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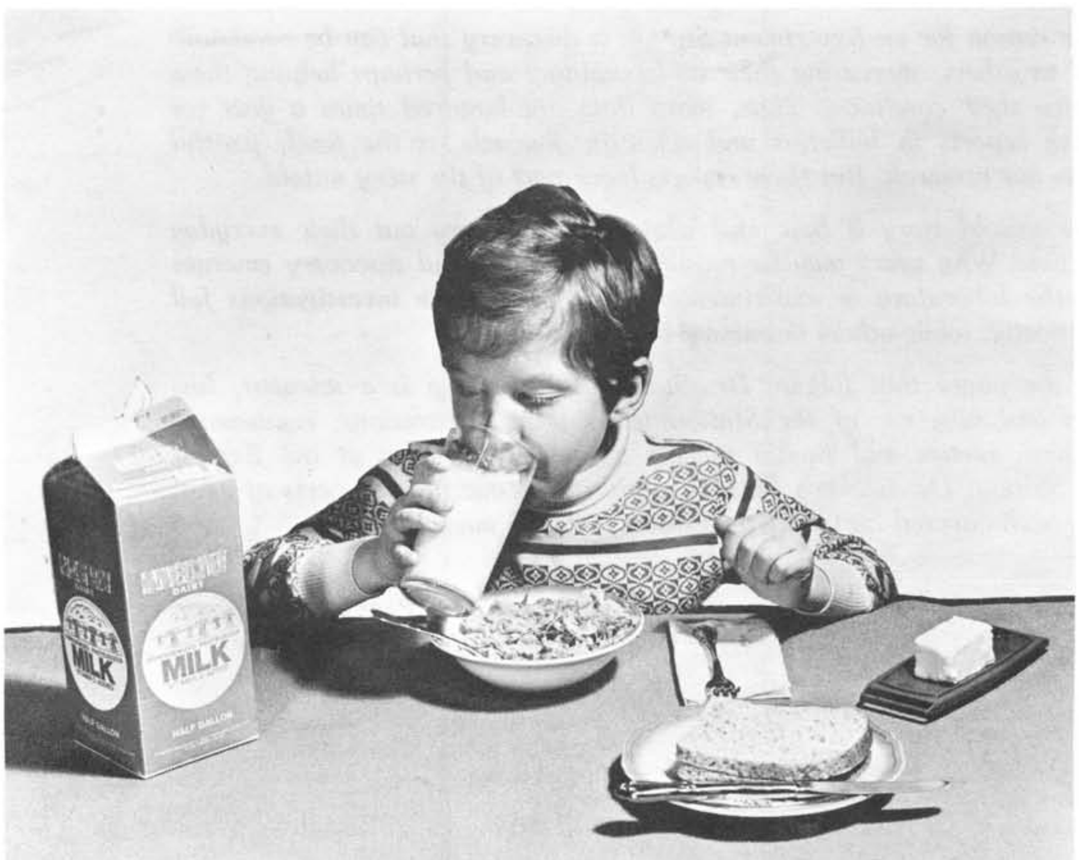
BUTTER MAKES THEM GROW

An Episode in the Discovery of Vitamins



By Stanley L. Becker

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION NEW HAVEN

FOREWORD

The reason for an Experiment Station is discovery that can be communicated to others, increasing their understanding and perhaps helping them improve their condition. Thus, more than one-hundred times a year we publish reports in bulletins and scientific journals as the final, fruitful step in our research. But these reports leave part of the story untold.

The untold story is how and why scientists carry out their everyday activities? Why years may be required before a useful discovery emerges from the laboratory or experimental farm? Why some investigations fail pathetically, while others illuminate our world?

In the pages that follow, Dr. Stanley Becker who is a scientist, historian and observer of the Station tells of the frustrations, excitement, drudgery, tactics and finally success of an investigation at the Experiment Station. Dr. Becker's bulletin supplements our usual reports of what we have discovered by telling how discoveries are made.

Paul E. Waggoner
Director

BUTTER MAKES THEM GROW

An Episode in the Discovery of Vitamins

By Stanley L. Becker

Today, millions of Americans spend vast sums of money on vitamins and special protein "health foods." The protein and vitamin content of foods are clearly labelled on most canned and packaged foods in supermarkets. Proteins and other "proper" foods are discussed in newspapers and magazines as well as on radio and television.

It was somewhat surprising to learn that the word vitamin did not exist until 1912 (and then it was spelled vitamine). It is also difficult to believe that the protein concept only became well-developed in the last quarter of the nineteenth century, while nutritional values and needs for amino acids (the chemical building-blocks of proteins) were just coming into recognition during the first decade of the twentieth century.

Historical records show that pioneering discoveries of nutritional requirements of proteins, amino acids and vitamins were made at two American Agricultural Experiment Stations: Connecticut (New Haven) and Wisconsin (Madison). It was at Wisconsin that the alphabetical naming of the vitamins, A, B, C, D, . . . was coined, originally as fat-soluble A and water-soluble B. In Connecticut, proteins and amino acids were brought into prominence as their different chemical compositions and nutritional functions came to be understood.

The Connecticut story (and only a small part at that) is the one to be told here. It is the story of the team of Thomas B. Osborne and Lafayette B. Mendel who set out to learn how proteins function in living animals. In the process they ultimately provided new information on the mineral requirements of animals and discovered a few vitamins along the way.

The belief that certain foods in very small quantities (trace nutrients) are needed by animals was a difficult one to hold or even conceive of in the early twentieth century.[1] How this uncertain belief was transformed into positive knowledge is the substance of this narrative.

Like many episodes in science, this is a mystery story complete with unusual clues and strange hints; of false trails and blind alleys; of errors in judgment; of unpredictable events. Yet, ultimately, in this case, a story of success.

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NUTRITION: 1906

In the early twentieth century it was reasonably clear that humans required proteins (for muscle and tissue growth), carbohydrates and fats (for energy); minerals such as calcium and phosphorus (for proper development of bones and teeth). Iron was required for red blood cells, while other minerals such as sodium, potassium, magnesium and iodine appeared necessary but their functions were only partially understood. A good guess was that they were needed to keep nerves, glands and other organs functioning normally.[2]

How, and in what forms these food substances got to where they are used in the body, was vaguely understood. How much of each is needed daily remained an open question. In simple terms, knowledge of how living animals utilize their food—their nutritional metabolism—was limited and fragmentary.

But the winds of curiosity and inquiry were blowing strong during the first decade of this century. In England, in Germany, in Russia, and in the United States, the number of researchers investigating nutritional metabolism was on the increase. And, to paraphrase F. Gowland Hopkins, the English physiologist, there was a lot more to an adequate diet than proteins, fats, carbohydrates, minerals and water.[3]

THE MEN

Thomas Burr Osborne was a true "Connecticut Yankee" whose family history extended into the pre-Revolutionary past.[4] He differed from his forbears by electing a career in science, specifically in chemistry. A Yale student in both undergraduate and doctoral studies, he came to work at The Connecticut Agricultural Experiment Station in May, 1886, at the request of Samuel W. Johnson, a pioneer agricultural reformer and at the time, station Director. That June, Osborne married Annah Elizabeth, Johnson's daughter.[5] In many ways this dual relationship with the Johnson family was to shape the form and character of Osborne's career.

In 1888, after spending two years analyzing dairy products, fertilizers and soils, Osborne was asked by Johnson to study the protein composition of plant seeds grown especially for agricultural purposes.[6] By 1909, more than 20 years later, Osborne had become, at age 50, an acknowledged world expert on vegetable proteins. Furthermore, he had commenced studies to determine the amino acid compositions of these proteins. It was a prodigious task, but by 1909 Osborne had nearly completed it and was faced with the problem of what to do next.

That problem was resolved by the formation of a partnership with Lafayette Benedict Mendel, a brilliant physiological chemist some 13 years younger than Osborne. Mendel's ancestry was "old country", his parents having emigrated to the United States from Germany and settled in Delhi, New York. Mendel's precociousness earned him entry to Yale at age 15. After he completed an undergraduate program in the humanities, he did graduate work in the relatively new field of physiological chemistry at Yale's Sheffield Scientific School, performing so well that he completed his doctorate in 1893 at age 21.[7]

Mendel stayed at Yale, attaining full professorship in 1903. His research in animal physiology, his special ability to get to the core of a problem, combined with a rare talent for working harmoniously with diverse personalities, made him the ideal scientific partner for Osborne, whose aloofness and domineering manner made life difficult for his co-workers on many occasions.

The "real" story of how such different personalities operated effectively for nearly 20 years will probably never be known. Suffice to say that they had done joint research earlier (1904/05)[8] and, in the relatively small scientific community of New Haven at that time, these gentlemen knew each other, so that when a mutual need arose, each was ready for collaboration.

Thus, Mendel seeking to understand more about protein nutrition and digestion, found Osborne with an abundance of proteins and amino acids on hand, looking for a way to measure their physiological properties. As Mendel's biographer states:

> The ways of two explorers who started at different points met in a common interest and thus Osborne and Mendel joined hands in the common objective of the study of the problems of nutrition based on the appraisal of the food values of pure chemical substances of various degrees of complexity.[9]

THE BEGINNING

In May, 1909, one of Mendel's graduate students, using himself and eleven others—all unidentified except by their initials—carried out an interesting nutritional study in which each participant subsisted on a different diet.[10] In keeping with contemporary procedures, each recorded his food intake and collected urine samples to determine the effects of diet upon the composition of body wastes. The information so obtained is revealing as a commentary on experimental methods of the time. The experiment covered only about three days. One person ate nothing at all for two days (this was called a starvation diet). Another had 16 egg yolks in three days (a high protein, high

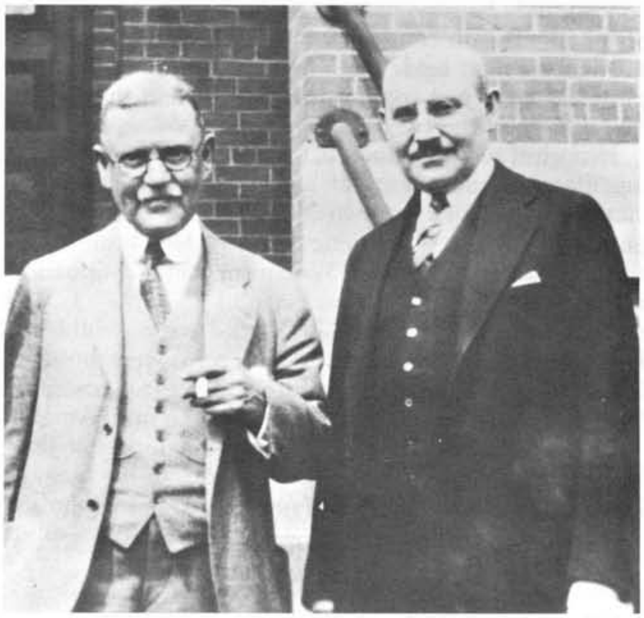


Fig. 1. Thomas B. Osborne (left) and Lafayette B. Mendel (right) on the steps of the Johnson Laboratory, May 1926.

fat diet). A third person ate nothing but pine nuts—one and one-third pounds of them. Others were on total carbohydrate diets—sugars and starches—and one poor fellow had what was called a high carbohydrate/high fat dietary consisting of bananas, candy and olive oil!

Needless to say, the results were inconclusive. Not much was learned other than to confirm what was already known: High protein diets generally produced an increase in urinary nitrogen compounds (*all* proteins contain nitrogen). Low protein or starvation diets resulted in a lower output of nitrogen compounds.[11]

This type of experiment had been part of the nutritional researchers' standard technique for many years. In truth, the *only* way to learn about food values was to feed animals different diets. The method had some obvious strong points but its greatest handicap was that it yielded little knowledge about *how* and *which* proteins were used in the living animal: What happened to proteins during and after digestion? Were all proteins alike in their nutritional values? Which amino acids had to be supplied in an animal's food? Which amino acids could be synthesized by these animals? Did all animals, especially mammals, require the same proteins and amino acids?

