parts of the plant were included in such proportions that the entire ration for each group of animals had the same composition as shown by the "Official" method of chemical analysis. A control group received food derived from all three plants, for the purpose of determining whether variety in source of nutrients was of physiological importance (1).

Heifers of 350 to 400 pounds weight had been placed on these rations about a year previously, and the animals in the three groups had differentiated remarkably by the time I first saw them. All were able to grow and maintain sufficient vitality to conceive; but the wheat-fed cows deteriorated in appearance, were small of girth, rough-haired, listless, and delivered their calves some weeks before term. Their calves were undersized and were dead when born. Early in the experiment the wheat-fed cows had become blind. The oat-fed cows were in much better condition, and although they carried their young to full term, the calves were dead or moribund, but one surviving. In marked contrast to these groups the corn-fed animals were in excellent condition. They produced vigorous calves.

My assignment was to find, if possible, the cause of the differences in the quality of the three rations. The chemical criteria accepted generally at that time indicated that they were of essentially equal value as sources of nutrients for cattle. I set to work with the enthusiasm and inexperience of youth to solve the problem.

Having cast my lot in research in animal nutrition without any knowledge of the experiences of investigators in that field, and without ever having analyzed a food by the method of the Association of Official Agricultural Chemists, my fitness for a critical appraisal of current practices and beliefs or for planning an experiment in which animals were used, might well have been assessed by an experienced man at near zero. This I realized and began at once to make good my deficiencies.

One very important source of instruction to me was my daily contact with Dr. Babcock. He was interested in everything in science, and among other things he talked about, in his visits to the laboratory where I was working, were the history of nutritional investigations, and the inadequacies of the methods used for feed and food-analysis. He was a man of wisdom and my association with him almost daily was a great privilege. He did not believe that the method of analysis which had been widely accepted by experimenters in animal nutrition for over forty years yielded any information of importance concerning the nutritive values of feeds. This method had been devised by Wm. Henneberg, Director of the Agricultural Experiment Station at Weende, near Göttingen. He combined elements in the technics used by various chemists during the preceding four decades, and his method became known as the Weende method. Eventually it was modified in various details, but these were of little practical significance. It was adopted by the American Association of Official Agricultural Chemists when they first organized in 1884. It yielded information about the content of moisture, ether-soluble matter (reported as fat), "crude protein," derived by multiplying the nitrogen content by 6.25, "crude fiber" (cellulose, lignin), "nitrogen-free extract," and ash of the sample analyzed. It was assumed that the substances expressed in these terms had the same nutritive value irrespective of the plant sources from which they were derived.

After about 1860 agricultural experiment stations multiplied rapidly in several countries, and notably in the United States. Chemists analyzed enormous numbers of samples of farm crops grown under different climatic and soil conditions. These were compiled and published in the various editions of E. von Wolff's book, *The Rational Feeding of Farm Animals.* "Wolff's standards" for calculating rations for livestock were based on these analyses. His book was the basis of teaching feeding practices during four decades, wherever animal husbandry was taught. But these "standards" were often found unreliable by feeding tests, and it became increasingly evident that the methods of chemical analysis of feeds were inadequate for supplying the desired information about them. Agricultural chemists became painfully aware of their shortcomings. At the meeting of the Association of Official Agricultural Chemists in Washington in 1890 (2) the report of a committee of the foremost agricultural chemists, under the chairmanship of Dr. Harvey W. Wiley, was discussed at length. It had to do with proposed improvements in the "Official" method of feed analysis so as to provide more worthwhile data. All the suggestions offered were favorable to the determination of specific chemical constituents of the sample, especially the "nitrogen-free extract." An opinion which was supported by some was that the discrepancies between expected and realized results of feeding studies based on chemical data could be accounted for on the assumption that nutrients which were enclosed in, or imbedded in cellulose or lignin were inaccessible to digestive juices. Nothing of any importance came of these discussions, and the method then current continued to be employed for another two decades.

