THOMAS B. OSBORNE
A MEMORIAL

Connecticut Agricultural Experiment Station
New Haven

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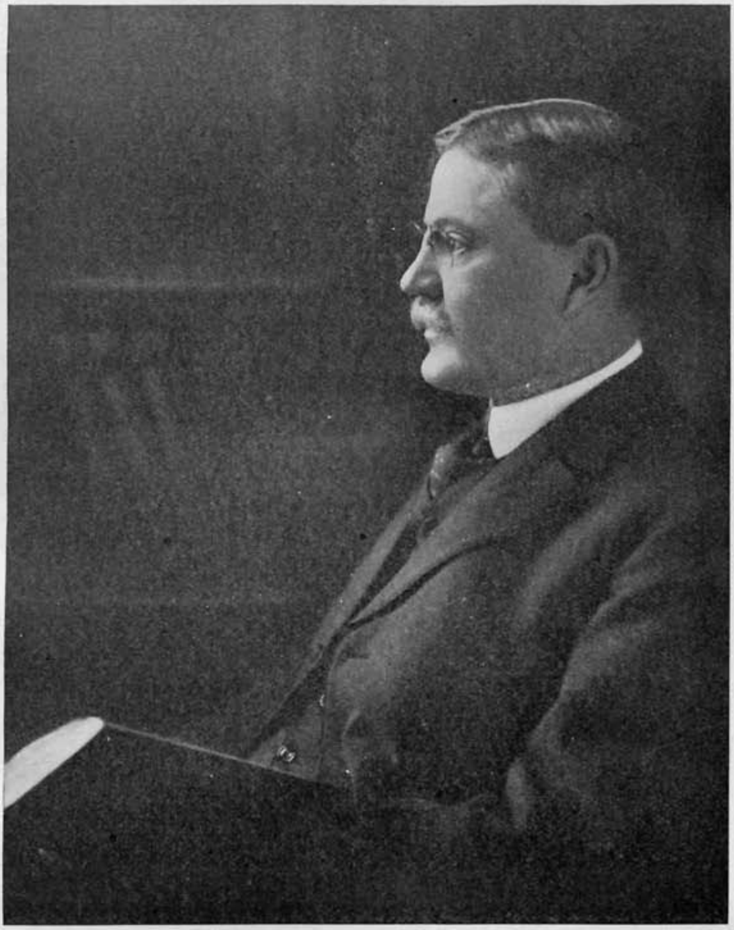

Thomas B. Osborne

FOREWORD

The outstanding contributions of Thomas Burr Osborne to the chemistry and physiology of proteins have brought honor to his name and have reflected lasting credit upon the institution he served so long and so devotedly. It seems fitting therefore that the Station should recognize his distinguished public service by some permanent testimonial of his worth. This bulletin aims to serve as a memorial of him by the colleagues with whom he labored daily, showing also something of his varied interests and activities aside from the problems of his life work, and something of those qualities which compelled the admiration of his associates and won their affection.

The committee gratefully acknowledges the courtesy of those editors who have permitted the reprinting of published articles, and desires to express its indebtedness to Miss Luva Francis who compiled the bibliography.

W. L. S.
E. H. J.
E. M. B.
H. B. V.

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DR. OSBORNE AS AN ASSOCIATE

The facts about Dr. Osborne's inheritance, his education, his long-continued work, its great results and its final public recognition are set forth in the papers which follow. I wish to speak very briefly of some of the personal qualities of the man with whom I was associated in the Agricultural Station for more than forty years.

But it is not easy to speak soberly and without feeling of a companion and loved friend whose devotion to his work and whose dogged perseverance in meeting its difficulties I had watched almost daily for many years and in which I had shared his disappointments and his successes.

The first impression of a stranger meeting him for the discussion of things in which both were engaged would probably be that Osborne was a severe and just critic of his own work and that of others. He made it very clear that he could not accept any scientific statement unless its proof was absolutely clear to him. He hated half truths and "hedging," "for men in earnest have no time to waste in patching fig leaves for the naked truth," but kindly always. He was quick with suggestion and help to his associates, as free and judicious with praise as with criticism. His steady devotion to his work, the originality of his thought and the inspiration of his example raised the tone of the work of all who were his associates in the Station. Each of his assistants was a fellow worker, helped and encouraged by him and shown how his work was an important part of the whole research. Anxious as he was to keep his staff intact, when he saw that one was fitter for other and better paid work, he would tell him and, if he wished, help him in getting a more satisfactory place elsewhere. In times of their disability or illness he was quick to help, using his own means—sometimes largely—if that was needed.

Dr. Osborne had no taste for poetry, the drama or noble prose. He was a realist. He did not find in these things much stimulus for his imagination or charm for his leisure time. But he found both in thinking of the great problem which possessed him, a study of those things which are the basis of all physical life. It was a work which needed not only an expert chemist but a man of great originality, with clear thought on all the possibilities and withal a trained imagination; not "mere delectation meet for a minute's dream" but for the grasp of a vision and then strict testing of its value. He rejoiced in the discovery of a vitamin, but to see the knowledge of it saving the health and life of thousands of children in Russia was worth a lifetime. On the walls of his study hung the pictures of Liebig and Johnson, on his desk was his wife's picture and near by on the other wall was a large diagram of a protein complex as he conceived of it, changed from time to time as his work went forward.

These were his visions. He spent forty years of his life in study and laboratory, devoted to a particular problem, putting aside tempting offers to engage in better paid work elsewhere and the other temptation to exploit his work in popular articles.

Such was his life work, but not his life, which had wider interests.

During his boyhood and youth he was greatly interested in the study of plants, insects and particularly of birds. He was a keen and accurate observer, not only recognizing their names but their nesting, feeding and migrating habits. He collected nearly one thousand specimens, some mounted, but chiefly prepared skins. In C. H. Merriam's Catalogue of the birds of Connecticut acknowledgment is made and there is frequent reference to the valuable contributions of T. B. Osborne. About 1880 his collecting was discontinued, as he was occupied with his chemical studies, and the specimens were given away. But his intimate knowledge of bird life remained a lifelong interest and pleasure to him. While walking with friends and discussing things of mutual interest the flutter of a small bird or a bird song would halt everything until he had seen and identified his friend and interrupter. When plagued with insomnia, as he often was, he would catch in the fall and spring the notes of migrating birds flying high at night and sometimes recognize the species. A love of nature was music and poetry to him.

His interest in the political questions of the day was keen and his knowledge of financial matters made him an active director of the Second National Bank, where he served for years.

But this is not all that specially endeared him to very many who were his intimates or were within his influence. A wholesome clean-minded man, quick, impulsive, generous and broadminded and in all ways companionable; these qualities are a large part of what made the world better for his life and our lives the poorer by his going.

EDWARD H. JENKINS.

THE WORK OF THOMAS BURR OSBORNE (1859-1929)[1]

It is given to few men to begin a scientific career with an investigation in an obscure and unattractive field, to continue their labors in it throughout a long and active life and ultimately to see this field become one of the most fertile and widely cultivated in their particular domain of science. The work of Thomas Burr Osborne on the vegetable proteins, continued from 1889 until his retirement in 1928, furnishes a striking example of a life devoted almost exclusively to scientific research upon a single group of substances and their derivatives. Owing to the diversified relationships of these substances this work has had a profound influence upon many phases of biochemistry.

Dr. Osborne was born in New Haven, Connecticut, on August 5, 1859. He was graduated from Yale University with the degree of B.A. in 1881, and received his doctorate from Yale in 1885. His dissertation was on "The Quantitative Determination of Niobium." From 1883 to 1886 he was an assistant in analytical chemistry at Yale and during this period published several papers dealing with analytical problems.

In May, 1886, at the invitation of Professor Samuel W. Johnson, professor of agricultural chemistry at Yale and director of the Connecticut Agricultural Experiment Station, Dr. Osborne became a member of the station scientific staff, forming a connection he retained until his death on January 29, 1929. Professor Johnson had become interested in Ritthausen's extensive studies of vegetable proteins. He was fully alive to their significance and suggested that further investigation was desirable. Accordingly, Dr. Osborne began in 1888 the labors that continued without interruption until his retirement.

Dr. Osborne's work on the vegetable proteins falls chronologically into three phases. From 1890 to 1901 the chief interest was in the preparation of pure specimens of the proteins of plant seeds. The initial investigation of the oat kernel, published in 1891, was followed by a series of papers in which the proteins from no less than thirty-two different seeds were described. Each of these was prepared, where possible, by a number of different methods; the criterion for purity and individuality was ultimate analysis for carbon, hydrogen, nitrogen and sulphur.

The properties of these substances were such as clearly to show the advantages, for scientific investigation, of the reserve proteins of seeds over the proteins of animal origin. Efforts to isolate proteins of definite properties from the complex mixtures in

[1] Reprinted from *Science*, **69**, 385 (1929) by permission of the editor.

animal tissues had been for the most part unsuccessful, and even as late as 1911 ovalbumin was the only animal protein that had been clearly characterized as a chemical individual. On the other hand, many seed proteins were early shown by Dr. Osborne to be chemical individuals and preparations possessing identical properties were reproducible at any time.

A careful investigation was made of the proteins which had been previously grouped under the terms legumin, conglutin and vitellin, and it was shown that many of the proteins which thus had been brought together were, in fact, distinct substances. Specific designations were, therefore, in many cases coined and the use of the older names was restricted to those proteins to which they had first been applied. This clarification of the nomenclature has been of immense assistance in bringing a semblance of order into an almost hopelessly confused subject.

A few proteins had been previously prepared in crystallized form by other investigators. Dr. Osborne crystallized many of the seed globulins, and the readiness with which this could be done emphasized the fact that these proteins were definite substances entitled to the serious consideration of chemists.

The second phase of Dr. Osborne's work was initiated in 1899 with a paper[2] in which it was shown that the crystalline protein edestin from hemp seed forms two compounds with hydrochloric acid, a mono- and a di-hydrochloride, that the solubility of edestin in acid increases in direct ratio with the amount of acid present and that a number of crystallized vegetable globulins behave as bases neutralizing definite proportions of acid. In other words, the behavior of these proteins was that to be expected of basic substances of fixed composition. This was the end towards which his careful descriptive studies had been directed, a demonstration that proteins were definite chemical individuals. The position here taken was strengthened by later papers in which it was shown that proteins in general behave towards acid like bases, that they form salts both with acids and with alkalies and show many evidences of a capacity to undergo electrolytic dissociation and enter into ionic reactions.