These were but a few of the questions being asked by nutrition researchers of the day. It was already known that proteins and amino acids were different in their chemical makeup and it was reasonably assumed that such differences meant that these substances had different nutritive properties. Thus, it all came back to the proteins themselves. The greatest stumbling block in research of this kind was that the experiments were carried out over periods of time inadequate to measure any long-term effects. It was here that Osborne and Mendel put their collective knowledge to work. Taking a few hints from the available literature and a specific idea from E.V. McCollum, a Yale graduate in organic chemistry who had worked with both scientists before going to Wisconsin, they planned extensive feeding experiments using the white (albino) rat as the experimental animal.[12] Mendel had previously used white mice in physiological studies and he understood the value of small rodents in this type of research. Albino rats, common as house pets in those days, were relatively docile and easily handled. They bred rapidly (about 20 days) and their small size meant that large quantities of expensive foods would not be necessary. In addition, any effects of nutritional imbalances would appear rapidly in a small animal—days or weeks—as compared to cows or pigs, which might require months or years of feeding and enormous quantities of food before significant imbalances could be detected.

Thus, on July 5, 1909, the first rats were started on experimental diets. In the years that followed the numbers would surpass 10,000 and, with Osborne and Mendel's persistent, cautious technique, these rats would be fed a variety of foods in known amounts for periods, in some cases, as long as two years. From the

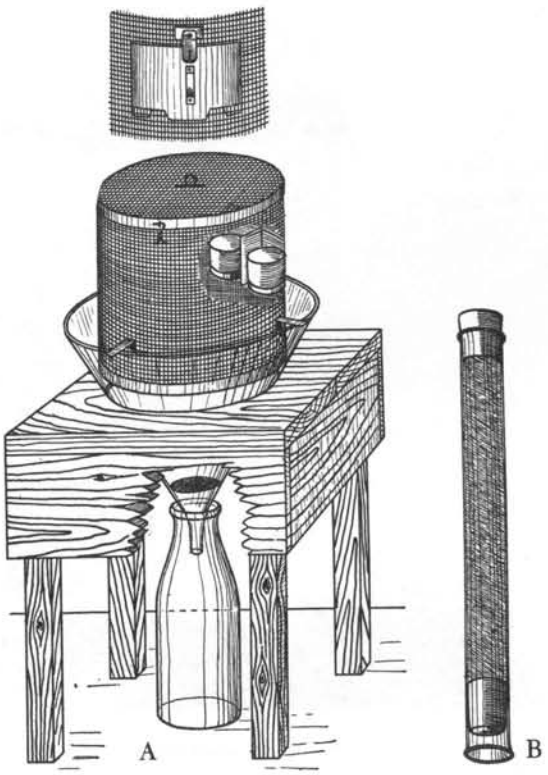


Fig. 2. Sketch of rat caging apparatus and food tube.

accumulated data would emerge a wealth of knowledge about rat nutrition and, at the same time, increased understanding of human dietary needs.

Before describing the methods used by Osborne and Mendel, it would be worthwhile to turn back the clock to that summer of 1909 and see what it was like to carry out experiments in animal nutrition in a time before scientific apparatus and techniques had become standardized and almost automatic; in a time before the elegant, sophisticated, modern-day laboratory had come into existence.

Consider the following list of equipment and supplies in the notebook of M.S. Fine, a student of Mendel's at Yale. It is a list that is revealing in what it tells us directly and in what it says implicitly.[13]

Bought:

2 lbs. "C" sugar @ 5¢ -	10	cents
3 lb. can Sperry & Barnes Leaf lard	55	cents
3 " " " " " " "	55	"
1 pr. pigskin gloves—	$1.00	
Express wagon—	$1.25	
Note book	$1.25	
Express on rats from N.Y.—	$0.60	
Express on rats from Miss Lathrop	$0.30	
Note book	$1.25	

Taken from Biological Lab.
1 12″ funnel
2 jars corn feed
1 jar dog crackers
Trip scale & wts.
cages & rats
dishes & insets
water bottles

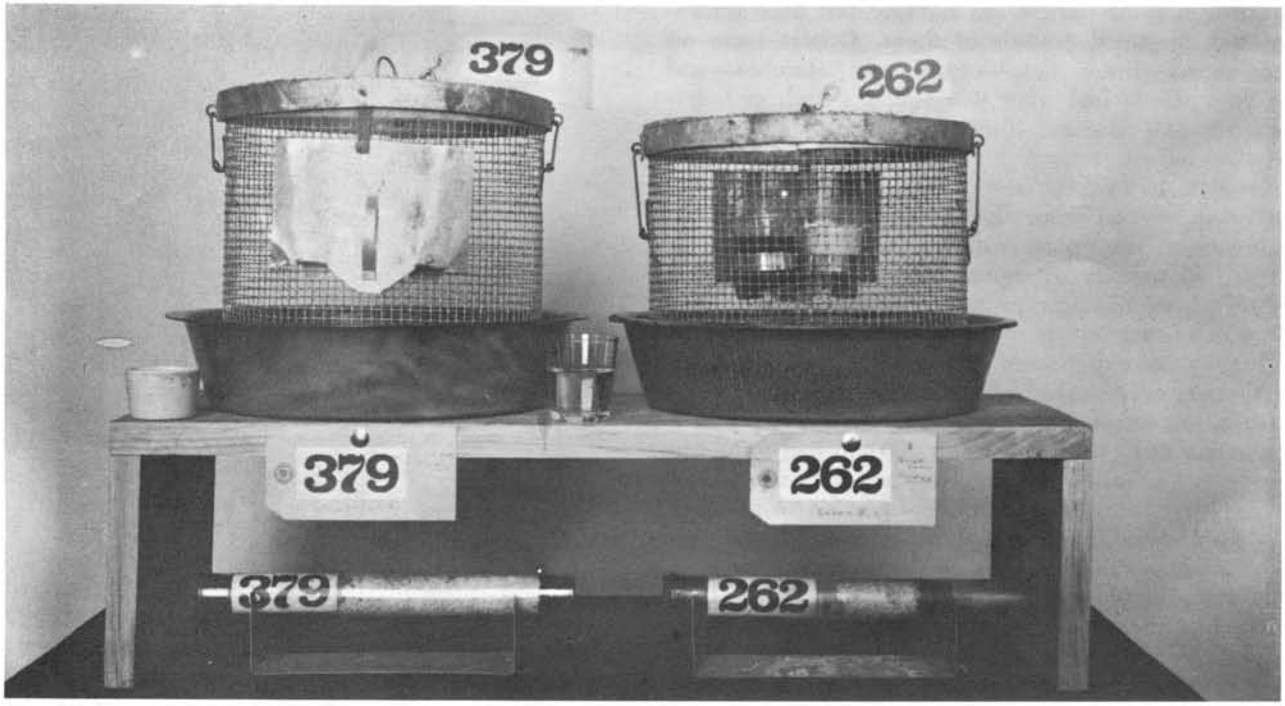


Fig. 3. Modified rat caging apparatus with food tubes in racks below the cages. 262 shows mounting of food cup and water cup. 379 illustrates the cup and whiskey glass.

Those were the days! Sugar at 5¢ a pound. Pigskin gloves, no doubt for use in handling white rats. But the most fascinating item is the Express wagon. Was it a "little red wagon"? What was it used for? To transport equipment around the laboratory? How does a modern scientist do without an Express wagon? (He has specially designed, multi-level carts with various attachments.)

And yet, that list, along with the data on the earliest rat experiments carried out by Osborne and Mendel, bear eloquent testimony to the humble beginnings of a revolution in nutritional science.

THE METHODS

> The best hope of success—if such be possible—rests at present on the method of trial and error in which each variable is gradually eliminated by successive comparative experiments.[14]

Osborne and Mendel wrote those words in 1911 to explain their methodology. They drew information from available literature on rat caging, feeding and hygiene. Using modifications to suit their specific plans they developed a simple apparatus illustrated in Fig. 2,[15] and described as follows:

The cage was constructed of wire mesh so as to allow maximum ventilation and to provide a convenient means of collecting urine and feces for chemical analyses. (After about two years, Osborne and Mendel gave up this latter aspect of the procedure as not worth the effort involved. From then on, they used the apparatus as pictured in Fig. 3.)

This cage was designed to hold one porcelain cup for food and a small glass for water. Ingenious as they were, Osborne and Mendel found a common, two-ounce whiskey glass to be the most useful water holder.[16, 17]

The glass tube, B, shown on the right in Fig. 2, corresponds to the tubes in racks below each of the cages, 262 and 379 in Fig. 3, and was used as a food storage container. It measured about 25 cm (10 in.) in length and 3 cm (1¹/₅ in.) in diameter. Food, in paste form, filled the tube.

> A rubber stopper inserted into one end can be moved forward like a piston head and the food expelled from the other end of the cylinder into the food receptacle. The exit end of the cylinder is kept stopped when the food is not being expelled and the entire apparatus with its food content can be preserved in an ice-box for long periods without deterioration of the diet.[18]

It certainly was convenient. All one had to do was weigh the tube and contents, push some of the food paste into the cup, then reweigh the tube. The difference in weight thus represented the rat's food supply for a given time.

A question naturally arises here: Why was a paste used at all? This type of food packaging seems to belong to the space age where it is eminently suitable in a weightless environment. The simple answer is that rats are messy eaters. They scatter dry food all about them. One could never be certain how much food a rat was consuming if portions of his (or her) meals fell through the wire mesh floor of the cage. Thus, the paste. Using a grinding mill, dry foods such as dog biscuits, purified proteins, starch, etc., would be pulverized into a fine powder, then mixed with melted lard. After cooling the mixture was passed through a meat grinder, forcing the paste through many small holes—almost like making spaghetti. The "strings" of paste were vigorously blended until the paste was uniform.[19] Virtually all the diets were prepared in this manner, then filled into the glass tubes described above.

For a time dog biscuits or dog crackers served as a standard or control food: something to compare the experimental diets against. It would have been fine except that Osborne and Mendel's rats did not do well on this fare. Eventually a powdered milk food or a mixed food diet was substituted as the control. More about these later.

The diets of experimental rats consisted of different proteins, minerals, starch, lactose (milk sugar) or sucrose (table sugar) and amino acids in a variety of combinations, all fed in paste form. Perhaps these were not gourmet meals but the rats appeared to enjoy them most of the time.

And, to guarantee a continuous supply of white rats, Osborne and Mendel set up a breeding program whereby they not only insured their own needs, but also became suppliers of experimental animals for an increasing number of nutrition research workers.[20]

A typical experiment would be done in the following way: After mating, the female would give birth in 20-21 days to a litter of from 4 to 15 young. At birth the rats normally weighed 4 to 5 grams (about $^1/_7$ to $^1/_6$ oz.) and would be nursed by the mother until weaning, at approximately 25 days, when their weights ranged from 25 to 40 grams (slightly less than an ounce to perhaps 1 and ⅓ oz.)