I read Henry's *Feeds and Feeding*, then the most popular textbook on the subject in America. From it I learned of the views of Liebig, Boussingault, Henneberg, Kellner, Rubner, Zuntz, and others who had investigated nutrition problems and feed values. I became familiar with the kinds of experiments in that field which had been employed during the preceding half-century. From these sources I learned nothing of value to me in my efforts to help Professor Hart solve the problem presented by the cow experiment. I soon came to share with Dr. Babcock his appreciation of the humor of his advice to Atwater, a story he often repeated with hearty laughter. W. A. Atwater was at that period the outstanding researcher and authority in America on human nutrition and human foods. He seems to have had no doubt that the standard food analysis, supplemented with data on calorie values and digestibility, sufficed for his purpose of determining food requirements, and for recommending economical food purchasing. Dr. Babcock told of recommending to Atwater that instead of feeding pigs on farm crops it would be cheaper to feed them soft coal. Bituminous coal, he said, when judged by the "official" method of analysis, was in itself a well-balanced ration. Dr. Atwater was annoyed by this treatment of a serious subject with levity.

The source of information which was of greatest value to me at that time was Maly's *Jahresbericht ueber die Fortschritte der Thier-Chemie*. The first volume appeared in 1872 and covered the entire literature relating to animal chemistry, and much of plant chemistry. It abstracted almost everything of importance in this field, including proteins, carbohydrates, fats and other lipids, blood, urine, digestion, pathological chemistry etc. There was a chapter under the heading *Gesamt-Stoffwechsel*, which contained abstracts of papers dealing with feeding experiments on men and animals, and their interpretation. Most of these papers described experiments designed to throw some light on nutritional needs of the body and the chemistry of nutrition. Although we had a file of this journal in the library, I bought a set of the thirty-seven volumes then published and spent many evenings at home studying their contents. It was a most profitable use of my time since these volumes made available to me the history of constructive thought and experiment in animal and plant biochemistry of the period which they covered.

It was there that I saw abstracts of the experiments of Forster (1873), Lunin (1881), Socin (1891), Hall (1896), Marcuse (1896), Steinitz (1898), Zadik (1899), Gottstein (1901), Röhmann (1902), Ehrström (1903), Falta & Nöggerath (1905), Jakob (1906), Tunnicliffe (1906), and Willcock & Hopkins (1906). These investigators restricted animals to mixtures of isolated proteins, carbohydrates, fats, and mineral salt mixtures for the purpose of comparing the nutritive values of proteins from different sources; the value of phosphorus-containing as compared with phosphorus-free proteins; the significance of feeding nucleoproteins; the effects of supplementing certain proteins with individual amino acids; or the effects of the inorganic salt content of the food on the health of the animals (3).

As I made notes on these studies I was astonished to find that every effort which had been made to feed animals on such mixtures had resulted in prompt failure of their health. It came to my mind that the most important discovery to be made in nutrition would be the elucidation of the cause or causes of these failures.

Reflection on this type of experiment as compared with the cow project with the single-plant rations—seed, leaf, and stem, which were extremely complex chemically, led me to conclude that we were not likely to succeed in accomplishing more than giving an account of what we did in this unusual study, and describing the physiological effects of the rations on the animals, but without discovering the causes, which obviously lay in chemical differences in the feeds.

It was such considerations that led me to conclude that the only promising course lay in the use of the simplest possible diets in the chemical sense, and of employing small animals, as some of the few men here recorded had done, and to make an effort to solve the problem of what, in chemical terms, constitutes the minimum quota of chemical substances on which an animal can function normally. The necessary chemical work in the preparation of the foodstuffs required precluded the use of large animals. Small animals have short periods of growth and mature early. Their periods of reproduction and suckling of the young, and their life span are such that the life history can be observed in two to three years.