These results emphasized the desirability of more complete chemical characterizations of the different proteins. The development of the methods of analysis of proteins at the hands of Hausmann, of Kossel and of Fischer furnished powerful means for supplying this and full advantage was taken of them. Furthermore, determinations of physical properties such as specific rotation, the heat of combustion and solubility in saline solutions contributed materially to the solution of the problem.

By 1908, when the paper on "The Different Forms of Nitrogen

[2] T. B. Osborne, *J. Am. Chem. Soc.* **21**, 486 (1899).

in Proteins,"[3] perhaps Dr. Osborne's most widely quoted contribution, appeared, data had been accumulated which indicated clearly that few proteins had been obtained that could not be completely characterized by the methods of amino acid analysis, coupled with a study of the physical properties.

Beginning in 1906 and continuing for about six years, Dr. Osborne, with the aid of a number of collaborators, carried out a series of analyses of the amino acid composition of proteins by the Fischer ester distillation method. These studies set a standard for such work which has been surpassed only since the introduction in recent years of greatly improved methods for dealing with certain of the amino acids. Characteristically, he returned again and again to the analysis of a few of the proteins, such as casein, gliadin and zein, which possess special economic importance, each time increasing the summation of the components by the use of more refined technique. These analyses laid the foundation for the extensive studies of the nutritive properties of proteins that were begun in collaboration with Professor Lafayette B. Mendel, of Yale University, in 1909 and continued until 1928. This aspect of protein chemistry had attracted Dr. Osborne's interest from the earlier part of his career; but he had realized that until pure and uniform material could be obtained in abundance and its composition established by chemical analysis, an investigation of the comparative nutritive properties of proteins was useless. The striking differences which now became evident in the composition of many of the proteins suggested that their biological values might be correspondingly unlike.

It may be worth while to point out that in 1911 the notion that proteins might differ widely in nutritive value was relatively new. The chemical methods showed that wide differences in amino acid make-up occurred and, where these failed, the anaphylactogenic relationships which had been studied in collaboration with Professor H. Gideon Wells, of Chicago, emphasized the difference in all save a few remarkable cases. But where wide chemical differences occurred, as between edestin and casein, both of which were found to be adequate for growth, it became necessary to suppose that the animal organism is capable of effecting far more elaborate and extensive chemical transformations than had generally been thought.

The investigation of the nutritive properties of the proteins involved the development of a technique for feeding individual small animals which would permit accurate measurements of the food intake. This was successfully accomplished, but the first experiments in which the pure isolated proteins were fed, together with sugar, starch, lard and an inorganic salt mixture, showed

[3] T. B. Osborne, C. S. Leavenworth and C. A. Brautlecht, *Am. J. Physiol.*, **23**, 180 (1908).

that normal growth of young animals did not take place, although mature animals, as well as young, could be maintained for considerable periods. Growth of young animals could readily be secured when dried whole milk powder was furnished together with starch and lard. This appeared to indicate that milk contained something other than protein essential for growth. The preliminary assumption was made that the missing factor might be supplied by the inorganic constituents of the milk, and it was found that excellent growth could be secured when evaporated milk serum, from which casein and lactalbumin had been removed, the so-called "protein-free milk," was added in sufficient amounts to a diet of isolated protein, starch and lard. With the assistance of this material an extensive investigation revealed wide differences in the alimentation of animals on different proteins. Animals rapidly failed on zein and gelatin, were maintained on hordein, rye and wheat gliadin but grew well on edestin, wheat glutenin, lactalbumin or casein. Further work showed that the failure of animals on a zein diet was due to the lack of tryptophane and lysine in this protein. When these amino acids were supplied growth occurred. Similarly, gliadin could be made adequate for growth by an addition of lysine in which this protein was conspicuously deficient.

The use of protein-free milk in diets was attended by certain difficulties. It is not entirely free from nitrogen and it could not be successfully replaced by an artificial mixture of salts made to imitate the composition of milk ash as closely as possible. Furthermore, animals nourished on this diet over long periods ultimately ceased to grow and declined rapidly in weight. In every case such animals could be brought to a normal rate of growth by changing to a diet containing whole milk powder, and the ultimate failure on protein-free milk could be postponed or averted by feeding whole milk powder for occasional short intervals. An examination of the composition of the two types of food revealed that the most conspicuous difference lay in the presence of milk fat in the dried milk food. Experiment soon showed that the addition of butter to a casein, starch and protein-free milk diet sufficed to permit normal growth to maturity. When butter was added to a diet of dried skim milk upon which it had been found that animals ultimately failed, complete realimentation occurred.

These results were published in 1913. The paper describing them was submitted to the *Journal of Biological Chemistry* about three weeks after a paper by McCollum and Davis in which similar results secured by the use of an ether extract of egg yolk and of butter, were described. The observations indicated that a substance occurs in butter which is essential for animal growth. This substance was later designated as vitamin A.

In the following year the important observation was made that the same stimulation of growth could be secured by the addition of cod-liver oil to a diet of purified food substances and protein-free milk, a discovery which served to focus attention upon the value of this oil, in particular as a curative agent for the peculiar eye condition known as xerophthalmia that was regularly encountered by Osborne and Mendel in animals on the deficient diets. At the close of the war the sight of many children in Europe was preserved by its use, a remarkable example of the application of scientific results to practical problems.

The later extensive contributions of Dr. Osborne and his associates to the science of nutrition can only be indicated. Much labor was devoted to the study of the nutritive value of the proteins of the commercially important foods and this work gave a rational explanation of many practices which empirical experience had shown to be advantageous. The distribution of vitamins A and B in natural food products was studied and considerable success was attained in an effort to prepare a vitamin rich concentrate from yeast. The phenomena of growth, its suppression and acceleration under various regimens, the effect of the individual inorganic constituents of the diet, these and many other topics received attention at different times.

The remarkable influence of minute traces of certain organic substances, the presence or absence of which in the diet determine success or failure of nutrition, drew attention to the importance of an investigation of the constituents of living cells. This led to a detailed study of extracts of the alfalfa plant and of yeast, both of which are valuable sources of vitamins. Much of the information secured did not reach the stage of publication, but a striking demonstration was obtained of the complexity of the chemical environment in which the life process takes place.

It would be incorrect to assume that Dr. Osborne's interest in the fundamental chemistry of proteins waned as he penetrated more deeply into the mysteries of animal nutrition. Innumerable chemical problems arose as a result of the feeding work and demanded solution. Such, for example, was the discovery in 1913 of lysine among the products of hydrolysis of gliadin: its presence had escaped the notice of previous observers, including himself. A study of the constituents of milk in 1917 revealed a new protein soluble in diluted alcohol, the first animal protein possessing this property to be found. Its anaphylactogenic relationships were worked out in collaboration with Professor Wells in 1921, and it was demonstrated to be distinct from the other three proteins of milk.

Dr. Osborne made a fundamental contribution to the chemistry of nucleic acids in 1900, when he announced the discovery of tritico-nucleic acid in the wheat embryo and observed that this substance yielded the purines, guanine and adenine, in molecular proportions. Subsequently he made it clear that the various nucleoproteins which could be prepared from the wheat embryo were in reality salt-like compounds of one and the same protein with variable proportions of nucleic acid. Generalizing from these observations he pointed out that the numerous nucleoproteins from animal sources that had been described were, very probably, also salt-like compounds of protein with nucleic acid.

Although all the preparation work and much of the chemical investigation of the proteins was done before the modern conceptions of acidity had been advanced, Dr. Osborne was aware of the influence of different degrees of acidity on his preparations. One of his early papers on the effect of small amounts of acid on edestin[4] contains the phrase "the concentration of the hydrogen ions in the solution," and it was his custom invariably to state the indicator which he used. It was not sufficient to neutralize a solution; the solution was neutralized to phenolphthalein, or litmus, or tropeolin, as the case might be, and the differences in behavior so indicated were fully appreciated. It is this meticulous attention to detail which gives Dr. Osborne's early work a value to the present-day physical chemist and renders it possible to furnish an interpretation in terms of modern theory, as has recently been done by Cohn.

Dr. Osborne was one of the most distinguished pupils of Professor S. W. Johnson, and through him traced his intellectual ancestry back to Liebig, the founder of agricultural chemistry. A painstaking, careful investigator who spared no effort, time or expense in the attainment of the truth, Dr. Osborne accepted no result until it had been subjected to the test of rigorous and repeated experiment and all his publications bear the marks of meticulous editing, lest a statement should to the slightest extent pass the bounds of ascertained fact.

To those who were privileged to be associated with him in his work he was a rare stimulus, a formidable opponent in argument and an ever genial but just critic. He frequently closed a discussion with the remark that facts were to be found in the laboratory, not in the books. Naturally shy and retiring, the delivery of a public address or of a paper was a severe trial to which he looked forward with trepidation. But among a small group of friends he showed himself as a gifted conversationalist, who was equally able to discuss the latest achievements of science, the current political situation, the intricacies of the world of finance or the faults of the modern educational system.

The first public recognition of Dr. Osborne's exhaustive work came from Germany. V. Griessmayer, in 1897, published a book

[4] T. B. Osborne, *Z. physiol. Chem.*, **33**, 225 (1901).

on vegetable proteins that contained many extracts from Dr. Osborne's papers and stated in the introduction that it was his object "to bring to light these treasures buried in their American publications." This encouragement at a time when few of his associates or scientific friends had any conception of what his work meant, was of great assistance to him.

In 1900 he was awarded a gold medal by the Paris Exposition. In 1910 recognition came from Yale University in the form of the honorary degree of Sc.D., and in the same year he was elected a member of the National Academy of Sciences. Two years later he was made an honorary fellow of the London Chemical Society, and in 1914 he was made a fellow of the American Academy of Arts and Sciences.