The rats were then placed on experimental or control diets in separate cages and a record kept of their size, general appearance and, most importantly, their

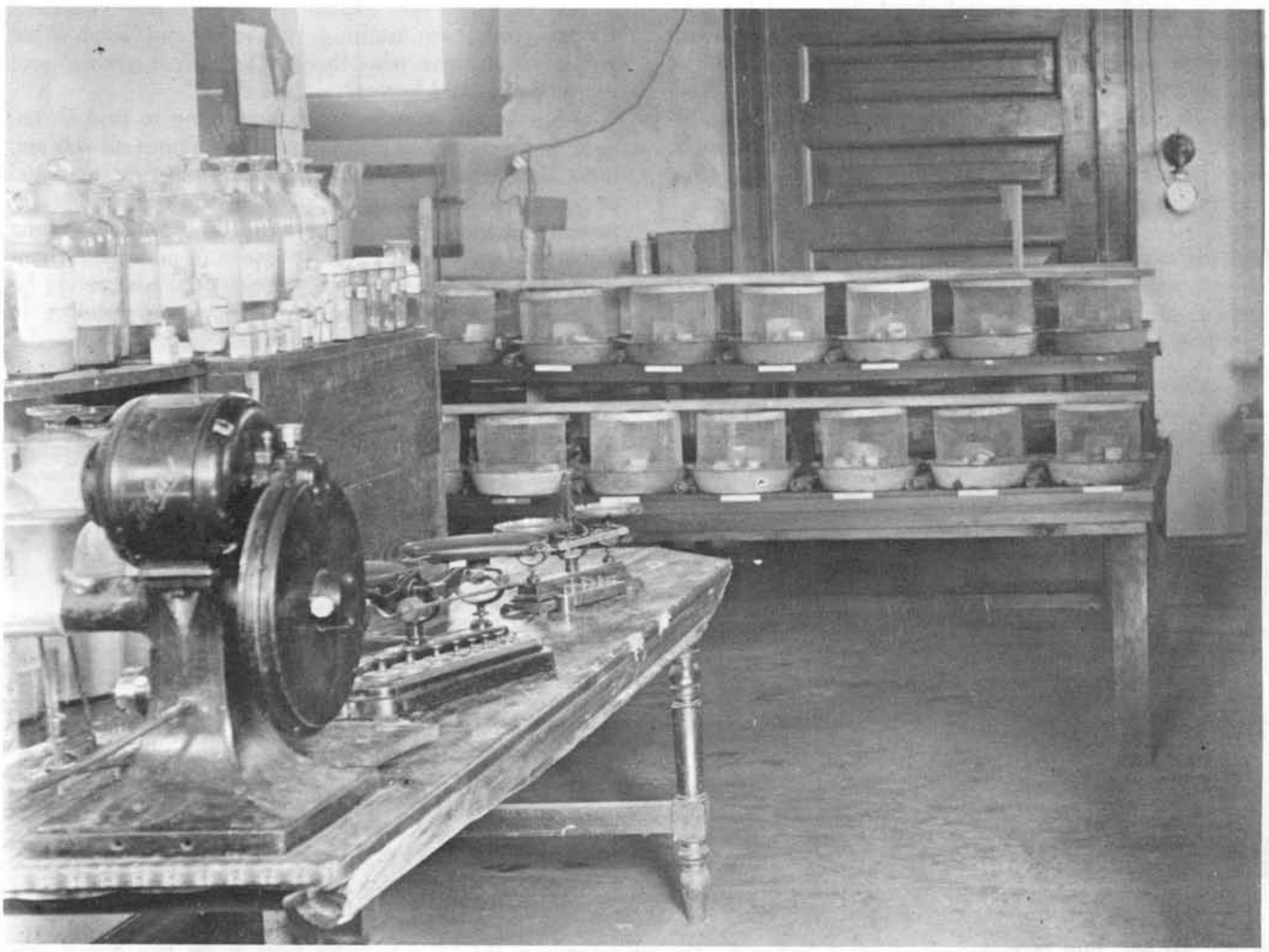


Fig. 4. Rat lab, 1919. Experimental cages. Note meat grinder for making food paste in left foreground.

weights. As we will see, the rate of increase of weight was to be the principal measure of growth and the determiner of any particular diet's adequacy.

Figures 4 and 5 are photographs of the rat laboratory as it appeared in 1919. The round cages seen in Fig. 4 were used for experiments and the long, rectangular cages in Fig. 5 were used for breeding.

While many diets were employed over the years, two are given here as examples:

Experimental Diet[21]	% Composition
Casein	18.0
Starch	29.5
Cane Sugar	15.0
Mineral Salts	2.5
Agar	5.0
Lard	30.0

Control Diet[22]	% Composition
Powdered Whole Milk	60.0
Starch	16.7
Lard	23.3

THE FIRST TWO YEARS: APPARENT SUCCESS

In 1911, nearly two years and about 300 rats later, Osborne and Mendel could look back upon their work with some satisfaction. Certain hypotheses and ideas had been verified; others had been discredited. New problems had arisen but they seemed amenable to solution if the trial-and-error principle was maintained, eliminating each variable by "successive comparative experiments".

Proteins and Amino Acids

As suspected, proteins *were* different in their nutritional values. Osborne and Mendel found that casein, a protein obtained from cow's milk, was almost a complete protein. When fed to mature rats as the only source of protein, casein appeared capable of supporting adequate nutrition for up to five months.[23] It was not, however, adequate to induce growth in young rats.[24]

Other proteins proved to be even less 'complete' than casein (Fig. 6). Growth of young rats or constant weight in mature rats (maintenance) were almost impossible to attain. Examples of such proteins were gliadin (from wheat), hordein (from barley) and zein (from corn).[25]

This was something that had been vaguely understood for centuries. Humans on inadequate diets often did not grow normally, yet they remained alive and reached maturity although never attaining the heights and weights of those who had been adequately nourished. Many reasons had been given to explain this phenomenon, and faulty diet was an obvious one. But why? Human cultural history (better known as common sense learned through generations of experience) had taught that a certain amount of meat, poultry or fish, milk, eggs, vegetables and fruits provided sufficient nourishment for growth in early years and maintenance of weight and health during adulthood.

Yet, how did one explain the health and vitality of Eskimos who ate nothing but meat and fish? What about vegetarians who thrived on cereal grains, root crops, fruits, and leaves?

Osborne and Mendel were beginning to find an answer to these vexing problems. If the albino rat was any indication, then essential proteins, meaning essential amino acids, had to be supplied in the diet and it apparently made no difference as to their source as long as certain amino acids were present in adequate quantities. *Which* amino acids were essential had yet to be determined but a number of clues were pointing in specific directions. Osborne and Mendel had opened a way to lead them in those directions.

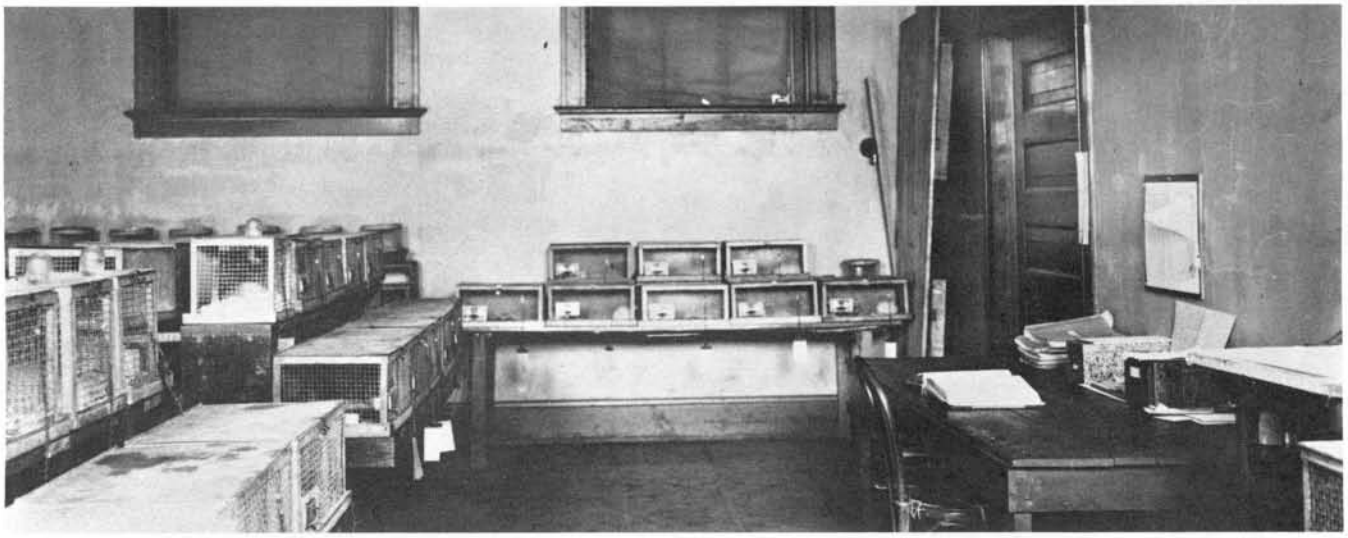


Fig. 5. Rat lab, 1919. Breeding cages against back wall. The other cages on the left with water tubes in the tops housed pregnant rats.

A few illustrations will demonstrate more vividly some of these points concerning growth. Figure 7 shows two growth curves. The dashed line is the result of many years' study of the white rat by Henry Donaldson at the Wistar Institute of Anatomy in Philadelphia. The solid line illustrates the growth of one of Osborne and Mendel's rats on a mixed food diet (dog biscuit; sunflower and other seeds; fresh vegetables, and salt). While not reaching the same absolute weights of Donaldson's rats, Osborne and Mendel's rats show the same general features of normal development: rapid growth in the first 100 days followed by a decreasing growth rate and then by a leveling out—reaching constant weight. Each dietary experiment was checked against this and one other growth curve, the one obtained on a powdered milk food diet, to determine the adequacy of the experimental diet.

Figure 8 illustrates the dramatic changes in growth of rats as the increasing knowledge of nutrition was put into practice.

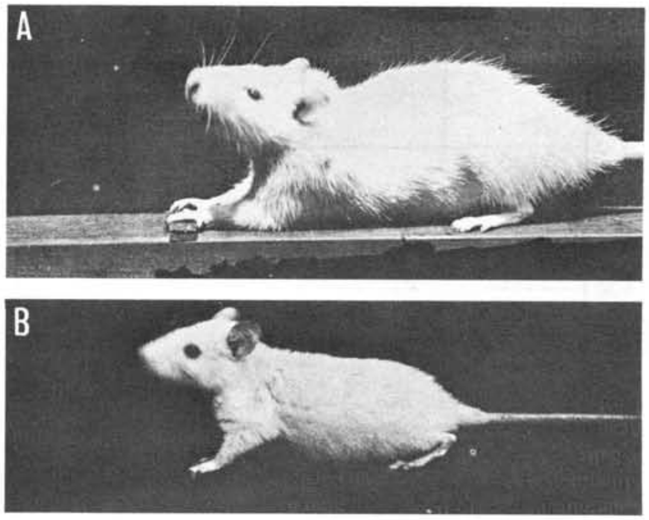


Fig. 6. Rat (A) and (B) on diets alike except that (A) had casein as a protein, (B) had gliadin. Both were 140 days old. Illustrates difference in ability of proteins to promote growth.