It was this plan which involved the use of rats, that I presented to Dr. Babcock on a Sunday morning in November, 1907. He was highly enthusiastic about the possible achievements which might come from nutritional research by following a plan in which we would proceed from the simple to the complex rather than attempt to find why complex natural feeds in certain combinations and from certain sources failed to sustain health. With his approval and support I was able to start experimenting with rats. Mine was the first rat colony in America maintained for nutrition studies. At first I tried to use wild rats, but they were so frightened under caged conditions and were so ferocious that I soon abandoned them for albinos which I bought from a pet-stock dealer in Chicago.

From the outset I sought to find whether the failures of earlier investigators who used diets of isolated food substances might have been caused by some deficiency of an organic phosphorus compound. Professor Hart was devoting much study to this aspect of nutrition. While at the Geneva Experiment Station, in cooperation with Director W. H. Jordan and A. J. Patten, he had investigated the nutritional significance of the newly discovered organic phosphorus compound, phytic acid, and its salts "phytin," for cows. They had already published their results, which seemed to show that this substance exerted specific beneficial effects on the physiology of the cow (4). In 1909 I published the results of a considerable number of experiments bearing on this subject, in a paper *Nuclein Synthesis in the Animal Body* (5). In it I brought forward evidence for the belief that, in planning experimental diets, all known organic phosphorus compounds such as lecithin, cephalin, nucleic acid, and phosphoprotein, which are prominent constituents of animal tissues, could be omitted, since they were all capable of synthesis in the animal body. In this paper I reviewed the work of the investigators listed above who had studied nutrition with simplified diets composed of more or less purified food substances (3).

At first I did all the work necessary for preparing foods, making rations and caring for the rats, but in the summer of 1909 Miss Marguerite Davis, who had just graduated at the University of California at Berkeley, became my student. She had not been long at work in that status when I told her what I was attempting to do with the rats, and she volunteered to take care of the colony for me. She remained with me on a voluntary basis without pay except during the sixth and last year of our work together. I continued to prepare the food materials and to plan the experiments and assist in weighing the animals in order to observe them carefully, while she otherwise had all the care of the colony. I owe her a debt of gratitude for her enthusiasm and loyalty to the undertaking. Without her co-operation it would have been impossible for me to have carried out so extensive an experimental pro as we did working together.

During my early years at the College of Agriculture I wrote letters to Dr. Mendel and kept him informed on what I was trying to do, since he seemed enthusiastic about my experimental work. He commended me for undertaking studies with purified diets and seemed greatly pleased when he read my paper in 1909 (5) in which I gave my reasons for believing that all organic phosphorus compounds could be synthesized in the body. In 1909 he and Dr. Osborne started their rat colony for the study of differences in nutritive values of proteins from different sources.

From the outset of my experiments with "purified" diets I met with little success, my animals failing to grow, and showing signs of malnutrition. In seeking to overcome the failure of the rats to eat these insipid mixtures I was influenced by the work of Pavlov on the psychic reaction of animals to food and the response of the digestive glands to the chemical composition of the food ingested. I sought to overcome the difficulty of anorexia by giving as great a variety of isolated and recombined nutrients as possible, changing the source of food from time to time and providing flavor by such means as adding daily to the diet freshly rendered bacon fat, the distillate from water in which cheese was immersed, employing carbohydrates from different sources, etc., but without much success. Curiously, I did not discern the possible significance of feces-eating by my rats, and this disturbing element, together with unsuspected impurities in some of my materials, especially in milk sugar, enabled the animals to grow sufficiently to keep me enthusiastic about eventually achieving success. By reason of a combination of defects in my technic my rats were able to distinguish clearly between the value to them of butter fat and egg-yolk fat, in contrast to olive oil and lard. They fared markedly better nutritionally on the two former than on the two latter adjuvants to the diets. This study Miss Davis and I published in

1913 (6). It afforded the first evidence that certain fats contain an indispensable nutrient hitherto unsuspected. Some months later Osborne & Mendel described experiments which confirmed our discovery that certain fats contain an unidentified nutrient essential for the nutrition of the rat. Of special interest was our transfer of this nutrient from butter fat to olive oil. Butter fat was saponified in alcoholic KOH and the resulting soap was dissolved in water and olive oil was thoroughly emulsified in the soap solution. The olive oil was of the same sample as had been tested on rats and found of no value to them. The emulsion was then broken with ether, and the olive oil was recovered in that solvent. After removing the ether the olive oil was found by feeding tests to have acquired the nutritive quality of the butter fat.