In 1922 he received the John Scott medal and in the following year was made a research associate in biochemistry of Yale University with professorial rank. In 1928 he was the first to receive the Thomas Burr Osborne gold medal founded by the American Association of Cereal Chemists in recognition of his outstanding contributions to cereal chemistry.

Dr. Osborne's extensive investigations would have been impossible without generous financial support and encouragement. Throughout the early years, when results came slowly and their application was by no means apparent, the directors of the Connecticut Agricultural Experiment Station, in the early years Professor S. W. Johnson, and after 1900, Dr. E. H. Jenkins, with the coöperation of an enlightened board of control, allowed no interference or distraction to hinder the progress of the work. Since 1904 a large proportion of the financial burden has been borne by the Carnegie Institution of Washington, D. C., of which he was a research associate. Dr. Osborne's connections with both the experiment station and the Carnegie Institution of Washington furnish a striking example of the value to science of a policy of non-interference on the part of those in control of the distribution of funds for research. Except for routine annual reports he was never asked for statements of progress or for outlines of projects. The relationship was always one of the utmost mutual confidence and esteem.

The results of Dr. Osborne's investigations were summarized in a monograph, "The Vegetable Proteins," which first appeared in 1909 and was extensively revised in 1924. This slim volume has become the classical publication in the field. His extensive studies of wheat proteins were reviewed in "The Proteins of the Wheat Kernel" (1907), now a standard text among cereal chemists. Including these and a few public addresses and popular articles a complete bibliography of his publications reaches 253 titles, of which about two hundred are journal reports of his personal scientific work.

Dr. Osborne's most marked characteristic was, perhaps, the thoroughness with which his problems were investigated. In the early preparation work each protein was isolated in as many different ways as possible, the composition finally ascribed to it was deduced from a large number of carefully conducted analyses and, where the economic importance of the protein warranted it, he returned again and again to its study. The wheat and maize prolamins received extraordinary attention and the methods of preparing even these well-known substances were recently, with the aid of his assistants, materially improved. Time and again he discarded the whole of his painfully acquired results to make a fresh start, this time to "do it right," as he expressed it. His death removes one of the great pioneers of American biochemistry, a man whose name will always be linked with the subject he made peculiarly his own. He was more fortunate than most men in that advancing years, distinctions and scientific recognition did not bring with them administrative responsibilities that deprived him of the opportunity to share in the daily work of the laboratory. His time was always freely available for discussion, not only with his associates, but with the innumerable investigators from all parts of the world who came to New Haven to see him and ask for advice. Ever kindly and courteous, with keen insight into the problems of others and an extraordinary wealth of experience upon which to form his judgments, he has left a memory that will long be treasured by those who had the privilege of knowing him.

HUBERT B. VICKERY,
LAFAYETTE B. MENDEL.

SELECTED PAPERS OF DR. OSBORNE

OUR PRESENT KNOWLEDGE OF PLANT PROTEINS[1]

To the biological chemist few substances present so many features of interest as the proteins of plants. These lie at the very foundation of the nutrition not only of plants but of animals, and from them are derived a multitude of products directly connected with physiological processes. The study of chemistry of plant proteins, although it early interested several of the leading chemists of their time, has received in the aggregate so little attention that to-day our knowledge of this subject is but slightly advanced beyond what may properly be called a beginning. How slow the progress has been may be shown by a brief review of the literature that is on record.

HISTORICAL

In 1746, Beccari announced his discovery of a peculiar substance which he obtained by washing wheat flour with water, that had all the properties which up to that time had been considered to be characteristic of animal life only. This substance, which we now know to be wheat gluten, appears to have been for more than fifty years the only form of vegetable protein that was known, for Beccari failed to obtain similar products from other seeds. In 1805, Einhof discovered that a part of the gluten of wheat was soluble in alcohol, and he described the existence of similar proteins in rye and barley. Einhof overlooked the fact that only a part of the gluten of wheat was dissolved by alcohol, and he considered this property to be characteristic of all plant proteins except the "Eiweiss," which he obtained by heating the aqueous extracts of seeds and other parts of plants. When, therefore, he later discovered in leguminous seeds another form of protein which was not soluble in alcohol, but had in pronounced degree the properties of "animal matter," he assumed that he had obtained a substance belonging to a distinctly different group but yet related to the gluten or "Kleber" that had been found in other seeds.

Taddei, in 1820, showed that only a part of the wheat gluten was soluble in alcohol and he applied distinctive names to each part; gliadin to the substance soluble, and zymom to that insoluble, in alcohol.

From this time chemists were more and more attracted to the

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[1] An address delivered before the American Chemical Society at New Haven, Conn., July 1, 1908. Reprinted from *Science*, **28**, 417 (1908) by permission of the editor.

study of vegetable proteins, and among those thus engaged are found many of the most distinguished chemists of the earlier part of the last century, such as Berzelius, Dumas, De Saussure, Boussingault, Liebig and many of his pupils.

In 1841, Liebig reviewed the work done in his and other laboratories on the properties and composition of plant proteins. The state of knowledge which then prevailed respecting this subject is well illustrated by the following quotation from his review:

> Another, in number very limited, class of nitrogenous compounds is very abundantly distributed. There are four of these substances, of which one occurs, without exception, in all plants, while the others are only constituents of certain families of plants. These are the nitrogenous food substances properly known under the names of Vegetable "Eiweiss," Pflanzenleim and legumin. . . . These substances, to which a fourth must be added, which I will name Pflanzenfibrin, are the true food substances of the plant-eating animals.

In discussing these four proteins, Liebig asserted that each was identical with the protein of animal origin bearing the corresponding name. The identity of legumin with milk casein was claimed and this protein he therefore named plant casein.

The work undertaken by Liebig was continued for twenty years or more by Ritthausen, who was one of his pupils. Ritthausen, in 1860, began the first serious study of these important substances and devoted much time and care to the production of preparations of the highest attainable purity, and to accurate determinations of their ultimate composition. His work greatly extended the scope of the prevailing knowledge of the plant proteins, and made it plain that these substances exist in much more diverse forms than had before been supposed. He also added much to our knowledge of the decomposition products of vegetable proteins by showing that they yielded many substances already obtained from proteins of animal origin, and discovered glutaminic acid which is now recognized as a constitutent of practically all proteins, whatever their origin. He was also the first to obtain aspartic acid from the products of protein hydrolysis.

In 1877 Hoppe-Seyler and his pupil Weyl applied to seeds the then recently developed method of extraction by solutions of neutral salts. They showed that a large part of the protein of a number of different seeds was soluble in such solutions, and had the properties of the so-called globulins of animal origin. While the experimental work of these investigators was hardly more than qualitative and of very superficial character, the conclusions which they drew and the criticisms of Ritthausen's work which they put forth were generally accepted as final by most physiologists, and threw it into general discredit.

Although Ritthausen afterwards showed that a large part of many of his previously described preparations, which had been obtained by extraction with dilute alkalies, was soluble in solutions of neutral salts, and that the composition of many of the proteins which he had previously analyzed was the same as that of preparations obtained by extraction with solutions of sodium chloride, nevertheless physiologists continued to repeat the criticisms of Hoppe-Seyler, and the work of Ritthausen failed to receive the recognition which it deserved.

REVIEW OF THE WRITER'S WORK

Since Ritthausen ceased his work with vegetable proteins little has been done in this field outside of my own laboratory. It is true that from time to time papers have appeared dealing with special questions in the chemistry of these substances, but no other connected and extensive investigation has been described, and as the work which I have been doing during the past twenty years has now reached a point where it can be profitably reviewed, I propose to take up some of its more important features and briefly discuss them.

As Ritthausen's researches were far from exhaustive and left the subject in such a state of confusion that it was impossible to form definite conclusions respecting much that he had described, it seemed best to me to direct attention chiefly to those seeds which had been previously studied by him and by others, and to try to clear up the existing uncertainties, rather than to add to them by describing new proteins. As a result, we now have about twenty-five different proteins of vegetable origin, the important characters of most of which have been studied by all means at present available. These proteins appear to represent the different types to be found in seeds and are, I think, sufficient in number to form a suitable foundation for the future study of their chemistry. All of these are constituents of seeds. A few of them represent constituents of the physiologically active embryo, but the majority represent the reserve food protein of the endosperm, and serve not only for the nutrition of the growing seedling, but also for the nutrition of men and animals. Of the protein constituents of other parts of plants very little indeed is known.

CHEMICAL INDIVIDUALITY OF PROTEINS

In considering the position of our present knowledge of the seed proteins, the question of chemical individuality should first be considered. We are now well past the time when agreement in solubility, ultimate composition and color reactions, are to be accepted as evidence of the identity of two preparations of protein. It is not necessary to explain why it is at present not possible to demonstrate the chemical individuality of any single protein, for the reasons are evident to all who will give this question the slightest consideration from the standpoint of the organic chemist. While it is not possible to establish the individuality of any protein, it is possible to show differences between the various forms which can be isolated, and to establish a constancy of properties and ultimate composition between successive fractional precipitations which give no reason for believing the substance to be a mixture of two or more individuals.

On the basis that agreement in ultimate composition affords no evidence of identity of two similar proteins, but that distinct and constant differences in composition are conclusive evidence that they are not alike, I have endeavored to differentiate the several seed proteins that I have studied, and have since subjected them to careful comparisons in respect to their physical properties and the proportion of their decomposition products, so that those which are alike in their more apparent characters have been still further distinguished from one another. Whether these are in fact chemical individuals, must await the development of new methods of study. For the present they must be accepted as the simplest units with which we can deal.

SUITABILITY OF SEED PROTEINS FOR A STUDY OF PROTEIN CHEMISTRY

The various proteins thus established furnish material for further study, and are characterized by wide differences not only in physical properties, but in the proportion of their decomposition products. They can be prepared in large quantity in a high state of purity, and, being a part of the reserve food stored up for the nutrition of the developing embryo, are by nature more stable than the animal proteins which form a part of physiologically active tissues. Furthermore, they are not associated with tissues and fluids rich in other forms of protein from which they are to be separated, and they are mostly obtained in the form of dense precipitates, often crystalline, which are little inclined to adsorb other substances from which they can afterwards be separated with difficulty. Although associated intimately in the seed with many forms of soluble and insoluble carbohydrates, they can, in many cases, be separated from every trace of the latter, as is shown by appropriate reactions.