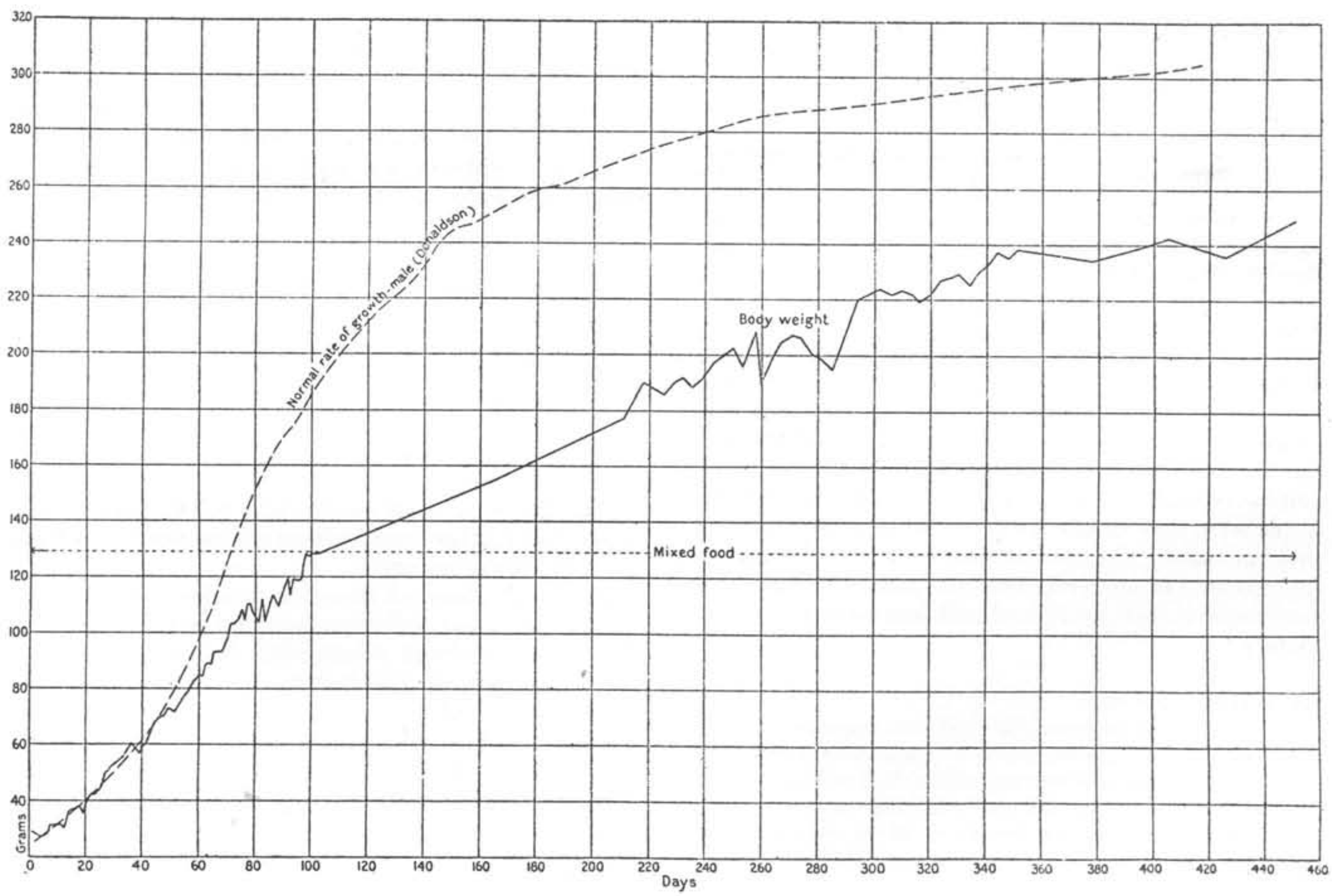


Fig. 7. Graph illustrating growth rate of male rats (avg.). Dashed lines—rats at Wistar Institute of Anatomy, (Donaldson was Director of program)—weight 300 g at 400 days. Osborne and Mendel's male rat shows similar growth pattern, but doesn't weigh as much at 400 days. (1910/11). See Fig. 8 for illustration of change in this curve.

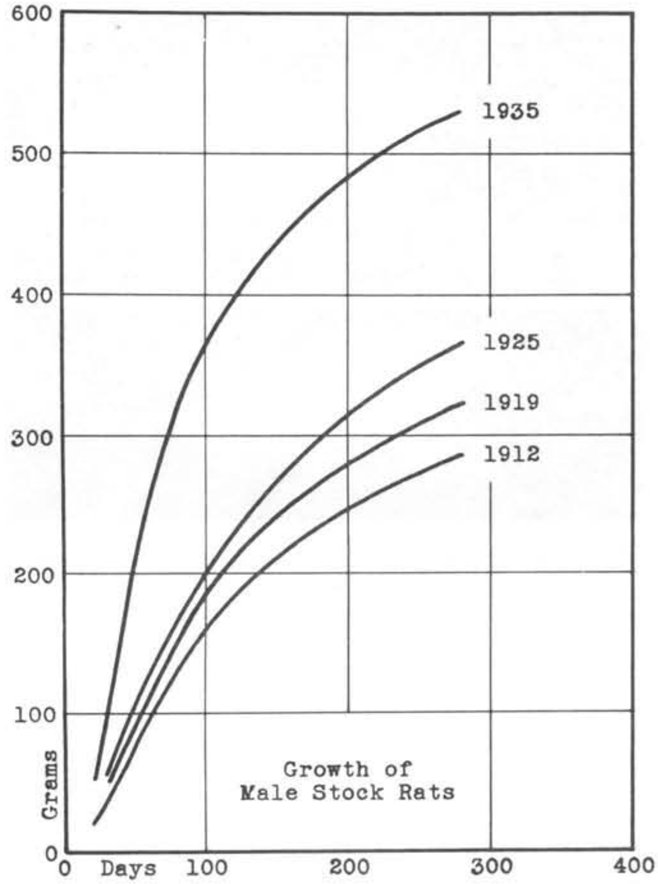


Fig. 8. Growth of male stock rats (for breeding) at C.A.E.S. Some of the change was due to selective breeding. Most was produced by improved knowledge about nutrition.

Milk

Another lesson drawn from human experience was the value of milk as a nearly complete food. After all, babies grow normally for months or even years on a diet of nothing but mothers' milk. To Osborne and Mendel, the literature provided adequate support of this bit of common knowledge. They, however, *used* the knowledge in planning their research work. Vast quantities of cows' milk provided a source of casein which was purified and incorporated in some experimental diets. In addition, as mentioned earlier, powdered milk was used in a control dietary.

Non-Protein Factors

> Remembering that our earlier trials with casein, the chief protein of the milk powder, and with combinations of casein and other proteins were at best successful only in maintaining nutritive equilibrium—and that not indefinitely—and were never adequate for the manifestation of real growth, we directed our attention to the non-protein constituents of milk.[26]

Osborne and Mendel had tried many combinations of minerals vital to the health of an animal, yet none had been completely successful. In other words, no matter what protein was used in the diet, *normal growth and maintenance did not occur except with the powdered milk or mixed food diets*. So, off they went to investigate the non-protein part of milk: lactose and mineral salts. Starting with fresh, skimmed milk, they removed the casein by chemical precipitation (you can do the same thing by adding vinegar or rennet to skimmed milk). After the casein was filtered off, the resultant solution was evaporated to dryness. "The product thus obtained formed a friable, pale yellow mass which was easily reduced to a fine powder by grinding in a mill."[27] Osborne and Mendel called this material protein-free milk or p.f.m. The results obtained by use of p.f.m. in experimental diets were dramatic at first. One rat, at constant weight for 83 days, doubled its weight in 35 days when given p.f.m.[28]

Success?

Osborne and Mendel must have been euphoric. In just under two years of research they had determined that plant proteins varied in the ability to promote growth and maintain the weights of albino rats. They had confirmed the positive nutritional value of milk and mixed food diets, and by doing so in a quantitative fashion, forced their and other investigators' attention upon milk as the key to successful nutrition. There seemed no way around the fact that milk apparently possessed all the nutrients essential to good health.

Osborne and Mendel had demonstrated that young rats could be kept in a stunted (no growth) condition for weeks or months on a maintenance diet, then resume growth when changed to a milk or mixed food diet.

> With such an ideal non-protein dietary component at hand, [p.f.m.] amino-acid substitutions can be attempted in the adult as well as in the growing animal. The protein minimum (or minima) is also open to accurate investigation. With a method of feeding devised which will permit a differentiation between growth and maintenance, which furnishes an energy-yielding protein-free component that is appropriate, and leaves the protein as the sole variable in the dietary, we believe that further contributions can be made to the problems of nutrition.[29]

(The last sentence is confusing. I think they intended to say that further contributions can be made to solving the problems of nutrition.)

The implications of these discoveries and confirmations with respect to human nutrition were all too obvious. While a direct relationship between nutritional needs of white rats and humans did not appear likely, the fact that both are mammals and eat similar if not identical foods, could not be ignored. As the future would amply illustrate, rat nutrition would be almost immediately translated into human nutrition.

INTERLUDE 1: THOUGHTS ON SCIENTIFIC WORKMANSHIP

I am sitting in the biochemistry library of the Johnson Building at The Connecticut Agricultural Experiment

Station. More than 65 years ago, Osborne and Mendel began their nutrition investigations in the basement on the opposite side of the building, for this library is part of an annex that had not yet come into being in 1909.

The structure has changed in many other ways during the intervening years. Not far from this room are laboratories equipped with apparatus that can do in one hour or perhaps one day some of the chemical work that required weeks or months in Osborne and Mendel's day.

The men and women who staff these facilities have much of the same drive to know; to understand the nature of living things that so motivated Osborne and Mendel. The directions of their work today are different. The emphasis is on living plants so that the extensive white rat colony is now confined to photographs, documents, and memories.

I need only to get up and walk 75 feet or so and I can stand where Osborne used to sit and argue with Mendel over procedures. The familiarity of the place—the aura created by those who lived and worked within these walls—is unmistakable. If I turn and walk down the stairs, I enter the area that used to be "home" for the white rats, Figs. 4 and 5. I can envision an ordinary day here: the poor lighting, the inadequate ventilation, the pervasive odor of the rats along with the sounds of their squeaking and rustling.

I can see Osborne with his severe expression, checking the data books on the rats, his mustache twitching over both good and bad results.

I can see Mendel after a full day's teaching at Yale bicycling the mile or so from Sheffield Scientific School to the experiment station, coming in to discuss the latest plans to unravel the mysteries of protein and mineral metabolism. A short, bald, gentle man whose incisive thinking provided so much inspiration to the partnership.

I think again of Osborne and Mendel's arguments —and they had some wonderfully loud ones—and at the same time I remember that they always addressed each other by their last names.

I try to relive their activities, even if only for a moment or two. Mendel, a bachelor until 1917, in the laboratories practically every day of the week, yet finding the time to edit a journal as well as participating in community affairs.

Osborne, the stern Yankee, returning home to his wife, Samuel Johnson's daughter, to discuss chemistry and nutrition.

I see Osborne and Mendel as insatiable readers of scientific literature and occupied almost constantly with the writing of yet another paper describing the results of their experiments.

I can almost recall Osborne's "hot weather" trips to the family's residence at Holderness, New Hampshire as well as Mendel returning to his family's home on the Delaware River in southern New York.

Whenever business or vacations intervened in the normal routine, Osborne and Mendel maintained contact with respect to their mutual problem: nutrition. Letters, often long and detailed, would come and go with great frequency. And always these men retained formal address, as if they were speaking to one another: My Dear Mendel or My Dear Osborne. If things were a bit hectic the salutations might be reduced to: Dear Osborne or Dear Mendel. If these men ever used first names it is beyond the recollection of anyone still alive.

As I wander in both thought and fact around the building, I stop for a moment outside one of the fireproof rooms that served as a storage vault for Osborne's proteins and amino acids as well as for the rat records and laboratory books, many of which are still here. It is when I look at one particular notebook that I think of the night of January 10, 1910, when a fire swept through the laboratory, destroying all the rats and much of the equipment. But the fireproof rooms had been locked so the chemicals and records were relatively unscathed. Yet even today I can find a notebook which bears the scorch marks of that mid-winter blaze. And, some of the journal articles that had been shelved close to the firewall carry the reminder even more strongly because every now and then a bit of charred paper breaks off in my hand. About the rats, I choose not to think at all.

Burnt paper is not the only reminder of the past, for all around, arrayed on shelves, are glass containers of many shapes and sizes, each with its content of protein or amino acid; the labels tell me that some of the chemicals were prepared when 1900 was still in the future.

On other shelves are the notebooks: paper and ink, recording more than 40 years of chemical manipulations; boxes of correspondence; idea and plan books. Alongside these are photographs and charts; manuscripts written but never published; ideas conceived but never put into practice. The overwhelming sensation that fills these vaults is one of frustration and excitement: the frustration of an experiment gone wrong and the excitement of discovery. There are other feelings that pervade these small rooms but the one I notice at the moment is one of an accomplishment well done and the workers now quietly at ease.

And then I step outside the vault and blink my eyes under the bright fluorescent illumination that shines on the up-to-date laboratory of 1976. As I begin to retrace my steps (and I did not forget to close the door of the fireproof vault) to the floor above, I remember something else about the fire of 1910. One of the more amazing aspects was the recovery shown by Osborne and Mendel. Far from being overwhelmed by the catastrophe, they cleaned up the mess, rigged temporary equipment, obtained a new stock of white rats and were back conducting experiments within two weeks.