In a short time we were able to demonstrate that this nutrient, now known as vitamin A, was present in kidney fat and in fats from other glandular organs and also in the ether extract of the leaves of plants, but was absent, generally, from the fats of adipose tissues.

Following up the idea of observing the effects of diets of the simplest possible composition, I restricted young rats to single kinds of seeds: maize, wheat, oats, barley, rye, peas, beans, millet, etc. To my surprise I found that young rats restricted to any one of these were able to grow but little or not at all. Even combinations of two, three, or more seeds in this list, as the sole diet, did not support growth. This type of ration was, of course, much simpler, chemically, than the rations derived from all parts of the plant, which had been fed the cows; it was also more satisfactory for critical study.

I had devoted considerable attention to the published analyses of the ash constituents of various food substances and was impressed by the fact that the seeds of plants were all low in their calcium content. They differed considerably depending on the type of soil on which the plants had been grown. The great activity in the study of the chemistry of proteins revealed their pronounced differences in yields of amino acids on hydrolysis, and this suggested that the deficiencies of seeds might lie, solely or partly, in the peculiarity and inferiority of their proteins. The absence of the fat-soluble factor from vegetable oils and fats obtained from parts other than leaves, afforded another clue to the planning of experiments with rats to reveal the nature and number of nutrients in which seeds were deficient.

Our first experiments of this type were conducted with wheat (7). The results were as follows: (*a*) Wheat alone: no growth, short life; (*b*) wheat +purified protein (casein): no growth, short life; (*c*) Wheat+salt mixture to give it a mineral content similar to that of milk: very little growth; (*d*) Wheat+a "growth-promoting" fat (butter fat): no growth; (*e*) Wheat +protein+salt mixture: good growth for a time, few or no young, short life; (*f*) Wheat+protein+butter fat: no growth, short life; (*g*) Wheat+salt mixture+butter fat: fair growth for a time, few young, short life; (*h*) Wheat+protein+the salt mixture+the "growth-promoting" fat: good growth, normal number of young, low mortality among the young, and long life span. Other experiments showed that by far the most important constituent in the salt mixture for making good the inorganic deficiencies of wheat was calcium.

On testing the maize kernel and the seeds of barley, rye, and millet, we found that as food for young rats, each had the same deficiencies as wheat, each requiring the same supplements of protein, salt mixture and "growth-promoting" fat. It was now clear that all of our common cereal grains are deficient in the same nutrients and approximately to the same extent. Oats are an exception since with the three additions named the response was less satisfactory than with the other three grains. Years later this was shown by others to be due to the low riboflavin content of oats.

These observations accounted for our failure to secure appreciably better results with young rats fed combinations of two or more cereals. Our experiments demonstrated that, nutritionally, all seeds have the same shortcomings. Hence when used in combination they do not make good each other's deficiencies.

Whole rice proved to be much like wheat in its dietary properties, but polished rice was not made complete nutritionally by the three types of nutrients which made wheat, maize etc. complete. But when a fourth adjuvant in the form of three per cent of wheat germ, or the water or alcohol extract equivalent to three to five per cent of wheat germ, was added, polished
with either water or alcohol also provided the necessary nutrients to render rice plus the three supplements complete (8).