It is my firm belief that a careful examination of them will ultimately afford a better knowledge of the chemistry of proteins in general than can be obtained from proteins of animal origin. Although the problems immediately connected with the animal proteins are of greater importance to physiology than those at present recognized as connected with seed proteins, there is no question but that definite knowledge of the chemistry of seed proteins will be directly applicable to many important problems of animal physiology.

THE DIFFERENT GROUPS OF PROTEINS FOUND IN SEEDS

The seed proteins for the most part can be grouped in much the same way as the proteins of animal origin, but in so doing, it is necessary to modify to some extent the requirements to which the animal proteins belonging to some of these groups are at present assumed to conform. The necessity of some scheme of classification for the proteins is recognized by all who write or teach about them, and although the present method of classifying proteins according to their solubility is wholly unsatisfactory from a purely chemical standpoint, it is practically the only one now available. On chemical grounds there is no more reason for dividing the proteins into two groups of animal and vegetable proteins, than there is for making a similar distinction between the carbohydrates. I have, therefore, endeavored to assign the various forms of seed proteins to the commonly recognized groups established for those of animal origin, and have proposed to slightly modify the definitions usually given for these groups, but only so far as this is necessary.

1. *Globulins* form much the greater part of the reserve protein of all seeds except those of the cereals. By globulin is meant protein soluble in solutions of neutral salts but insoluble in water. This definition does not strictly apply to many of the seed proteins assigned to this group, for these behave as globulins only under certain conditions. As these conditions prevail during the extraction and isolation of these proteins and depend on the presence of free acid in the extracts, it is important to consider the relations of the proteins to this acid.

The behavior of seed globulins toward acid is shown by studies made in my laboratory on edestin. Crystallized preparations of edestin, obtained by dialyzing or cooling sodium chloride extracts of hemp-seed, frequently contain protein in three forms; one, soluble in pure water and also in strong saline solutions, another, insoluble in water but soluble in strong saline solutions, and still another, insoluble either in water or in saline solutions. The proportion of these products varies with slight differences in the conditions under which the edestin is isolated, and plainly depends upon changes in the protein which take place during its preparation.

The explanation of these changes has been found in the presence of a small amount of acid extracted from the seed together with the protein. The part of the edestin preparation just referred to, which is insoluble in neutral saline solutions, has been found to be a product of the hydrolytic action of the acids of the extracts. This product is not the result of a profound splitting of the edestin molecule, for the changes leading to its formation are so slight that they can be detected only by the altered solubility. For such primary products of protein hydrolysis, which were designated "albuminates" by Weyl, I have proposed the name "protean" and for the products derived from the individual proteins, a corresponding name ending with the affix *an*. Thus the product derived from edestin may be called *edestan*. The part of the edestin above referred to which is soluble in water contains more combined acid than the part insoluble therein. The preparation, therefore, contains a mixture of salts of edestin. These edestin salts contain some of all the anions present in the solution at the time of precipitation, that salt being predominant whose free anion was most abundant in the solution from which the edestin was last precipitated. When freed from this combined acid by making the preparation neutral to phenolphthalein, the edestin is wholly insoluble in water, but soluble in neutral saline solutions. Edestin has, consequently, the properties of a true globulin. Other seed proteins behave towards acids in a similar way, except that some of them, when neutral to phenolphthalein, are soluble in water. Many of these latter behave as globulins when in the form of salts, and as they are obtained as salts by the methods employed in preparing them, I have, for convenience, placed them among the globulins.

The fact that our protein preparations, as usually obtained, are protein salts is fundamental for a correct conception of their behavior under the conditions of isolation and purification, and for an explanation of many of their physiological relations.

The seed proteins which are described as globulins differ in some of their properties from some of those that are commonly assumed to characterize the globulins of animal origin. In this connection, however, the fact should not be overlooked that our knowledge of animal globulins is relatively small, and it is probable that further study will modify our present conception of them. It has become customary for physiologists to consider that all *globulins* are precipitated by adding to their solutions an equal volume of a saturated solution of ammonium sulphate, and of recent years it appears to have become an almost universal practise to designate as globulin all of the protein that can be thus precipitated. This practise is unfortunate and leads to confusion, for it wholly ignores the original conception of a globulin, namely, a protein soluble in neutral saline solutions but insoluble in water.

Globulins are commonly described as proteins that are coagulated by heat. This is doubtless true of the globulins of seeds if sufficient acid is present in their solutions. It is, however, difficult to add to the saline solutions of most seed globulins a sufficient amount of acid to cause complete coagulation on heating, for even a very minute quantity of acid in a strong saline solution alone precipitates a large quantity of the globulin.

The deportment of edestin in this respect is well illustrated by the experiments of Chittenden and Mendel,[2] who showed that a saline solution of edestin was only partly coagulated by boiling,

[2] Chittenden and Mendel, *J. Physiol.*, 17, 48 (1894).

that the edestin remaining in solution could be recovered unchanged and in well-formed crystals, and that addition of acid to the solution filtered from the coagulum gave rise to a new coagulum on again heating.

2. *Prolamins*[3] form a unique and sharply differentiated group of proteins which occur in quantity in the seeds of cereals, but not in those of any other plant yet examined. These are soluble in all proportions in alcohol of 70-80 per cent., and are not affected by boiling their alcoholic solutions, even for a long time. They are practically insoluble in water and saline solutions, but are soluble in dilute solutions of acids and alkalis.

The prolamins are better characterized, from a chemical standpoint, than any of the other groups of seed proteins, for on hydrolysis they all yield a very small amount of arginine and histidine and no lysine whatever. On the other hand, they yield from 20 to 30 per cent. of their total nitrogen in the form of ammonia and also contain relatively large amounts of glutaminic acid. Gliadin from wheat and rye, and the related hordein from barley, yield about 37 per cent. of glutaminic acid, which is very much more than that found in any other protein yet examined, and zein yields nearly 20 per cent., which places it among the proteins relatively rich in this amino-acid.

Prolamins have been found in the seeds of all the cereals examined in my laboratory, namely—oats, wheat, maize, rye, barley and sorghum. That this form of protein is characteristic of the seeds of all grasses is rendered improbable by the recent report of Rosenheim and Kajiura,[4] who found none in rice. A detailed statement of their results, however, has not, to my knowledge, yet been published.

3. *Glutelins* constitute a large part of the proteins of all cereals that have been studied, and possibly occur in seeds of other kinds. These proteins are insoluble in all known neutral solvents, but are easily dissolved by very dilute acids or alkalis. Only one member of this group is known which is accessible to satisfactory investigation. This is the glutenin of wheat which forms nearly one-half of the gluten. Owing to the fact that glutenin can be separated from the other components of the seed as a constituent of the gluten, it is possible to make preparations of it of a fair degree of purity.

As the seeds of the other cereals yield no coherent gluten, the

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[3] I propose this name for the group which heretofore has been simply called alcohol-soluble proteins. The name refers to the relatively large proportion of proline and amide nitrogen which they yield on hydrolysis. The English committee on protein nomenclature has very recently proposed to call these proteins gliadins, but as this name has long been used to specifically designate the prolamin obtained from wheat it seems to me important to have a distinctive name for this group.

[4] Rosenheim and Kajiura, *J. Physiol.*, **36**, liv (1903).

protein corresponding to glutenin can not be isolated from them, for the alkaline extracts of these seeds are too gummy to filter and the small amount of the preparations that can be obtained is very impure.

Whether glutelins are constituents of other seeds is a question not yet settled. Most seeds, when exhausted with the several neutral solvents, still contain a small amount of protein which can be extracted with alkaline solutions. It has not yet been definitely determined whether these products are residues of the other proteins extracted by the neutral solvents, or are actually different substances. It is quite conceivable that a part of these other proteins may form combinations with other constituents of the seeds which are not soluble in neutral solvents but are extracted by alkalies, and also that a part of the protein is enclosed in tissues which are dissolved by the alkali and the protein then brought into solution. It is also possible that much or all of this protein is the result of a change, whereby a part of the protein originally soluble in neutral solvents is converted into less soluble products, such as those which have been designated as proteans. The quantity of nitrogen which remains in the thoroughly extracted residues of the seeds is small in the case of most seeds other than the cereals, and it is not probable that many of these contain much, if any, protein belonging to the glutelin group.

4. *Albumins* are probably present in *very* small amount in nearly all seeds, and in none of those that I have examined are they present in large amount. The albumin is probably a part of the physiologically active embryo, and resembles the proteins of animal origin in properties, ultimate composition and proportion of the various products of hydrolysis, more closely than do most of the reserve proteins of the endosperm. While the albumins of seeds are like those obtained from animals in the essential properties of solubility in water and coagulability by heat, they differ in their precipitation relations towards strong solutions of inorganic salts.

It is at present almost universally assumed that albumins are not precipitated by adding to their solutions an equal volume of a saturated solution of ammonium sulphate, but this is not the case with all of the albumins from seeds. Many of them are also precipitated by saturating their solutions with sodium chloride or with magnesium sulphate, in which respect they differ from animal albumins.

5. *Proteoses* similar to those of animal origin have been obtained from all the seeds examined, and from some, proteoses closely resembling hetero-, deutero- and proto-proteose were separated. The amount of such proteose is small in all the seeds which have come under my observation, and it is possible that all of this is formed by enzyme action during isolation of the other proteins of the seeds.

There are several groups of proteins which occur in animal tissues, which have not yet been proved to exist in plants. The most important of these are those which contain phosphorus. In the yolk of eggs and in the milk of mammals, a large part of the protein which nourishes the developing animal contains phosphorus which appears to be intimately concerned in the structure of its molecule. No similar phosphorus-containing proteins have been found in seeds, although by analogy such might be expected to occur. It has been asserted that such proteins are found in leguminous seeds, but an examination of the literature will show this assertion to be founded on very little experimental evidence. Czapek, in his "Biochemistry of Plants," describes the proteins of seeds as vitellins which doubtless contain phosphorus. This view, however, has no evidence to support it and is quite incorrect.