Now, as I return to the library to take up the story of these later investigations, one question still lurks in my thoughts: What happened to that $1.25 Express wagon? Was it worn out and then thrown away? Was it destroyed in the fire? Perhaps. But I prefer to believe that it survived to continue a function that will remain a mystery forever.

FAILURE AND PROMISE: 1911-1913

Trouble

Osborne and Mendel appeared to have a clear road ahead of them. They had established an effective experimental technique that allowed them to vary, one at a time, each component of the diets of their animals. In November 1911, they could state with conviction that milk contains *all* the nutrients essential to growth; growth that had not been satisfactory with any of the artificial food mixtures.[30] Why was milk so special? Was it the combination of inorganic salts found only in milk? Was it a small quantity of an unidentified mineral that gave milk such nutritive value? Or were traces of essential organic compounds responsible for the adequacy of a milk diet? Whatever the cause, its nature ". . . remains to be ascertained."[31]

Earlier in 1911, on July 28th to be exact, Osborne wrote Mendel that the experiments were going along nicely except for an apparent infection of the eyes of many of the rats.[32] Osborne's description of the afflicted rats is vivid: "The eye lids are stuck tightly together in the bad cases and [the] eye ball appears dry . . . There [is] considerable inflammation and some swelling of the eye lids."[33] (Fig. 9a).

Two important aspects of this disease should be pointed out. First, only rats being fed protein-free milk were affected. Second, without realizing it at the time, Osborne was describing the classic symptoms of a disease called xerophthalmia, now known to be caused by a lack of vitamin A in the diet!

Osborne and Mendel were on the edge of a major breakthrough in nutritional science. It is, however, in the nature of things that even the best of scientists will overlook the unusual simply because it appears to be something quite ordinary. To Osborne the disease was conjunctivitis, probably caused by bacteria. Since no further comments were made in the ensuing days other than to report some improvement in a few rats, we can only assume that Osborne and Mendel came to accept it as one more problem associated with raising so many animals in confined quarters. Whatever the case, however, the disease returned with regularity. In time they would recognize it for what it was—a trace nutrient deficiency.

Planning a New Attack

Experiments planned by Mendel and recorded in a letter to Osborne in August 1911, showed the confidence and excitement Mendel had at the time. Nothing seemed impossible. Thus, he could speak of studying the effects of individual amino acids on growth and maintenance; an investigation to determine the nutritive values of proteins not yet examined; and the special value of lactose.[34]

Osborne replied with his usual caution:

> It is hardly possible to begin any new feeding experiments until we have fully and finally decided on just what we want to do next. Our opportunities are limited by our space as well as by our funds and we must be careful not to do more than we can do well."[35]

Mendel did not really intend to carry out in one year all the ideas he had suggested. More likely he was making certain that ideas in his head were transferred to paper before they vanished into the world of forgetfulness. Thus, Mendel wrote to Osborne, apologizing for not making his intentions clear.

> When you look forward to the possibility of a synthetic food supply which will keep animals in normal health throughout their lives you set a pretty high standard for us at the start. We are so far from knowing what the "little accessories" are.[36]

Failure

There they were: The unknown "little accessories." On previous occasions Osborne and Mendel, in company with other investigators, had asked the question about what substances were needed for proper nutrition other than the conventional ones. At the time (1911) such unknowns were often referred to as accessory substances, accessory food substances, food hormones, growth hormones, growth promoters, etc. To Osborne and Mendel they were little accessories. We know from their records that they considered these accessories to be, most likely, small quantities of specific amino acids or trace amounts of certain minerals in specific proportions, or perhaps a combination of both.

That they were correct in these assumptions is important. That still other, yet unsuspected (by Osborne and Mendel) accessories existed, organic in nature, was something they would learn the hard way. They had missed an opportunity to discover vitamin A in the eye disease problem. That oversight would not be repeated.

In the meantime other troubles were appearing. Rats that had lived successfully for many months on experimental diets were beginning to fail. In almost every dietary using different proteins, normal growth of rats would continue for 80-100 days, then slow or cease. "In most of the experiments now in progress (the body weights) are beginning to fall."[37]

Something was wrong and protein-free milk was involved just as it had been with rats that developed the eye disease. Take the case of rat 133, fed with edestin (a protein from hempseed). After being on this diet a while, the rat began to lose weight so he was given edestin + p.f.m. for a few days, then returned to the edestin diet.

To everyone's surprise, the rat continued to grow as rapidly on the edestin alone as he had when given p.f.m. as a supplement. "It may be," wrote Osborne, "that our rats which have been growing normally on protein-free milk until they reach a certain size and then cease to grow . . . are getting too much of the substance which stimulates growth. . ."[38]

To say the least, confusion reigned. Mendel's response to the problem of rat 133 was, in effect: growing rats probably need more of this unknown substance than adults.[39] Any way you looked at it, the number of possible 'correct explanations' was large. As time went on the problem would become acute. Over long periods it would prove impossible to keep a rat growing and nearly impossible to maintain an adult one without loss of weight and a decline in health.

In the cages next door, white rats on mixed or milk food continued to live happily, sleek and in good health; constant reminders of the hidden "accessories" in their diets.

Strategy for Success

From September 1911, through March 1913, Osborne and Mendel's experiments were conducted along the two major routes planned in advance: growth and maintenance. To these ends single proteins and combinations of proteins were fed with the basal diets of starch, sugar, lard plus mineral salts or protein-free milk. In order to solve the problem of what makes animals grow, Osborne and Mendel continued to make numerous variations in dietary composition, always searching for that particular one that would be the equal of a milk or mixed food diet. One that, somehow, would contain the mysterious accessory substance.

Yet, the troubles persisted. A few rats could be kept in good health for about 500 days, but as Osborne remarked in a letter to a colleague: "At the present time we are not concerned with the length of life of the white rat for our experiments are not yet reaching the time at which a rat should die of old age."[40]

An examination of the work carried out through March 1913, offers a bewildering array of data which, upon closer scrutiny, illustrates an elegant simplicity and order.[41] While the results might be confusing and uncertain in interpretation, the experimenters' methods were not. The near future would demonstrate that Osborne and Mendel's trial-and-error technique with three innovations as described below, would ultimately lead them to success on more than one front.

First, they increased the use of specific amino acids to determine whether an incomplete protein could be made complete, thereby becoming nutritionally adequate. (Details of this innovation will be presented later.)

Second, Osborne and Mendel developed a new dietary supplement which they termed, "artificial protein-free milk." Having observed repeatedly the positive nutritive qualities of milk and the effects of "natural protein-free milk", it occurred to them in November or December 1911, to duplicate as closely as possible, the composition of natural p.f.m. Why did they go to the trouble of doing this? Remember, *Osborne and Mendel were primarily concerned with proteins and the development of a totally artificial diet that would be the equivalent of milk food*. Artificial p.f.m. was a step in that direction. It included all the minerals to be found in the natural variety insofar as these could be chemically determined, as well as a quantity of lactose equal to that in natural p.f.m.

The first mention of this dietary supplement was made in April 1912,[42] and a more elaborate description appeared in a German publication later that year.[43] Early results obtained by feeding rats with artificial p.f.m. indicated that in some experiments growth was about the same as that of animals fed with the natural form although, in general, the artificial p.f.m. was not equal to the growth-promoting activity of natural p.f.m.

Another vital clue to accessory food substances thus emerged from use of artificial protein-free milk. Osborne and Mendel commented that: "To our surprise the artificial protein-free milk, III, made with these purer chemicals failed in every case but one to promote more than slight growth. . . ."[44]

It certainly must have been quite a surprise to say nothing about it being a rude shock. Osborne had more than twenty years experience in preparing the purest compounds available, yet feeding experiments convincingly showed impure materials to be more nutritious!

> The chemicals used in making the first lot of "artificial protein-free milk" (designated I) were ordinary laboratory preparations of good quality. Later we used Kahlbaum's (a commercial producer) preparations. . . . (which we designate II), (and) in many cases we obtained growth quite comparable with that previously secured with foods of similar character but containing the natural "protein-free milk."[45]

How could this anomaly be explained? It must have been embarrassing to have a relatively crude product more effective than the finest preparations from the laboratory. In reality there were only two choices: Either the artificial p.f.m. was not an accurate duplicate of the mineral and lactose content of the natural material or else natural p.f.m. contained something, in small quantities, that had not yet been chemically isolated and identified.

Osborne and Mendel did not realize it as yet, but they were within reach of discovery of the "B" vitamins.

The third innovation was the fat-free diet. Use of lard, while providing both energy and a means of producing food paste, was always suspect in that the quantity of fat eaten by experimental animals was generally much greater than what they would consume under normal conditions. Also, information from other researchers tended to show that, contrary to older beliefs, fats were not alike nutritionally.[46] Diets with food that had been washed and extracted with solvents that dissolved fats (hot alcohol and ether, e.g.) appeared to lose much of their nutritive value.

In December 1911, Osborne and Mendel began rat feeding experiments with fat-free diets. Using casein, sucrose, starch and p.f.m., all extracted with ether, they reported success in obtaining normal growth of white rats on foods devoid of fats (to be specific, devoid of ether-soluble fats).[47] In a footnote, however, Osborne and Mendel said:

> In these, as in all our other experiments in which very young animals exhibited a normal rate of growth on mixtures of isolated

> food substances, we have not yet succeeded in bringing the animals to their maximum normal size on the dietaries employed. This failure to attain complete growth involves some factor in nutrition other than the fat and is at present under investigation.[48]

Clues and hints were fast piling up. Fat-free diets were not very successful. Artificial protein-free milk was not as valuable nutritionally as natural p.f.m. Inadvertently, the fundamental question had shifted from: What diets will make rats grow? to Why won't rats grow on diets that are supposedly complete?

This change in emphasis or point of view had become, without their being aware of it, Osborne and Mendel's primary strategy for success.

INTERLUDE 2: VITAMINES

In my attempt to reconstruct Osborne's and Mendel's thinking in those critical days of 1912 and 1913, I walked over, late one night, to the experiment station's general library, the Osborne Library, to locate specific documents that I believed would be of assistance. Switching on the light, I was startled for a moment by the large portrait of Thomas B. Osborne that hangs opposite the entrance. For just a moment it seemed to me that Osborne was looking directly at me. Now, I had seen that portrait hundreds of times in my journeys through the library and I had never experienced a sensation of being looked at. Why then, had this occurred that night? Was it because the building, the oldest on the station grounds, used to be the chemical laboratory where Osborne had started his long-term studies of plant seed proteins? I told myself not to be silly; probably I was just overtired. But I couldn't reconcile myself to the fact that I had been really startled by that "look". I promptly rationalized the episode by thinking that my glance at the strong-willed Osborne, seated in an armchair, had reminded me that I had overlooked something in my story.

Well, I searched and did not find what I needed so I strolled down to Yale's Sterling Hall of Medicine to use the library. And there, on one wall is a portrait of Lafayette B. Mendel standing next to a table on which rests a cage containing three rats.

This was more than I was prepared to face. I had seen that portrait on almost as many occasions as Osborne's. Why were these paintings suddenly perplexing me?

To make a bewildering story a short one, I soon found myself scanning the pages of an article published in 1912 in an obscure British journal; an article written by the Polish chemist, Casimir Funk. I had read that paper a number of times and was aware that it contains the first printed reference to the word vitamine. It was when I re-read the article that I had one of those sudden illuminations of understanding. Of course! Osborne and Mendel had read this paper when it was new in 1912. That's what had triggered their change in procedures, a change that would lead them to a major discovery in nutrition.