By the time we had reached the study of rice I was familiar with the contents of Funk's book *Die Vitamine* (9). Among other information new to me it contained an account of the effects of water, or alcohol, extracts of rice polishings on polyneuritic birds, and the value of rice polishings as a supplement to polished rice in nutrition.

One of our most interesting findings of those years was the high nutritional value of mixtures of seed with leaf as against the slight improvement of the dietary value of mixtures of seeds of plants (10). Our studies made it clear that irrespective of what chemical analysis might show, the seed is inferior to the leaf as a source of nutrients. This was in harmony with the observation which is as old as history, that animals flourish when confined to good pasture grasses and to good hay.

My observations of the effects of such diets as those just described afforded the basis for reflection on the quality of human dietaries in different parts of the world—the coldest, the wettest, and the dryest regions. The new knowledge of the dietary properties of seed, leaf, milk (which we found to be an excellent supplement to seeds), and some observations of the dietary deficiencies of muscle meat, together with the new information about polished rice and the superiority of the germ as a source of nutrients, led me to make some important generalizations on human dietaries. I criticized the typical

American's diet of that period as being of poor quality because it was derived too largely from white flour or cornmeal, muscle meats, potatoes, and sugar. Sugar, I asserted, when eaten to the extent of an average of more than 100 pounds per capita per annum, crowded out from the diet significant amounts of better constituted foods. The foods listed, I declared, were not constituted to supplement each other by making good their deficiencies. I recommended a diet containing more milk and leafy vegetables, and extolled the glandular organs of animals as superior to the muscle meats as sources of nutrients. Milk and leafy vegetables I distinguished as "protective foods" because they were so constituted as to make good the deficiencies of whatever else we were likely to eat. The planning of menus to include sufficient of these "protective" foods was recommended in my Harvey Lecture of 1917 (11), and in my Cutter Lectures at Harvard University in 1918, which were published as the first edition of *The Newer Knowledge of Nutrition* (12).

Recent practices in menu-planning stem from the principle of making the daily menus dietetically complete by the use of foods and food combinations which supplement each other. This viewpoint superseded that of Atwater which was based on the economic principle of the purchasing of those foods which would provide at lowest cost the necessary amounts of protein and available calories to meet the individual's needs.

Even in 1911, after four years of experience with feeding "purified" diets, I was still deluding myself with the idea that such success as I had achieved was the result of inducing my rats to eat enough of unpalatable mixtures to enable them to grow to some extent, and that this was the only impediment to further success in this type of study. I was awakened to my error in 1911 when Osborne & Mendel published the results of the first two years of study of nutritional differences in the values of proteins from different sources (13). Dr. Osborne had accumulated a superb collection of many highly purified proteins from vegetable sources, and in 1909 he and Mendel had undertaken to evaluate these by feeding studies in which they employed diets containing but a single protein. Their early efforts were based on diets derived from a purified protein, a source of carbohydrate, fat, and a salt mixture. They had the same experience as their predecessors in securing but minimal amounts of growth in their young rats. Metabolism studies on individual rats showed that positive nitrogen balances could be achieved over a period of three weeks, but their animals failed nutritionally before many weeks, and rapid and sustained growth was never observed. It became evident to Osborne & Mendel that some other type of basal diet must be employed for the realization of their objective.

They then turned to the use of a basal diet consisting of 28 per cent of a deproteinated whey made by coagulating the lactalbumin from acidified whey by heat, and evaporating the filtrate to dryness. This material they termed "protein-free milk." It was a mixture of the salts of milk, lactose, and of the numerous non-protein constituents of milk. When they employed this material together with starch and lard, and certain individual proteins they were successful in inducing growth and maintaining health, and in some cases reproduction, in rats. Certain proteins, fed in this manner, were inadequate, but were made adequate by a supplement of one or more amino acids. The extraordinary differences in nutritive values of proteins from different sources were first dramatized by them.