The existence of nucleoproteins, that is of compounds of nucleic acid with protein, is a different question and involves consideration of the chemical nature of these substances as now described. The nucleoproteins are, as far as I know, always described as phosphorus-containing proteins, and the phosphorus seems to be generally considered as a constituent of their molecules. This, of course, is strictly true even if the combination be only that of a base and an acid, but from the standpoint of protein chemistry it makes a great deal of difference whether this union is that of a salt, or one in which the phosphorus-containing groups are in intimate organic combination within the protein molecule. That true nucleic acids exist abundantly in seeds has been demonstrated by investigations made in my laboratory on the wheat embryo. In these investigations a great deal of attention was devoted to the protein compounds of the nucleic acid, but all the evidence obtained indicated very plainly that these were simply protein nucleates, the composition of which depended solely on the conditions prevailing at the time of precipitation. That *similar* combinations exist within the embryo is practically certain, but that any of the combinations actually isolated were *identical* with the combinations that exist in the seed, I consider highly improbable. Whether the nucleoproteins that have been described from animal tissues are more definite and intimate combinations between the nucleic acid and the protein than are the protein nucleates just mentioned, I am not prepared to say, but I think that nucleoproteins deserve more consideration from this point of view than they have received.

Whether, or not, true glycoproteins are contained in seeds, remains to be demonstrated. That a large part of the seed proteins are entirely free from any carbohydrate yielding group, is proved conclusively by the fact that these yield no trace of the Molisch reaction. That those that give the Molisch reaction contain a carbohydrate group as a constituent of their molecule is seriously to be questioned, for it is not possible to obtain even traces of furfurol from them.

If one considers how small an admixture of carbohydrate gives a very strong reaction with the Molisch test, it may well be asked whether this reaction is not sometimes caused by a slight contamination. The possibility of this is so great in the preparations of proteins extracted from seeds containing a great variety of carbohydrates, glucosides and nucleic acids, that conclusions drawn from the results of the Molisch reaction have value only when this turns out negatively.

In plants no representative has yet been found of the group of albuminoids which form in animals so large a part of the skeleton, connective tissues, the skin and its appendages.

Although the nucleated cells of the wheat embryo are rich in nucleic acid which closely resembles in its properties and structure the nucleic acids obtained from the nucleated cells of animals, no substances have yet been found in plants which resemble the protamines which, in combination with nucleic acid, occur so abundantly in the spermatozoa cells of animals. Such substances are to be sought in the pollen cells of plants, but as yet no attempt has been made to isolate them, owing to the difficulty of obtaining a sufficient supply of material, and the fact that the nucleus of the cell forms so small a part of the whole structure.

PRODUCTS OF HYDROLYSIS OF SEED PROTEINS

Of the known primary products of protein hydrolysis all but one (diamino-trioxydodecanic acid, as yet obtained only from casein) have been isolated from seed proteins, and there is no indication that any essential difference exists in the general character of the structure of the proteins from these two forms of life. Some of the seed proteins, like some of those from animals, lack one or more of the amino-acids; and zein, from maize, lacks glycocoll, tryptophane and lysine. The crystalline globulins, which are possibly more definite chemical individuals, have yielded on hydrolysis as complicated a mixture of amino-acids as any of the amorphous preparations. These, therefore, furnish no ground for the assumption that the several proteins, as we now know them, are mixtures of less complex substances.

Of twenty-three different seed proteins which have been hydrolyzed, all have yielded leucine, proline, phenylalanine, aspartic acid, glutaminic acid, tyrosine, histidine, arginine and ammonia. Five have yielded no glycocoll. Two yielded no alanine which could be positively identified, but did yield impure products which left little doubt but that this amino-acid was present. Four yielded no lysine, and one, no tryptophane. Four of these proteins yielded extremely small quantities of cystine, three others, none. The remaining sixteen have not yet been examined for this amino-acid on account of the difficulties encountered in separating small quantities of it. No attempt has yet been made to isolate isoleucine. If this amino-acid is yielded by the seed proteins it will probably be found in the mixture of undetermined substances from the third fraction of the esters, which has not been converted into products suitable to weigh. Glycocoll, lysine and tryptophane are the only amino-acids that have been proved lacking in any one of these proteins.

In respect to the quantitative relations of the amino-acids, the fact must not be overlooked that the determinations of many of them are to be regarded only as approximations to the amounts actually yielded by the protein. The determinations of the mon-amino-acids by Fischer's method are doubtless comparable within certain limits, if sufficient care is exercised in conducting the analysis. The quantities of these amino-acids recovered are unquestionably less than those actually present, for esterification is never complete and the losses incident to the separations by fractional crystallization are not inconsiderable. Under uniform conditions, however, losses are nearly uniform and the figures representing the quantities of amino-acids found give a good idea of differences and similarities between the different proteins. Such figures are in most cases comparable to within perhaps one per cent. of the protein, and probably represent about seventy-five per cent. of the total quantity of the amino-acids which are determined that were originally formed by hydrolysis, providing that these amino-acids are first subjected to two well-conducted esterifications. Most uncertainty attaches to the results obtained for valine and serine, which are separated from associated substances with such difficulty that the determinations of them must be regarded as simply qualitative. Alanine also is difficult to separate in a condition fit for weighing, and no importance is to be attached to differences in the amount of this amino-acid unless these are pronounced.

A very extensive experience, however, with determinations, of arginine, histidine and lysine has convinced me that it is possible to make quantitative determinations of these bases which are very accurate. The results of these determinations can be controlled by comparing the amount of nitrogen contained in the quantities found with that precipitated by phosphotungstic acid under definite conditions which have been worked out in my laboratory. The amount of ammonia yielded by hydrolysis can be determined with such accuracy that differences of only a very few hundredths of a per cent. occur between determinations made on different preparations of one and the same protein. These four determinations are the most reliable that we now have for comparing proteins with one another, and make it possible to detect differences between them which would otherwise escape notice.

Glutaminic acid can be determined in most cases with a close approximation to its true amount, but there are some proteins, especially those from leguminous seeds, from which it is not easily obtained. Experience has shown that by the ester method alone about seventy-five per cent. as much glutaminic acid is usually obtained as by direct separation as the hydrochloride. It is possible, therefore, to control to a certain extent the results of direct determinations by comparing them with those obtained on a larger scale by the ester method.

Although the methods available for thus quantitatively analyzing the products of protein hydrolysis leave much to be desired, it must not be forgotten that only recently have we been able to make any comparison whatever of the proportion of these products.

A striking feature of these analyses, to which Professor Chittenden directed attention in his address before this society last January, is that the total quantity of the substances determined falls far short of one hundred per cent. The majority of successful analyses foot up between sixty and seventy per cent., and of this a part is made up of water which has been introduced by hydrolysis. Calculation shows this amount of water to be approximately equivalent to the losses that may be assumed to occur through incomplete esterification and separation of the acids, so that the summation of well-conducted analyses may be taken as representing somewhere near the total quantity made up by all the different substances determined.

Nothing is known of the undetermined residue which forms from twenty-five to thirty-five per cent. of the protein. There is no reason to believe that, in the seed proteins, undetermined carbohydrate forms any part of this, for those proteins which give no Molisch reaction give no higher summation than do those that do.

If the amount of nitrogen contained in the quantities of the amino-acids stated in the analyses is subtracted from the total nitrogen of the protein, it is found that this undetermined nitrogen forms about fourteen per cent. of the undetermined part of the protein. This is a higher proportion of nitrogen than is found in any of the monamino-acids that are known to be yielded by proteins, except glycocoll, alanine, serine and tryptophane, even if the proportion of nitrogen is calculated for them as united with one another in polypeptide union. It is improbable that this undetermined residue is made up of these four amino-acids, and we may expect to find still undiscovered substances among the protein decomposition products.

COMPARISON OF THE PROTEINS OF DIFFERENT SEEDS

The results of this comparative study of the seed proteins shows that no two seeds are alike in respect to their protein constituents. Similar proteins are found only in seeds that are botanically closely related.

The cereals are alike in the proportion and general character of their proteins. The seeds of each of these, with the probable exception of those of rice, contain a small amount of proteose, albumin and globulin, and relatively considerable quantities of prolamin soluble in alcohol, and of glutelin insoluble in neutral solvents. With the exception of the nearly related wheat and rye, the proteins soluble in alcohol from each of the cereals are distinct substances. Although no certain difference has yet been detected between the gliadin of wheat and of rye, their glutelins are not alike.

The leguminous seeds are similar in the general character of their proteins, but marked differences exist between the proteins of the various groups. Thus *Lupinus, Vicia* and *Phaseolus* present marked differences in their proteins, whereas the proteins of the species of each genus are very much alike. The proteins of *Lupinus luteus* and of *Lupinus angustifolia* differ slightly but in their physical properties are clearly distinguished from any of the other seed proteins. Although similar proteins are obtained from the horse bean, lentil, pea and vetch, these are distinctly different from the proteins obtained from other leguminous seeds. These seeds are not alike, however, in the proportion of their several proteins. The chief protein of *Phaseolus vulgaris* appears to be identical with that of *Phaseolus radiatus,* but the small amount of other protein was found to be different in properties and composition in each of these seeds.

The cow pea (*Vigna*) and soy bean (*Glycine*) contain distinctly different proteins which, however, are similar to but different from those of *Vicia.* The globulins of the seeds of *Corylus* and *Juglans* are much alike, but not identical, while those from *Juglans regia, nigra* and *cinerea,* so far as they have been compared, show no differences. The proteins of other seeds show marked differences, but the botanical relations of these seeds are not such as to permit of further discussion of this subject.

Although the data for generalizations are as yet few, those that are available plainly indicate a close connection between the chemical constitution of the seed proteins and the biological relations of the plants producing them.

That similar differences exist between homologous proteins of different species of animals is becoming evident from the facts which are gradually accumulating, and these strongly suggest a chemical basis for the multitude of diverse forms of animal and vegetable life.

THE CHEMISTRY OF THE PROTEINS[1]

The recent advances made in our knowledge of the chemistry of the proteins have attracted wide attention and excited much interest on account of the important connection which they have with many of the fundamental problems of physiology and biochemistry. These newer discoveries have been the subject of many excellent reviews and are now familiar to most of those who are interested in the various branches of biology. On the other hand, there are aspects of protein chemistry which have received but little notice during recent years, although they have an important bearing on the application of these discoveries to physiological problems. These seem well worth bringing before you this evening.