And then my excitement came to an abrupt halt. How did I know if Osborne and Mendel had read the article? Certainly I knew of its importance for it was, in retrospect, one of a small number of documents that served as a turning point in the history of nutrition. In this paper Funk made sweeping generalizations about accessory food substances and their relationships to deficiency diseases. Such diseases, Funk claimed, as beri-beri, scurvy, pellagra, and rickets were caused by the lack of small amounts of organic bases (amines) in the diet. I was aware that Funk had extracted from rice polishings—the outer husks and bran layers of rice seeds—a chemical substance or substances that cured a disease (polyneuritis) in birds similar to beri-beri in humans. Funk claimed, in addition, that all of these diseases could be cured or prevented ". . . by the addition of certain preventative substances . . . we will call vitamines."[49]

Furthermore, with respect to F. Gowland Hopkins' nutrition experiments in England as well as Osborne's and Mendel's work, Funk wrote: "I suppose that the substance facilitating growth in milk is similar if not identical, with the vitamines described by me."[50]

At this point I was, to say the least, disheartened. Here I thought I had discovered how and why Osborne and Mendel shifted their view for a time from proteins and minerals and turned to the idea of accessory organic substances. Their experimental plans for the fall of 1912 mentioned Funk's activities. I knew—just as I suspected Osborne and Mendel knew—that Funk's claims and discoveries were crucially important to their research. Yet I could not prove it.

I was really upset. Those portraits had started something and I wasn't about to give up the search. Somewhere the evidence existed, so off I went again, this time to examine Osborne and Mendel's correspondence files. I also began digging frantically through thousands of articles collected by Osborne. And then, only one night later, after midnight, I found what I had been seeking. Specifically, there were four items that practically shouted at me to be read:

1. *A publication by Hopkins, July 15, 1912, describing rat feeding experiments with and without milk in the diets.*[51]
2. *A letter from Funk to Osborne, March 20, 1912:* "Several days ago I send (sic) you my copies of papers on the curative substance in rice polishings which is closely analogous to the substance acting on growth of rats in milk."
3. *A paper published by Funk, August 2, 1912, describing the elaborate chemical techniques used to prepare the curative substance:*

> I have suggested the name *vitamine* for it as being one of those nitrogenous substances, minute quantities of which are essential in the diet of birds, man and some other animals . . . The curative substance was also isolated from milk.[52]

4. A strip of paper, five feet long and five inches wide, made up of eight sheets of paper glued together, on which Osborne had scrupulously outlined, in pencil, Funk's technique for preparing the 'curative substance'.

There they were. The missing pieces had fallen into place. Osborne's critical analysis of Funk's chemical manipulations was not the kind of thing one does with an unimportant paper.

Hopkins clear-cut demonstration that small amounts of whole milk fed to rats on otherwise deficient diets produces an almost miraculous rejuvenation, could only have forced Osborne and Mendel to take notice.

I relaxed for a while to enjoy the marvelous feeling that an act of discovery can bring. I mused over what Osborne and Mendel's feelings were in that summer of 1912. Or Funk's, when he isolated the curative substance. Well, whatever those feelings were, the Experiment Station chemist and the Yale professor, unaware as yet, were travelling a new path.

In the meantime, back in Great Britain, Osborne and Mendel's German publication,[43] *in which they had virtually disclaimed the need for organic growth substances or growth hormones, produced astonishment if not outrage. As Hopkins wrote later:*

> I have done so much work in this (and very successful) endeavor to separate the unknown substances which affect growth, that when your paper . . . . came out I suffered from an attack of nerves! I was at the British Association (for the Advancement of Science) in Dundee at the time, when Casimir Funk brought me a copy . . . and we were both somewhat overcome.[53]

Yes, I can imagine Funk and Hopkins in that old Scottish city, far from their laboratories, examining a respectable publication that contradicted much of their research to say nothing of the work of others in Europe. There would be a fuss about it alright, but once again fate would step in and the contradictions would melt away. That, however, is something I can see, for I know how it will happen.

As to the problem of my reactions to Osborne and Mendel's portraits that started this chain of thought I can only say that it will probably never be resolved. I could explain it all by virtue of the fact that I had been examining their letters, their notebooks and miscellaneous items related to their work for so long that I simply responded to any cue concerning either of them. Certainly one can understand how a painting could serve as such a cue.

On the other hand I know that Osborne and Mendel were remarkably persistent men. But just how *persistent can anyone be? And for* how *long? I don't for a moment believe that either was trying to get my attention. No, it was all a simple matter of being involved in this study for a long time.*

At this point I am content to live a while in 1912 and believe what Casimir Funk believed: that certain diseases are caused by a lack of vital-amines or vitamines in the diet and that animal growth is dependent upon vitamines.

As I rest here in the Medical Library where these ideas finally came to life in words, I possess one bit of certain knowledge: Osborne and Mendel, amongst others, would prove Funk to be prophetic.

SUCCESS: 1913

Osborne wrote to Mendel in the summer of 1912, that it was becoming more and more evident that rats were getting an *essential* substance from *milk*, ". . . . the nature of which we do not yet know. We ought to get at this problem as soon as possible and it ought to be made our first work in the fall."[54]

Indeed, the plans for the coming year were extensively oriented towards isolating the unknown "essential substance", just as Funk had isolated a curative material. And these plans were, unwittingly, developed around the question proposed earlier: Why won't rats grow on seemingly complete diets? That such diets were inadequate was painfully obvious. Rats would grow normally for about 80-100 days on an experimental food using both types of protein-free milk, after which they would either decline in weight or barely hold to a constant one.[55]

And, while these and earlier plans were being transformed into experiments, Mendel went off on a "business vacation" that included a stop at Wisconsin where his former student, E.V. McCollum, had been carrying out rat-feeding experiments since January 1908.

Back in New Haven, Osborne was writing two major papers: one on the role of gliadin in nutrition and the other on nutritive aspects of feeding rats on maintenance (no growth) diets. Both papers were complex and Osborne despaired of getting them finished in reasonable time. He wrote Mendel of his concern and, in addition, noted:

> I have made one or two minor changes in the text which I think you will approve of especially one in the first sentence which has cured some very bad grammar which you invented and I failed to discover before.[56]

Mendel replied with grace and humor, making a number of comments and suggestions and telling Osborne not to worry:

> There is no necessity of rushing these papers unduly . . . Don't break into your vacation. No one will steal our experiments. I became convinced of that when I saw under what conditions McCollum has to work. He has rats in his office! Fortunately, he has, like us, a competent woman to exercise the chief care of the animals. His working conditions are very bad. He has no paid assistants![57]

Later in the summer of 1912, Osborne and Mendel exchanged correspondence with F. Gowland Hopkins, in England, on the subjects of feeding experiments, accessory substances, proper diets, and nutrition in general. The major outcome of all this was increased emphasis on the part of Osborne and Mendel to discover the nature of any such growth-promoting accessory substances. Mendel was still not convinced of the absolute nutritional necessity of these substances. Proteins, amino acids, and minerals were the key ingredients.

> However, these 'accessory' extracts may be an invaluable aid to us in studying the comparative role of proteins if they permit us to keep our animals in better form. The point in our paper which Hopkins questions is very important, i.e., the quantity of p.f.m. that is sufficient, and it needs to be repeated perhaps."[58]

Evaluation of Results: The Correct Turn

By early fall of 1912, the atmosphere around the rat cages was one of discouragement. The fat-free experiments were "manifestly all failures". And to make things worse, Osborne noted that the breeding colony was inadequate to provide necessary animals for the extensive experiments that had been planned.

Discouragement was not new to these men. In more than three years of collaborative efforts, Osborne and Mendel had overcome equally difficult periods. The problems of 1912 were no exception. But, as 1913 approached, Osborne and Mendel could look back upon their intensive nutritional studies with satisfaction.

They had confirmed earlier hypotheses that animals build their protein tissues from food proteins digested to amino acids.[59] Furthermore, it was now obvious that any food lacking an essential amino acid "must lead to serious nutritive disturbances."[60]

On the other hand, Osborne and Mendel had reason to believe that animals could synthesize some amino acids from their food. It therefore appeared that rats (and probably humans) needed only certain amino acids in their diets. The question to be resolved was one of determining *which* amino acids were essential and in what quantities.

Osborne and Mendel had also worked out many of the difficulties encountered in mineral nutrition and in so doing, progressed further in a search for small amounts of unknown, but necessary, food substances.

They had successfully examined the factors influencing growth and maintenance: energy intake (fats and carbohydrates); inorganic salts (minerals); protein differences—in quality and quantity. With respect to the undiscovered "growth hormones", Osborne and Mendel could state: "Indeed, some writers at the present time believe that in the latter, as yet unknown factors, rests the secret to nutritive success."[61]

Perhaps the most significant aspect of their experimental procedures had been the trial-and-error method which had led them to the conclusions mentioned above. Evidently, however, Osborne and Mendel were sensitive about this methodology and reacted strongly to unidentified critics by writing:

> We believe that experiments such as those reported in this paper show the possibility of approaching certain of the problems of nutrition by new and hitherto discredited methods of study . . . If we had been content to discontinue the experiments after a reasonable period many of the declines . . . would have escaped attention."[62]

With respect to most of the literature of the day,

> None of the records, so far as we are aware, extend over periods even half as long as some of ours which ultimately ended in nutritive failures.[63]

If imitation is the sincerest form of flattery, then Osborne and Mendel would be flattered indeed. Scores of laboratories, using similar apparatus and similar techniques with white rats as experimental animals, would be opened in many areas of the world during the next 10-20 years, to solve nutritional problems.

The Path to Butter

If Osborne had been worried about having too few rats for experiments, that worry was soon dispelled. Whether by breeding or purchase (or both), the rat colony increased rapidly: 192 new experiments were started in the months of September through December, 1912.[64]

Into these experiments were incorporated many of the ideas set forth in Mendel's plan books: Fat-free diets; artificial p.f.m. vs. natural p.f.m.; using sucrose in place of lactose; purified lactose vs. commercial grades; different salt mixtures; different proteins and amino acids. Variations and combinations were tried in keeping with the initial concept of varying one item at a time in any individual diet. The technique, cumbersome as it was, and despite some criticism, was truly a productive one, even if it was slow in yielding results.

As winter gave way to Spring 1913, Osborne and Mendel increased the scope of their activities. Diets were served cooked and uncooked; milk was heated. or boiled before addition to a diet; evaporated milk was utilized. In addition, organic solvent extracts (fat-soluble materials) of milk and milk products were incorporated into some of the diets.

A number of these experimental designs had been influenced strongly by correspondence with Hopkins in England, in Feburary and March 1913. Early results inescapably pointed to lactose and some other component of milk as being involved in an adequate diet in ways hitherto unrecognized. Unlike proteins and amino acids, whose functions were being clarified by a host of investigators, lactose was an enigma. It was a simple sugar. Why did impure lactose provide better nutrition than the purified form? In fact, as Hopkins mentioned in one letter, even proteins were suspected of containing something other than amino acids—something necessary for growth.

> But I would make much of the fact that very thorough extraction with alcohol is, in my experience, the only way of completely removing the growth (fraction). The ordinary methods of purifying proteins do not necessarily remove them. I had, once,

> some recrystallized edestin on which rats grew well when it formed part of an artificial mixture. It yielded however quite a definite alcoholic extract, and when this was removed, growth wholly failed.[53]

(I wonder at Osborne's reaction to an intimation that his proteins were not really purified.)

Osborne and Mendel's reply to Hopkins was a lengthy one and represented a brilliant summary of their results to date. With Hopkins' letter, this correspondence, plus an earlier letter from Osborne and Mendel, provides an elegant picture of nutritional science in 1913.

Osborne and Mendel ended their second letter on an intriguing note, one that testifies to the thoroughness of their research.

> Your hope that you might welcome us both at the International Medical Congress in London makes us both wish that you could do so for nothing would give us greater pleasure. How many things we could talk over together and what a good time we could have.
>
> Rats, however, eat every day—Sundays, holidays, and vacations all the year round and year after year so how can we do it? We must work first and have our pleasure after but with our present rate of progress and the rapid growth of biochemistry, we fear that the time for pleasure will come when we are too old."[65]

Butter

On April 17, 1913, three rats were started on a casein and protein-free milk diet with one addition: butter.

During the same month, 15 rats on other experimental diets were abruptly changed over to food that included butter.