At the time it seemed to me that "protein-free milk" was more than a source of dietary adjuvants other than protein. I pointed out that when they fed 18 per cent of a purified protein with 28 per cent of "protein-free milk," the latter supplied 9 per cent of the total nitrogen of the diet in uncharacterized substances, some of which were presumably amino acids, peptides etc. which could supplement amino acid deficiencies in purified proteins. Hence what they were accomplishing was a comparison of a purified protein plus the amino acid supplement in the "protein-free milk," with another purified protein with the same supplement. This, of course, did not detract from their demonstration of the fact that proteins differ enormously in their adequacy as sources of amino acids, a fact which was in harmony with much chemical data, especially those which Dr. Osborne had published.

Their success in improving diets by the inclusion of the non-protein constituents of milk, led me to re-examine my milk sugar as a possible source of nutrients other than lactose. It at once emerged that lactose purified by re-crystallization was less valuable to rats restricted to my experimental diets than was the crude sugar, and that addition of the mother liquor from crystallization of milk sugar had an easily observable beneficial effect on the animals.

In 1913 Osborne & Mendel reported their experiences with an "artificial protein-free milk," prepared from milk sugar and the salts of milk. With this they achieved considerable success for a few weeks but the rats failed in health within about one hundred days, whereas with the natural "protein-free milk," they remained in good health far beyond this age.

Osborne & Mendel (13) rendered a service to animal experimenters in the field of nutrition by calling attention to the beneficial effects to rats fed "purified" diets, of feces-eating, a practice to which this species is addicted. They observed that a small allowance of feces, more especially from animals normally fed, was of considerable value in improving their well-being. They were led to try feeding feces by the recently reported studies of Herter & Kendall (14) which afforded strong evidence that certain types of bacterial flora in the intestines are physiologically beneficial, whereas others are harmful.

The investigations here described represent the principal ones which I carried out in the decade under consideration, which embodied new and novel features. They were well received by biochemists and physiologists. The comment on my work in 1917 by Professor Graham Lusk gave me great pleasure. He said to me: "You have entered a well-worked field and have brought forth new and astonishing facts." My investigations increased my visibility as a researcher sufficiently to prompt Dr. Wm. H. Welch and Dr. Wm. H. Howell to invite me to take charge of the department of chemistry in the newly established School of Hygiene and Public Health which the Rockefeller Foundation had made possible as a part of the Johns Hopkins University. I have never ceased to marvel that these two distinguished medical men should have risked appointing me to a professorship when I had no medical training and was a chemist working in an agricultural experiment station.

Since this is intended to be an historical account of my first decade in research, it seems desirable that I should mention the more important investigations previously and currently, which, in addition to those mentioned, contributed to an understanding of the problem of what constitutes an adequate diet.

Lunin (3) was inspired by his teacher, v. Bunge, to study the physiological importance of inorganic elements in nutrition. His specific problem was to find whether it was important to take into account the acid-base balance in foods. To this end he attempted to keep mice on a diet composed of what he believed to be the essential ingredients in milk, viz., casein, milk sugar, fats, and the ash of milk. His mice died within a few weeks on this mixture, whereas when given milk to drink they remained in health for at least sixty days. He wrote:

> Mice can live well under these conditions when receiving suitable foods (e.g. milk), but as the experiments show that they cannot subsist on proteins, fats and carbohydrates, salts and water, it follows that other substances indispensable for nutrition must be present in milk besides casein, fat, lactose and salts.

He contributed nothing further to this subject.

Pekelharing, in 1905, restricted mice to a diet of bread made of casein, albumen, rice flour, lard, and a mixture of all the salts which he thought should be present in their food. When they were given this ration with water they grew thin and died within four weeks. When they were given milk in addition to the bread they remained in health. He further showed that a whey allowance with the experimental diet would keep the mice healthy. He wrote:

> My purpose is to point out that there is a still unknown substance in milk, which, even in very small quantities, is of paramount importance to nourishment. If this substance is absent, the organism loses the power properly to assimilate the well-known principal parts of the food, the appetite fails, and with apparent abundance the animals die of want.