Attention has of late been largely centred on the salient features of the constitution of the proteins as revealed by the brilliant work of Fischer and of Kossel, and many elaborate attempts have been made to apply the ideas suggested by their discoveries. These have at last given us a conception of the constitution of these substances which, while far from complete, has been of the greatest help in dealing with chemical and physiological problems in which the proteins are involved.

The two most important of these new discoveries in protein chemistry are that the proteins consist essentially of combinations of amino-acids joined with each other by a union between the carboxyl group of one with the amino group of another, and that many of the various forms of protein differ widely in their constitution. That the proteins are composed, at least in large part, of combinations of amino-acids can be accepted as proved by the fact that the only substances which have been isolated from the products of their complete decomposition are amino-acids, and a small proportion of ammonia. That these amino-acids are united in the way just stated is proved by the presence among the products of partial hydrolysis of combinations of amino-acids which are identical with synthetic products of known constitution. Whether those amino-acids which are now obtained from the proteins are their only constituents is still undetermined, for in no case, if we except one or two of the protamines, have the recovered products of hydrolysis been even approximately equal to the amount of protein which yielded them. All who are familiar with the methods employed in making these analyses have regarded the values obtained for many of the amino-acids as minimal, but some of these, as, for instance, those for glutaminic

[1] A lecture delivered February 4, 1911, before the Harvey Society and reprinted with the permission of the J. B. Lippincott Company, Philadelphia, publishers of the Harvey Lectures, and of the secretary of the Harvey Society.

acid and tyrosine, have been considered, notably by Abderhalden,[2] as approximately correct. So long as much of the protein cannot be accounted for in products of definite character, uncertainty will prevail as to whether many of the apparent differences between the individual proteins are in fact real or only due to imperfections in the analytical methods, and the uses to which these analyses can be applied will be very greatly restricted. Thus to give a concrete example: from gliadin, which constitutes a large part of the protein of wheat, upwards of thirty-five per cent. of glutaminic acid can easily be isolated, whereas from milk casein, under similar conditions, only about eleven per cent. can be obtained. Since about one-half of the products of casein are as yet unknown it is quite possible that among these is a quantity of glutaminic acid that, for some undiscovered reason, is held in solution, and this may be quite sufficient to make up for the apparently great difference between gliadin and casein.

We have now come to the point where the chemical individuality of our protein preparations is a matter of much importance for future studies, both chemical and physiological, and it will be well to review briefly the data which can help us to form some conclusions concerning this question. As this is the logical starting point for a discussion of the chemistry of the proteins I will take it up first.

A large number of protein substances are now on record to which special designations have been given, but about many of these we know at present comparatively little. To a few of them much attention has been directed, but unfortunately many of those who have worked with them have had little experience with such substances and their efforts have led to so much confusion that it is almost impossible to form any estimate of the value of the recorded data.

As the animal organism consists for the most part of protein, and as each individual protein occurs associated with many others, it has been especially difficult to isolate products of definite properties from such tissues. The animal proteins are the ones with which the majority of those interested in protein chemistry are familiar, and it is not surprising that the definite character of our so-called individual proteins is regarded with much more than suspicion. Very little really serious work has been done with any protein of animal origin except ovalbumin. This is the only one which has been subjected to careful fractional crystallization, whereby the constant chemical and physical properties of successive fractions have been established. In regard to crystallized serum albumin less is known than of ovalbumin.

In the case of the hæmoglobins the matter stands even worse,

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[2] Abderhalden and Samuely: *Z. physiol. Chem.*, **46**, 196 (1905).

for no extensive study of the products of fractional crystallization has yet been made, and some of the recorded data as to ultimate composition are manifestly wrong in consequence of analytical blunders. These, however, are still quoted as evidence of the uncertain composition of this substance, and it seems to be generally believed that it is impossible to make two preparations of these beautifully crystallizing substances which are alike.

The seeds of many plants afford the best material from which to obtain preparations of definite character, for in these we find a relatively large proportion of reserve protein, which is in a sense the excretory product of the protoplasm of the cells of the ripening seed. This reserve protein is far more stable than that from animal tissues, and usually can be subjected to extensive fractional crystallization, or precipitation, without showing any detectable change in properties. As such protein has the characteristic structure shown by the animal proteins it is the best material now at our disposal for a study of the chemical and physical characteristics of proteins in general, and apparently furnishes the best preparations for experimental studies of nutritional and other physiological problems. The knowledge gained by such study will to a large extent be applicable to proteins of animal origin.

In regard to the chemical individuality of any of the proteins nothing definite can be said, but there are good reasons for believing that many of those from seeds consist of but a single substance, as the methods which have readily shown the presence of two or more proteins, in preparation from some seeds, have not given the slightest evidence of an admixture in those from others.

The crystalline globulin edestin, which is obtained from hempseed, has been the subject of a most extensive fractionation under a great variety of conditions, and has yielded fractions which have not shown any differences which exceeded the limits of error of observation in ultimate composition, in the partition of nitrogen, or in specific rotation.[3] These facts are important, for edestin yields on hydrolysis just as complex a mixture of amino-acids as do any of the proteins yet analyzed, and hence affords no basis for the belief that the individual proteins, as we now know them, are mixtures of relatively simple polypeptides, each containing but a few of the amino-acids. It is impossible after my experience to believe, as Fischer suggests, that many of the seed proteins are mixtures of several substances of simpler constitution. If this view were correct, it would seem improbable that a substance which crystallizes readily in definite form would resist all possible efforts to break up the mixture to at least some extent. This

[3] Unpublished results obtained by the author.

question deserves still more critical study, and such work is now in progress in my laboratory.

I have gone into this question of the possibility of obtaining preparations of probable chemical individuality, because there is a widespread belief, largely founded on the indefinite character of the preparations of animal origin, that it is impossible to make protein preparations which are worthy of the consideration of the chemist.

To secure a few well characterized proteins is important to the future progress of protein chemistry, for our next task is to subject some of the best defined of these to exhaustive investigation of all their properties. It seems to me that only in this way can we ultimately acquire definite information which can be applied to proteins in general and thus obtain a secure foundation for physiological experiments and conclusions. In the process of differentiating the proteins the efforts of the earlier investigators were directed toward discovering similarities between those of different origin, for it was assumed that there were in nature only a comparatively small number of forms. As the means for differentiation were developed it gradually became apparent that the number of individual proteins was very great. The recently discovered precipitin and anaphylaxis reactions made it probable that an almost infinite number of chemically distinct forms occur in different animal and vegetable tissues, but the evidence was not conclusive that these reactions were actually due to the proteins contained in the different fluids and extracts which had been used in obtaining these reactions until Wells reported his anaphylaxis experiments, made a short time ago with carefully recrystallized ovalbumin. Some experiments with plant proteins, just published by Wells and myself, fully confirm this conclusion, and indicate very strongly that the specificity of the anaphylaxis reaction is intimately connected with the structure of the protein.

One series of our experiments are of direct interest in this connection, as they indicate that this reaction can be used to determine the relation of one protein to another, when these are so nearly alike that differences between them cannot be recognized by a most careful comparison of their physical and chemical properties. Preparations of globulin from the seeds of hemp, flax, squash, and castor-oil plant are obtained which are so nearly alike in ultimate composition, crystalline form, solubility and physical properties that only the most minute examination has revealed any differences whatever between them. We found that while all these proteins showed a strong anaphylaxis reaction, only those from the flax-seed and castor-bean showed any tendency whatever to react with one another, and between these the reaction was of a doubtful character. On the other hand, gliadin from rye reacted strongly with gliadin from wheat, a result in accord with the fact that by chemical or physical means no differences have been detected which were sufficient to indicate that these gliadins were different substances. Likewise legumin from the pea reacted with the apparently identical legumin from the vetch. No reactions were obtained between proteins of distinctly different structure nor between those from seeds botanically unrelated.

From these facts it is evident that structural differences exist between very similar proteins of different origin and it is interesting to note that chemically identical proteins apparently do not occur in animals or plants of different species, unless these are biologically very closely related. In this respect the proteins are in marked contrast to the other constituents of plants and animals, for not only do identically the same sugars and fats occur in many species of plants and animals, but many of these are common to both forms of life. It thus appears that the chemical constitution of the proteins is closely connected with the biological relations of the forms of life which produce them, and that the morphological differences between species find their counterpart in the protein constituents of their tissues. A similar differentiation has recently been made by Reichert and Brown[4] on the basis of the crystalline form of the hæmoglobins, since the measured angles of the crystals show close generic relations.

We have thus far merely considered the fact that differences exist between the proteins, but for the present problems of physiology the extent of these differences is of much more importance, for it is only within the last ten years that we have come to realize that the differences in the structure of many of the proteins, especially some of those extensively used for food, are so great as to require a complete change in our views of digestion and assimilation.

The first observation of an important qualitative difference was made long ago on gelatin, which by its failure to give more than feeble Millon's reaction, was known to contain no tyrosine. This protein was, however, not regarded as a typical one, but was assigned to the group of albuminoids containing those protein-like substances which compose the greater part of various specialized tissues having little, if any, physiological activity. Since none of these substances were regarded as capable of supplying protein to the tissues when taken as food, no special importance was attached to the fact that gelatin was deficient in one of the common constituents of food proteins, although it was known that it was capable of replacing a part of the protein in a maintenance diet.

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[4] Reichert and Brown: Carnegie Inst. Wash. Pub. No. 116 (1909).