In May 1913, 11 rats were started on dietaries containing butter or butterfat while two were given the fat of egg-yolk as a supplement, and 26 others were taken off experiment and given new meals—all containing butter or butterfat.[66]

On June 19, Mendel wrote to Osborne who was on a short vacation:

> You will be interested to know that today's weights uniformly show the sort of changes which we are expecting. The butter is still doing its work, while the butterfat gives no evidence of success as yet."[67]

On the following day, another letter went out from New Haven:

> . . . not only are the butter rats shooting upward toward the sky line, but the rats which had exhibited evidences of spring fever or summer disease or whatever we may care to call it, are almost without exception beginning to grow.[68]

It had been a long time in coming, but a major breakthrough had at last occurred. All the hints and clues from the literature: the correspondence with Hopkins, Funk's vitamines, McCollum's research at Wisconsin, the daily reminder that milk was a complete food; coupled to the persistent, patient, rat-feeding program had brought Osborne and Mendel to this point. They finally examined that part they had previously neglected: the cream—the fatty portion of milk. Step-by-step, Osborne and Mendel had eliminated every other known component of milk (except lactose which still has a story to tell) in terms of its ability to promote growth. All the balancing of mineral salts; all the variations in protein; the changes in carbohydrates; the heating of p.f.m. and milk powder had not produced a diet upon which rats would grow to adulthood and attain a maximum possible weight. In the end, there was only one direction in which to turn.

> In seeking for the 'essential' accessory factor we have, therefore, been led first of all to supply the cream component in the form of butter, to rats which have ceased to grow on the 'protein-free milk' foods . . .
>
> It would seem, therefore, as if a substance exerting a marked influence upon growth were present in butter, and that this is largely, if not wholly, removed in the preparation of our natural 'protein-free' milk . . .[69]

What a remarkable, restrained understatement of a major discovery:

> It would seem . . . as if a substance exerting a marked influence upon growth were present in butter. . . .

In simple language, butter made rats grow.

They did not grow on diets that in all other respects were adequate, containing as they did correct amounts and types of proteins, carbohydrates and minerals.

The addition of butter to these diets produced startling results. Rats that had declined in weight began to grow at prodigious rates. Some doubled their weights in as little as 40 days. Others showed weight increases of 80% in a month.[70] There were no significant failures.

Osborne and Mendel had found what in later years would be called vitamin A, an organic substance absolutely essential to animal growth.

The fact that McCollum at Wisconsin made the same discovery at almost the same time only reinforced an old scientific truth: simultaneous discoveries are common.

It is not necessary to belabor the point. Suffice to say that 1913 witnessed an "explosion" in nutritional science. True, Funk had earlier found something that cured a disease in birds as well as in humans but he had not shown the necessity of the "curative substance" for animal *growth*. As later experimental work was to illustrate, Funk's curative substance contained a number of vitamins, one of which, vitamin B_1, was *the* curative material in beri-beri and was also necessary for normal animal growth.

Whatever the terms applied—vitamines, growth promoters, accessory substances—the fact remains that vitamines (or vitamins) had been discovered and, like the rats that proved their existence, vitamins would multiply rapidly. The road was open to the "B" group as well as "C", "D", and a host of others.

INTERLUDE 3: THOUGHTS ON SCIENTISTS AND VITAMINS

In retrospect it is easy to ask why Osborne and Mendel did not discover the growth promoter earlier. They certainly had plenty of opportunities to do so.

This is of course, a nonsense question. Science is not conducted by absolute techniques leading to absolute solutions of absolute problems. The discovery of a substance that makes rats grow occurred only after those who were studying nutrition were forced, by conditions of their work, to concentrate on an aspect previously overlooked. Human decisions, human fallibilities and human errors necessarily came into play, much as they do in other activities such as sports, business, medicine or law.

No, instead of asking a nonsense question we ought to sit back and marvel at the realization that anyone discovered the vitamins at all. McCollum was a mere three weeks ahead of Osborne and Mendel in publishing the fact that butter makes rats grow. But others were in position to make the discovery even earlier. Hopkins had suspected, had forewarned nutrition researchers, and had written about such substances but he had not isolated an active growth promoter or vitamin. Funk had prepared a crude extract of rice polishings that cured or prevented polyneuritis in birds. He took a giant mental leap when he invented the word and concept of vitamines; he even predicted their discovery. But he did not do what McCollum, Osborne and Mendel had done: demonstrate the necessity of such a substance for growth.

It is here in the process of discovery itself, that the non-measurable qualities of luck, insight, diligence and a host of other indefinable human traits come into action. It is here that the exceptional scientist succeeds while his equally-trained colleague does not.

Osborne and Mendel had their share of luck, both good and bad. They began by asking: what foods make rats grow? and wound up asking: why won't our rats grow? Every bit of instinct and training told them that growth should be possible. Men of lesser faith would have long since given up in frustration.

Osborne and Mendel's primary interest from their first experiment to their last, was the function of proteins in animal nutrition. A secondary interest was the role of minerals in nutrition. Only when they had failed in using every conceivable means to keep rats growing normally on foods developed from their knowledge of protein and mineral functions, did they turn to an aspect of the problem that they had discarded earlier, the role of fats in the diet. In this respect, their perseverance and meticulous trial-and-error approach "paid off" handsomely.

VITAMINS AND AMINO ACIDS EMERGE: 1913-1917

Vitamins

Osborne and Mendel continued their study of butter as a growth promoter. In so doing, they discovered that the active substance was in the oily portion of butterfat itself. Although the exact chemical nature of this material, vitamin A, would not be determined until 1931, its presence as a growth promoter was established beyond doubt in 1913/14. Still, Osborne and Mendel were not ready to place this substance in the category of Funk's vitamins. An accessory it was; a vitamin, no.[71] A few years later, with more evidence they would change their position and adopt the vitamin hypothesis.

Other aspects of this accessory substance were brought out. First was its ability to prevent or cure the "summer disease" or "spring fever" that affected the

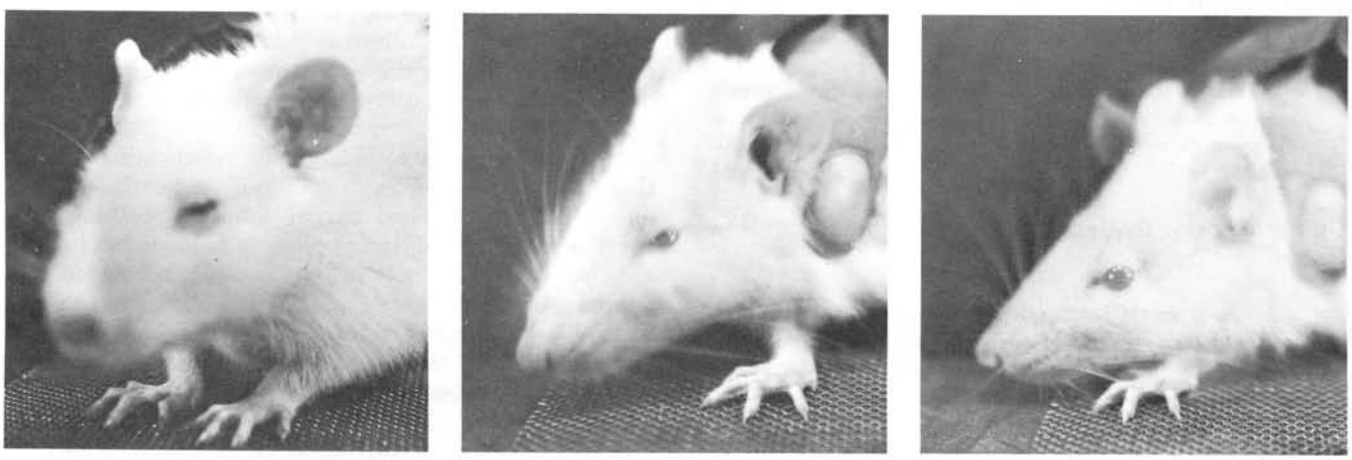
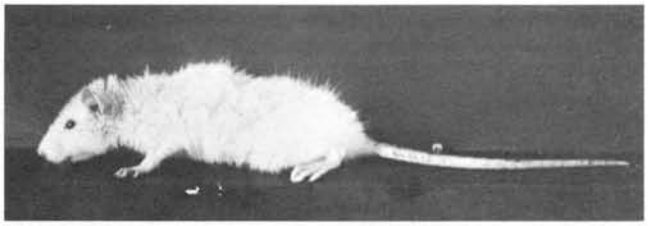


Fig. 9A, left, rat 81 days on Vitamin A-deficient diet. Note bad eye. Fig. 9B, center, same rat after 13 days on original diet and watercress as a source of Vitamin A. Note improved condition of eye. Fig. 9C, right, same rat after 17 days on original diet and watercress for Vitamin A, fully recovered.



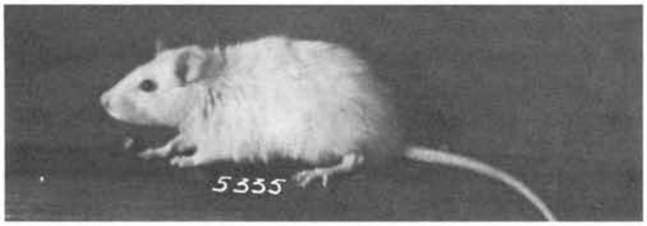


Fig. 10A, top, rat on a diet deficient in B vitamins. Note the rough, scraggly coat and emaciated character of the rat. Fig. 10B, bottom, the same rat as in 10A, after 12 days on a diet that included B vitamins in a water extract of yeast. Note the marked changes in the coat.

growth of young rats in particular. Second, was the curative ability of butter in the treatment of xerophthalmia, the eye disease first noted by Osborne in 1911, and evidently recurring at frequent intervals.[72] *(Today when everyone knows that vitamin A is "good" for the eyes, a glance at rats afflicted with xerophthalmia will make this cliché more meaningful.)* Figs. 9a, 9b, 9c.

By 1917, Osborne and Mendel had come to accept the vitamin hypothesis. Reluctant at first, their own experimental results, in concert with a vast literature from other laboratories, left them no choice. Whatever the substance or substances were chemically, they were necessary for growth and maintenance of rats.[76]

Osborne and Mendel, along with numerous other nutrition investigators, recognized the existence of at least two vitamins, one being fat soluble, the other water soluble.[77] Eventually they would agree with McCollum's claim that commercial lactose and even laboratory grade lactose contained some of the water soluble vitamin[78], probably adsorbed from milk during preparation of dry lactose. (Figs. 10a and 10b.)