I did not learn of the study of Pekelharing until it was brought to my attention about 1923 by his countryman Dr. van Leersum. It was not recorded in Maly's Jahresbericht.

In 1906 F. G. Hopkins wrote (15):

But further, no animal can live upon a mixture of proteins, carbohydrates and fats, and even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either on plant tissues or on other animals, and these contain countless substances other than proteins, carbohydrates and fats. Physiological evolution, I believe, has made some of these well nigh as essential as are the basal constituents of the diet . . . ."

The studies of Grijns (19) corrected the initial error of Eijkman (18) in interpreting his famous experiment on the production of polyneuritis in fowls by restricting them to a diet of polished rice. Eijkman proposed to explain the observed phenomena on the presence in the endosperm of rice of a nerve poison for which there was in the outer layers of whole rice a substance which neutralized this in the pharmacological sense. Grijns (19) was the first to interpret correctly the connection between excessive consumption of polished rice and the etiology of beri-beri.

In 1902 Holst & Froelich (16) made the momentous discovery that any diet which was thoroughly dry or thoroughly heated would induce scurvy in guinea-pigs, whereas fresh, unheated vegetable foods would prevent or cure it. Hitherto, views about the cause and cure of scurvy had been based on human experience, and well-controlled studies were out of the question. But with the guinea-pig as a subject for experimental scurvy, progress was to be rapid in securing sound knowledge to replace the divergent views which had hitherto prevailed as to the etiology of the disease. At this period boiled milk and barley water formulas were commonly fed to artificially reared infants, and infantile scurvy was common. On the suggestion of Holst & Froelich's experiments A. F. Hess in 1914 (17) substituted potato water for barley water and promptly cured scurvy in an infant. Dr. Hess was very active in educational work which resulted in the practically universal provision of some fresh fruit or vegetable juice to bottle-fed infants. The incidence of infantile scurvy was markedly reduced.

The greatest impediment to progress in nutrition studies up to 1917, or even somewhat later, was the biochemists' lack of training in pathology and the pathologists' lack of training in chemistry. Knowledge of the meaning of symptoms exhibited by experimental animals in states of malnutrition due to confinement to diets from different sources, and deficient in different nutrients, would have shed much light on the interpretation of feeding studies which caused specific kinds of malnutrition. I keenly realized my deficiency in this respect and sought assistance from medically trained men and from professional pathologists and veterinarians. None of these men manifested much interest in the meaning of the photographs of experimental rats, cows, calves, pigs, and chickens which I carried about. To questions about the meaning of abnormal posture, skin and eye lesions, etc. I received no helpful replies. The pathologists of that time were informed in morbid anatomy, the natural history of disease, in the roles which bacteria, fungi and the protozoa played as agents in the causation of disease, and in immunology. None had sufficient training in chemistry to enable him to conceive of a diseased state arising from a deficiency of some essential chemical substance which the diet must supply. Disease at that time was generally regarded as due to some positive agent.

Practical feeders had long known that certain feeds were better than others as supplements to some farm crop, e.g. hay or silage. Feed-lot tests at many experiment stations had revealed various combinations of feeds which produced better results than other combinations apparently similar. The reasons remained unknown until the decade here discussed.

From the account here given of prior investigations it will be apparent that feeding studies on laboratory and farm animals afforded a number of lessons of importance which up to 1907 had not been studied with much profit. Lunin's experiments were twenty-eight years old and nothing had been done to advance knowledge beyond the facts which he recorded. The time was ripe for more systematic experimental inquiry, prosecuted to an extent which would confirm, clarify, extend, and unify the isolated observations of importance which were known to practical feeders, or were recorded in scientific journals. Some of these were of importance, but had not been followed up as they should have been by further study. I was fortunate to have opportunity and resources for extensive experimental studies in animal nutrition at a most opportune time.

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