Attention was first directed in 1900 to the chemical constitution of food proteins, in their relation to nutrition, by Kossel and Kutscher,[5] who found that the alcohol-soluble proteins, which form a large part of the protein of wheat and maize flour, contained no lysine. These had long been used with success in feeding both men and animals, and the question of their nutritive relations was at once raised. I also found that zein, the alcohol-soluble protein of maize, which Kossel had found to yield no lysine, fails to give the Hopkins-Cole reaction, and therefore contains no tryptophane.[6]

In respect to quantitative differences between food proteins a series of determinations of the partition of nitrogen, made by Harris and myself[7] in 1903, showed that such wide differences in their structure existed that if these had equal values in nutrition a very considerable change in constitution must be effected in the process of assimilation which would involve much more elaborate synthesis than, at that time, was supposed to take place in the animal organism. A striking discovery, made in my laboratory, which showed to what extent quantitative differences might occur in proteins of recognized food value, was that gliadin from wheat flour yielded over 35 per cent. of glutaminic acid.[8] This observation was shortly followed by analyses of the products of hydrolysis of a large number of proteins from many sources, which have given us a general picture of the main peculiarities of most of those which are commonly present in our foods. In these analyses important differences in the proportion of each of the amino-acids have been revealed, especially among the reserve proteins of seeds. Thus through the gradually developed recognition of the fact that our food proteins differ widely in their constitution, entirely new aspects of the problems of digestion and assimilation have been raised, which are still the subject of investigation and controversy.

The physiologist may well ask, are these differences between the various food proteins as great as they appear to be? This question is justified, for, as I have already said, in these analyses hardly more than one-half of the total protein has been recovered in the form of definite products. Although there is no doubt that, in many respects, very considerable structural differences actually exist between many of the proteins commonly used for food, the degree of the accuracy of our present analyses should be considered in detail, so that the actual quantitative value of the determination of each of the amino-acids can be definitely ascertained.

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[5] Kossel and Kutscher: *Z. physiol. Chem.*, **31**, 165 (1900).
[6] Osborne and Harris: *J. Am. Chem. Soc.*, **25**, 853 (1903).
[7] Osborne and Harris: *J. Am. Chem. Soc.*, **25**, 323 (1903).
[8] Osborne and Harris: *Am. J. Physiol.*, **13**, 35 (1905).

As a critical study of these methods has been in progress in my laboratory for some time, it may be a matter of interest to you to know some of the results already obtained. Not only our own work, but that of other laboratories, has shown that the amount of ammonia yielded by hydrolysis is uniform for each protein, and can be determined with accuracy. The proportion of the protein nitrogen obtained as ammonia has thus been found to be four times greater in some proteins than in others. The amount of arginine, histidine, and lysine can be estimated by Kossel's method with a relatively high degree of accuracy and can be controlled by determinations of the quantity of nitrogen precipitated by phosphotungstic acid under definite conditions. The results of the direct estimations of the basic amino-acids have been found to be very nearly equal to the amount of these substances actually present, and to be strictly comparable with one another. The only uncertainty attaching to carefully made determinations is caused by incomplete hydrolysis of the protein; this can be controlled by estimating the proportion of nitrogen precipitable by phosphotungstic acid, after hydrolyzing with hydrochloric acid for different periods of time.

From the results that we have obtained from a number of food proteins it has been found that the proportion of arginine or histidine is about ten times as great in some as in others. The proportion of lysine varied from none to 6.43 per cent. of these proteins while the lysine, obtained from the crude muscle substance of chicken and halibut, reached nearly 7.5 per cent. of the dry material, indicating that the pure proteins of these tissues yield an even greater quantity.[9]

Tyrosine, which has always been isolated by direct crystallization, can probably be estimated with a reasonable approximation to accuracy, although, according to my experience, it can never be so completely separated from the mixture of the amino-acids as Abderhalden assumes, who has stated that it is possible to thus separate it completely and regards its determination as one of the most accurate of those employed in analyzing the products of protein hydrolysis. If only pure tyrosine is weighed it seems possible to determine its proportion with a close approximation to the truth, but it is not always possible to determine the purity of the products weighed by some of those who have published such estimations. The amount of tyrosine obtained from food proteins falls between 2 and 4.5 per cent., although as much as 10 per cent. has been obtained from some of the albuminoids.

It has been commonly assumed that glutaminic acid in the form of its hydrochloride can be almost completely separated from

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[9] Cf. Osborne, Leavenworth and Brautlecht: *Am. J. Physiol.*, **23**, 180 (1908).

the mixture of decomposition products by the method of Hlasiwetz and Habermann. Abderhalden has used this determination in order to show the relations of various proteins to one another, and has stated that the results are more nearly quantitative than those obtained for any of the other mono-amino-acids, with the exception of tyrosine. Confidence in the accuracy of these determinations has been largely founded on the close agreement between different determinations. Thus several investigators independently obtained from 10.5 to 11 per cent. of glutaminic acid from casein, and a like agreement was also obtained with several other proteins. In making these determinations the question whether the protein was completely hydrolyzed or not received little attention, for it was formerly assumed when the products of hydrolysis ceased to give the biuret reaction that the union between the amino-acids had been completely broken down.

Some time ago I found that a considerable quantity of an insoluble product was formed by hydrolyzing gliadin for several hours with twenty-five per cent. sulphuric acid, and that this, on subsequently boiling with strong hydrochloric acid, yielded relatively large quantities of glutaminic acid and cystine. The most direct evidence of the existence within the protein of a highly resistant union between two amino-acids was exhibited by the di-peptide of proline and phenylalanine which was obtained by Clapp and myself[10] from gliadin which had been boiled with 25 per cent. sulphuric acid for many hours. This di-peptide was completely hydrolyzed only by heating in a closed tube to a relatively high temperature for some time with strong hydrochloric acid. We thus have every reason to expect combinations of amino-acids which will require very energetic treatment with acids before they can be completely decomposed. These observations have led me to determine the output of amino-acids after prolonged boiling with strong hydrochloric acid, and as a result I have obtained from several proteins much more glutaminic acid than had formerly been isolated from them. Thus after doubling the time of hydrolysis of casein I have recently found, in five closely agreeing estimations, 15.5 per cent. glutaminic acid, or 50 per cent. more than was previously isolated. Zein, gliadin, and edestin have also yielded distinctly larger quantities than after the shorter hydrolytic treatment to which they had been previously subjected.

Before the results, thus far reported, in respect not only to glutaminic acid, but also to all of the other amino-acids obtained by Fischer's ester method can be accepted as final, they must be confirmed by new determinations made after boiling the proteins with strong acids until the hydrolysis is complete.

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[10] Osborne and Clapp: *Am. J. Physiol.*, **18**, 123 (1907).

Heretofore we have had no means whereby we could satisfy ourselves that the union between all of the amino-acids had been broken apart and the acids set free. Sörensen's method of determining this by adding methylene to the amino-nitrogen, and estimating the proportion of free carboxyl groups by titration, does not yield satisfactory results in practice, as the endpoint of the titration is too uncertain. The method recently proposed by Van Slyke[11] for determining the proportion of free amino groups yields good results, and by its use the progress of the hydrolysis should be easily and accurately followed.

Although the yield of amino-acids can be increased by making the hydrolysis complete, the errors incident to the processes employed in isolating them involve losses which contribute largely to the deficit shown by the total of the products obtained in definite form. As no data were on record from which these losses could be even roughly estimated Jones and I[12] made a mixture of amino-acids in the proportion in which they had been obtained from zein, and analyzed it according to Fischer's ester method. We recovered only a little more than 80 per cent. of the leucine, about 70 per cent. of the proline and phenylalanine, and 40-50 per cent. of the alanine, valine, and aspartic acid, and none of the serine. In making this test analysis, ill-defined products similar to those observed in the course of an ordinary protein analysis were formed, showing that decomposition of the esters took place during the distillation.

By correcting our analysis of zein for corresponding losses 92.7 per cent. was accounted for as consisting of those amino-acids which are now recognized as protein decomposition products. Hence nearly all of the deficit shown by the analysis of zein may fairly be attributed to analytical errors, for the presence of 0.6 per cent. of sulphur shows that a part of the substance, still unaccounted for, belongs to some sulphur-containing complex, and also a part unquestionably to serine which, we are convinced, must have been present in much larger amount than that isolated. We cannot apply corresponding corrections to the analyses of other proteins until these have been subjected to further careful study, for differences in the constitution of the mixture of amino-acids may lead to losses quite different in extent. From the experience gained in this case, however, the way appears open for further investigations which should give us a more definite conception than we now have of the constitution of some of the more important food proteins.

Proteins which, unlike zein, give a strong Molisch reaction and contain carbohydrates, deserve especial consideration, for in the

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[11] Van Slyke: *Ber.*, **43**, 3170 (1910).

[12] Osobrne and Jones: *Am. J. Physiol.*, **26**, 30 (1910).

analysis of these the carbohydrate complex may have a pronounced influence on the results. It is generally assumed that carbohydrate complexes form an integral constituent of the molecules of many of the proteins. An amino-carbohydrate has been obtained from ovalbumin, ovomucoid and some of the mucins, but attempts to isolate any such substances from many other proteins have failed. The fact that most protein preparations give the Molisch reaction has led to the assumption that these contain some carbohydrate, but my experience has led me to believe that in most cases this reaction is caused by impurities in the preparation and not by a constituent of the protein molecule. This is certainly true for many of the plant proteins, from some of which every trace of carbohydrate can be easily removed, while from others this can be done only with difficulty.

Of the proteins which contain phosphorus we have two types—the nucleoproteins and the phosphoproteins.

Little need be said concerning the constitution of the nucleoproteins, other than that these are natural or artificial combinations of simple proteins with nucleic acid. The constitution of the nucleic acids has been revealed largely by the brilliant work of Levene and Jacobs,[13] but as these form no part of the protein molecule a discussion of their decomposition products lies outside the scope of this lecture.

The phosphoproteins, which like milk casein or ovovitellin, contain phosphorus, but not nucleic acid, present a wholly different problem, for in these the phosphorus appears to bear some relation to the protein molecule. No non-protein, phosphorus-containing group has yet been obtained from these proteins, and it cannot be assumed that these are combinations of phosphorus-free protein with such a complex, although the fact that the total quantity of decomposition products accounted for in analyzing both casein and ovovitellin is small indicates that this may be the case.

This brief review will have little interest for you unless in connection with it I consider some of the problems which have been presented to the physiologist by the recent investigations in the field of protein chemistry.