The years of World War I, 1914-1918, witnessed a phenomenal growth of the science of nutrition, and Osborne and Mendel were in the thick of it. They published numerous papers on vitamins, and continued this research into the 1920's. But their first and principal affection was for proteins. Osborne started his chemical studies of plant seeds in 1888 and Mendel began his career studying protein metabolism in the 1890's. It seems most fitting that their last collaborative paper in 1927 was entitled "Physiological Effects of Diets Usually Rich in Protein or Inorganic Salts."[79]

Amino Acids

Earlier it was remarked that one of the innovations in the experimental technique adopted by Osborne and Mendel was an increased use of amino acids to determine which were essential to health. The Question was

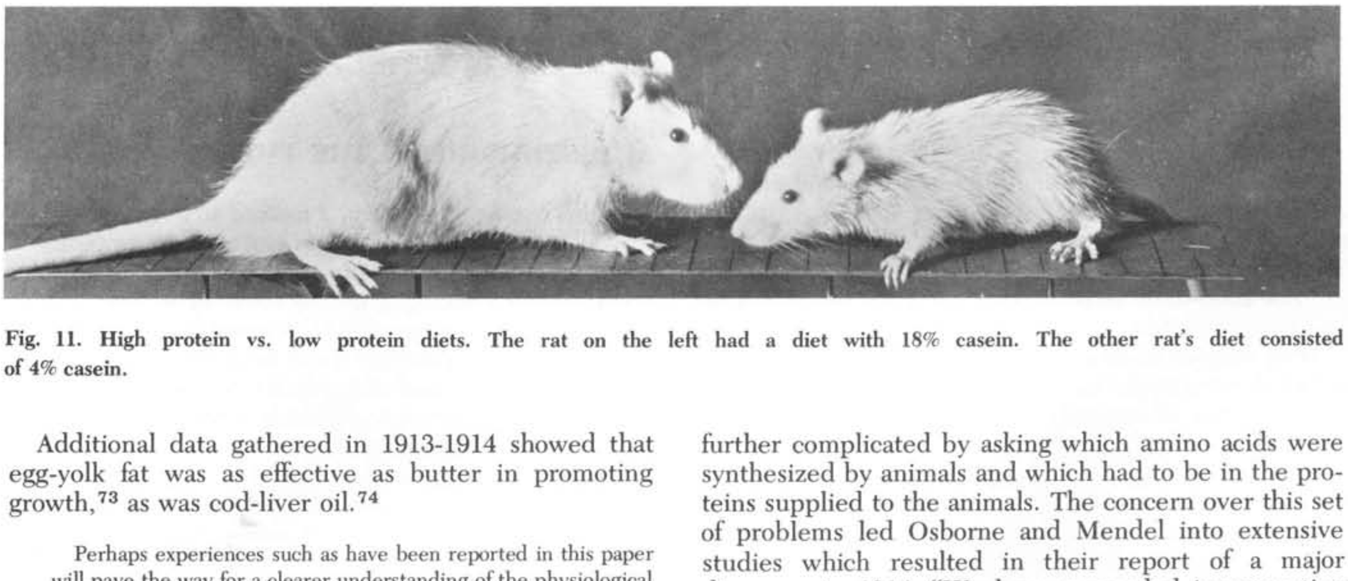


Fig. 11. High protein vs. low protein diets. The rat on the left had a diet with 18% casein. The other rat's diet consisted of 4% casein.

further complicated by asking which amino acids were synthesized by animals and which had to be in the proteins supplied to the animals. The concern over this set of problems led Osborne and Mendel into extensive studies which resulted in their report of a major discovery in 1914: "We have succeeded in promoting growth at a normal rate when a maintenance ration containing gliadin as the sole protein was supplemented with lysine" (an amino acid).[80] The feeding experiments were conclusive in demonstrating that lysine was absolutely necessary for growth, but not for maintenance.

Additional data gathered in 1913-1914 showed that egg-yolk fat was as effective as butter in promoting growth,[73] as was cod-liver oil.[74]

> Perhaps experiences such as have been reported in this paper will pave the way for a clearer understanding of the physiological potency of natural products like butter, egg yolk, and cod-liver oil, which have long enjoyed a popular, yet inexplicable, reputation for unique nutritive potency."[75]

(Once again, recognition of common sense in terms of centuries of human experiences with various foods.)

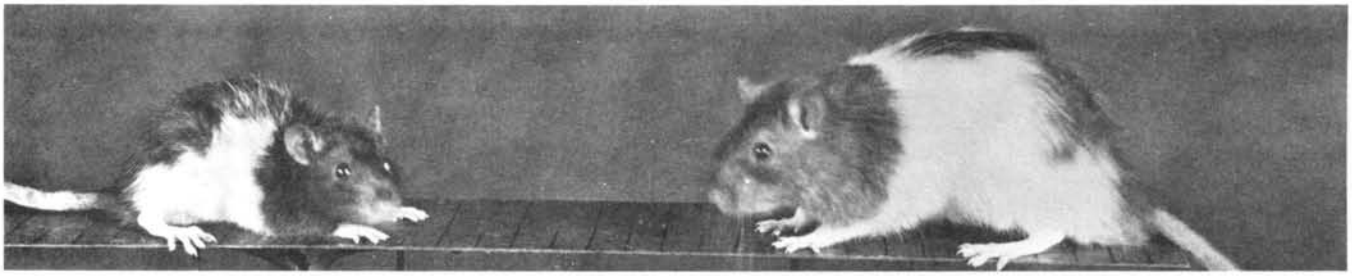


Fig. 12. 1930's. Rat on left had B vitamins obtained from a packaged hot breakfast cereal. Other rat was supplied B vitamins from whole wheat. [Today, most cereals are enriched with vitamins.]

In addition, Osborne and Mendel had confirmed earlier observations that an incomplete protein, zein, when fed as the sole protein in a diet, produced no growth or maintenance. However, by adding the amino acid tryptophane to a zein dietary, experimental rats could be maintained in weight for about 80 days or longer.

The next logical move was to supply lysine to the zein and tryptophane diet. The results, as predicted, were excellent. Rats fed with this mixture grew at a normal rate. If the lysine and tryptophane were removed from the rat's diet, an immediate weight loss occurred. Readdition of the two amino acids brought about resumption of growth.

> These are, we believe, the first successful attempts to grow animals on a diet in which zein forms the sole protein.
>
> The growth of rats on a food of zein, lysine, and tryptophane has not always been as rapid and prolonged as we might expect. We are by no means prepared to maintain that the final solution of the proportion of amino acids requisite for the growth of rats has been determined . . . other adjustments may be required to promote growth in this or different species. The way to successful investigation has been opened."[81]

SUMMARY

Osborne and Mendel's scientific research, deliberately arranged to evaluate one nutrient at a time, clarified and corrected numerous existing ideas about nutrition. The belief that many proteins were nutritionally equal in value turned out to be wrong. The same was found to be the case for fats.

With respect to minerals, Osborne and Mendel contributed extensively to the growing awareness that a large number of minerals, usually in small quantities, were required for normal animal health and growth.

And, while they were slow in recognizing the fact, they ultimately came to the conclusion that certain unidentified organic substances, in trace amounts, were necessary for growth and maintenance in animals—the vitamins.

Their research into amino acid requirements also produced excellent results. Once the functions of lysine and tryptophane were understood, the door was open to evaluation of other amino acids using similar experimental techniques.

Certainly one of the most significant aspects of their work was the recognition that chemical analysis by itself could not determine the nutritional value of a given food. While this was not an original discovery on their part, the meticulous character of their research could only strengthen the concept immeasurably.

The nature and quality of Osborne and Mendel's experimental results left little doubt that the animal feeding experiment was, at the time, the only way to determine dietary adequacy. While such knowledge had been part of cultural history in all parts of the world, Osborne and Mendel, amongst others, changed this common-sense knowledge to a form in which measurable quantitites of known substances could be evaluated for their nutritional properties and functions.

In the final analysis, Osborne and Mendel's experiences demonstrated to anyone who cared to follow (and many did) that there was no adequate substitute for the trial-and-error principle used in animal feeding experiments if one was seeking to understand nutritional metabolism. For decades to come this technique would produce an enormous flood of nutritional data that, in essence, we live with today.

AFTERTHOUGHTS: THE FUTURE

Tonight, acting on a whim, I walked to downtown New Haven and stopped in front of the house where Osborne lived the greater part of his adult life. It is no longer a private home, having gone the route of so many older dwellings in our cities: offices or apartments.

Not far in the distance I can hear the sounds of automobiles, trucks and motorcycles as they pour along two interstate highways. And only a few blocks away is a vitamin and health food store.

It is all very different from July 1913, a hot and sultry month, when Osborne and Mendel were confirming the existence of something in butter that makes rats grow. They both lived long enough to witness the emergence of the vitamin industry and I often wonder what their reactions were to seeing their findings transformed into marketable commodities.

After my sojourn I had dinner—nutritionally balanced, of course—then returned to the experiment station to record these thoughts.

The past has shown us what can be done to improve the human condition. True, some of our discoveries have been to our disadvantage but most have benefitted us in ways of which we are hardly aware. As far as diet is concerned, there probably has never been a time when all humanity had sufficient food to be adequately nourished. Today, the wealth of knowledge makes it possible to hope that the future will witness a balance between population and the food supply.

In the meantime, all about me in the buildings of this institution and in the hundreds, perhaps thousands similar to it, there exist the counterparts of Osborne, Mendel, McCollum, Funk and Hopkins. At this very instant, experiments being carried out by these people are meeting the ultimate test of reality: Do they work?

Amongst the men and women conducting these experiments are a few who will have the skill, the persistence and the luck to see what others have not seen.

The excitement of it all, for me, is knowing *these people are out there struggling to bring ideas to life, while the fun of it is guessing who and what will be in the history books of tomorrow.*

The Connecticut Agricultural Experiment Station
28 July 1976
10:29 P.M. (E.D.T.)

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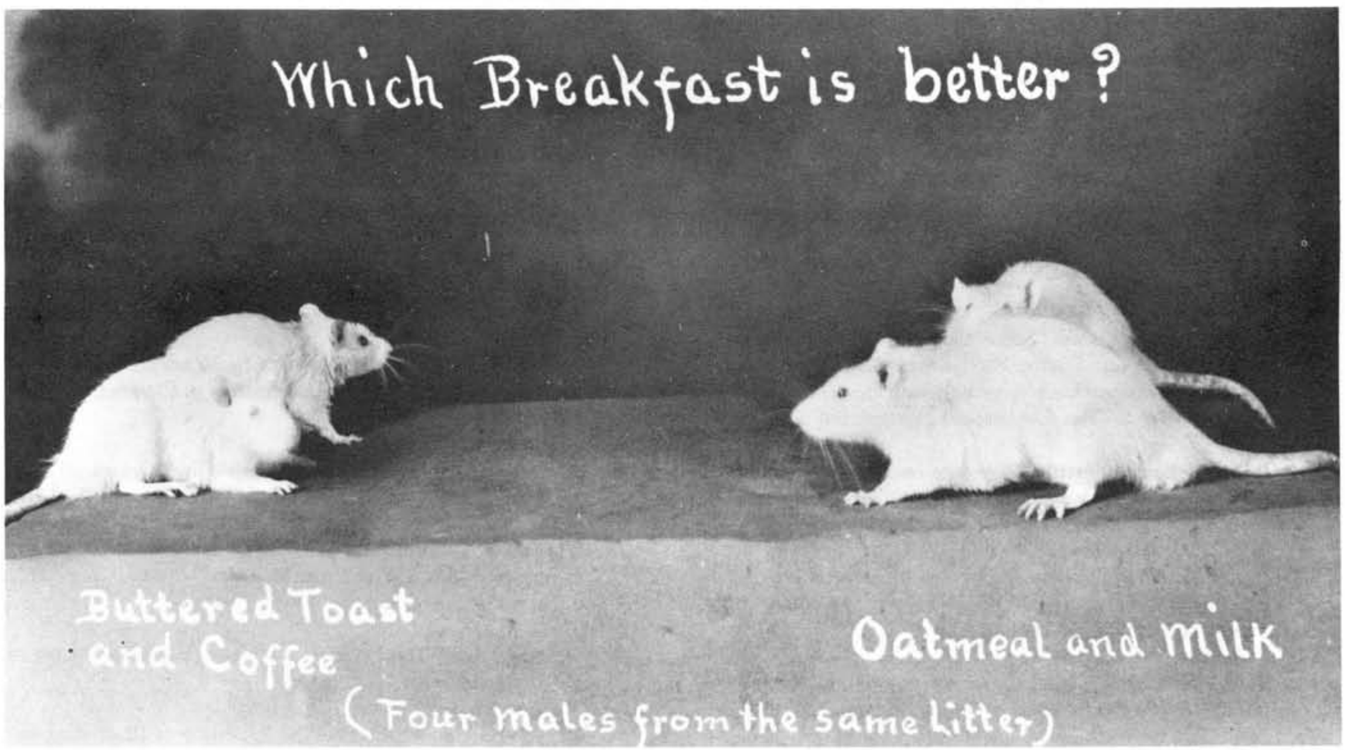

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I wish to thank the Director and staff of The Connecticut Agricultural Experiment Station for making my year's leave from college teaching both an enjoyable and enriching experience and Dr. Ross Gortner for reading the manuscript. I am deeply indebted to the Station's tradition of maintaining an historical record of its accomplishments, for without such a tradition the story presented here could never have been told.

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