The most important of these problems have been raised by the discovery that many of the proteins which are extensively used for food differ much in constitution, not only from the tissue proteins of the animal, but also from each other. In consequence of this the older views of protein assimilation have been abandoned, and a multitude of new questions now demand an answer. Since we can no longer assume that the food protein is but slightly changed in the process of digestion before conversion into the

[13] Levene and Jacobs: *Ber.* **43**, 1 (1910).

animal tissues, we must consider in how far it is decomposed by the digestive enzymes and to what extent a re-synthesis is effected in the process of assimilation.

A number of distinct and independent problems are thus raised, each of which must be settled before these questions can be answered.

First: To what extent is the protein decomposed by the normal process of digestion?

Second: To what extent does the animal synthesize the products of protein hydrolysis?

Third: What is the minimal protein requirement of the animal? In other words, how much of the food protein actually replaces protein waste in the tissues, and how much is burned, without ever being converted into tissue substance?

Fourth: To what extent do intestinal bacteria take part in these processes?

All these questions have been the subject of investigation and discussion, but a conclusive answer has not yet been obtained to any of them.

It has long been known that some of the ultimate products of protein hydrolysis occur in the intestine, although the importance of the earlier observations has only recently been recognized. Thus Koellicker and Müller,[14] in 1856, found leucine and tyrosine there, and later Kühne[15] confirmed their observation, but considered these to be by-products of the action of trypsin. As you all know, Kühne assumed that under the action of trypsin the proteins can be decomposed into nearly equal parts, which he called respectively hemi- and anti-peptone, and by the continued action of trypsin the former is completely converted into amino-acids, but the latter is resistant to any further action of this enzyme. He, however, did not believe that in the normal digestion the decomposition of the hemipeptone was carried to the amino-acid stage.

In 1901 Kutscher and Seemann[16] found that protein was normally converted into amino-acids in the intestine and supposed that these served as the material from which the new body protein was constructed. At the same time Cohnheim[17] showed that the intestine contained the enzyme, erepsin, which, although without action on native proteins, converts the proteoses and peptones formed by pepsin and trypsin completely into simpler products, among which amino-acids are abundant. Thus almost at the very

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[14] Koellicker and Müller: *Verhandl. physik.-med. Ges. in Würzburg*, **6**, 507 (1856).
[15] Kühne: *Arch. path. Anat. (Virchow's)*, **39**, 155 (1867).
[16] Kutscher and Seemann: *Zentr. Physiol.*, **15**, 275 (1901).
[17] Cohnheim: *Z. physiol. Chem.*, **33**, 451 (1901).

time when chemical investigations made it necessary to assume that the food protein is decomposed into amino-acids and new protein reconstructed from these products of hydrolysis, physiological investigations showed that the animal organism was equipped with enzymes able to accomplish this result.

However, it still remains undecided whether decomposition of the food protein, under normal conditions, is actually carried to a complete conversion into amino-acids, for Fischer and Abderhalden[18] consider this to be highly improbable, and suggest that it is possible that, after a certain proportion of amino-acids are split off, a nucleus may remain to serve as a basis for the construction of new protein.

The question of in how far the animal is able to synthesize protein from the products of protein hydrolysis has been put to a direct test. Loewi,[19] Lesser,[20] Abderhalden and Rona,[21] Henderson and Dean[22] and others have found that not only nitrogen equilibrium but even a distinct nitrogen retention could be obtained by feeding animals with the products of tryptic digestion, but not with the products of acid hydrolysis.

It has been thought that the products of tryptic digestion serve to maintain the animal better than do those of acid hydrolysis because they contain undecomposed polypeptide complexes which may serve as a nucleus to which amino-acids of the proper kind and in the right proportion are added. It has also been supposed that the products of acid hydrolysis fail to maintain the animal because of the destruction of some one, or more, essential constituent of the protein through secondary decomposition caused by the acid, for it is known that such occur, notably in the case of cystine and tryptophane.

The question of the dependence of protein synthesis on the structure of the food protein has been investigated by Abderhalden and Samuely,[23] who sought to detect a change in the glutaminic acid content of the serum albumin of a horse after feeding large quantities of wheat gliadin, from which several times as much glutaminic acid can be obtained as from serum albumin. As no change in the composition of the blood albumin could be discovered they concluded that the composition of the food protein had no influence on the composition of the blood proteins.

The same question was also experimentally tested by Michaud,[24]

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[18] Fischer and Abderhalden: *Z. physiol. Chem.*, **39**, 83 (1903).
[19] Loewi: *Zentr. Physiol.*, 590 (1902); *Z. Biol.* **46**, N.F. **28**, 113 (1904); *Arch. exp. Path. Pharmakol.* **48**, 303 (1902).
[20] Lesser: *Z. Biol.* **45**, N.F. **27**, 497 (1904).
[21] Abderhalden and Rona: *Z. physiol. Chem.*, **52**, 507 (1907).
[22] Henderson and Dean: *Am. J. Physiol.*, **9**, 386 (1903).
[23] Abderhalden and Samuely: *Z. physiol. Chem.*, **46**, 193 (1905).
[24] Michaud: *Z. physiol. Chem.*, **59**, 404 (1909).

who founded his experiments on the assumption that the animal requires for the construction of its body proteins a definite proportion of each of the amino-acids which enter into their constitution. He therefore thought that an animal could be maintained in nitrogen equilibrium by a smaller amount of protein consisting of a mixture of the tissues of an animal of the same species than of protein of distinctly different constitution. He accordingly fed dogs on a mixture of minced dog tissue, and compared the nitrogen balances with those obtained with food mixtures which contained the same quantity of nitrogen in the form of gliadin, edestin, nutrose, casein, dog serum proteins or horse flesh. The results which he obtained were very striking. The minced dog tissues were far more effective in preventing loss of nitrogen than either gliadin or edestin, and distinctly more effective than casein. The differences obtained with dog serum proteins, or with horse flesh, were too slight to lead to any definite conclusions.

The question of protein synthesis by the animal has also been studied by aid of the so-called "incomplete proteins." The numerous older experiments with gelatin, which lacks tyrosine, have led to the belief that this alone cannot support life, but that when added to a food containing other proteins it is capable of replacing a considerable part of the protein required to maintain nitrogen equilibrium.

Wilcock and Hopkins[25] found that zein, which lacks glycocoll, lysine, and tyrosine, failed to keep mice alive for more than a few days, but that if tryptophane was added they lived somewhat longer.

Henriques[26] fed rats with zein and also with gliadin, which lacks glycocoll and lysine, and found that he could not secure nitrogen equilibrium with zein but could do so with gliadin if fed in sufficiently large quantities. He concluded that probably the absence of tryptophane rendered zein incapable of supporting an animal, but the absence of lysine from gliadin was not of essential importance.

Definite data concerning the minimal protein requirement of the animal are lacking. In complete starvation a considerable quantity of nitrogen is eliminated which is much reduced if the energy requirements of the animal are satisfied by feeding sufficient carbohydrate. Michaud's experiments have shown that this quantity can be still further reduced by interposing a period of feeding with a minimal quantity of protein and then again feeding with nitrogen-free food, but his experiments left him in doubt as to whether or not the output of nitrogen thus found corre-

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[25] Wilcock and Hopkins: *J. Physiol.*, **35**, 88 (1906).
[26] Henriques: *Z. physiol. Chem.*, **60**, 105 (1909).

sponded to the destruction of tissue actually necessary to maintain the physiological functions of the body. This is of importance in connection with the question of the synthesis of body protein from food protein, for we must know how much of the latter is required for this purpose if we are to interpret the results obtained in feeding animals with proteins of different constitution. From Michaud's results it is evident that only a small part of the food protein commonly consumed is necessary to supply the tissue waste incident to the performance of the purely physiological processes required to support life, and that consequently, under normal conditions, a synthesis of new protein probably occurs to only a small extent.

The part which intestinal bacteria may play in the synthesis of body protein from food protein deserves much more attention than it has received. It is well known that bacteria are present in large numbers in the intestine, for from 30 to 40 per cent. of the fæces may consist of the bodies of these organisms.

Before we can accept as conclusive any of the evidence that has been offered that the animal actually synthesizes tissue proteins from the constituents of its food, we must carefully consider the data just set forth, not only in connection with each other, but also in connection with some other facts which have been recently discovered.

From Michaud's experiments it is evident that only a small part of the food protein normally consumed is used for the construction of tissue protein, and that consequently when the animal receives an abundant supply of food a deficiency in one or more of the essential constituents of the protein may not become apparent for a long time. Furthermore, such a deficiency may be supplied by the animal's own tissues, for we know that the muscles form a reserve supply which furnishes the necessary protein required during long-continued starvation and also serves for the construction of new tissues, under normal conditions, as shown by the development of the reproductive organs of the salmon.

It is, consequently, probable if the synthesis of the animal proteins is affected by a recombination of amino-acids that the deficiency of any one of these in the food protein will become apparent only when the experiments are carried on for much longer periods than has thus far been done in testing the proteins in respect to their relations to protein synthesis in the animal body.

All the experiments in this direction which have been made with the incomplete proteins are wholly inconclusive because similar experiments made with complete proteins have likewise failed, with the exception of one by Röhmann, in which he used a mixture of several proteins.

The fact that a nitrogen balance is not obtained in such experiments is no evidence that the fault lies in the constitution of the protein, as I shall soon show. Also a retention of nitrogen obtained for a time with a protein or its decomposition products is not evidence that these have been utilized in the construction of new tissue.

That the products of tryptic digestion maintain the animal for a short time while the products of acid hydrolysis fail to do so is not evidence that some essential amino-acid has been destroyed by boiling the protein with strong acid, for the failure of the latter may as well be due to the presence of some more or less toxic secondary decomposition product, as is indicated by the digestive disturbances noted by all those who have fed such substances.

Michaud's discovery that proteins which in constitution closely resemble the body proteins, prevent loss of nitrogen from the body better than do proteins which differ widely in their constitution from the body proteins, is the best evidence that we have that amino-acids from the food protein are actually used for the construction of the tissue proteins. This, however, is not wholly convincing, for Michaud's experiments are, in fact, a comparison of the nutritive effect of animal tissue substance with the nutritive effect of a mixture of isolated protein and other substances. It is also to be noted that he makes no mention of the addition of inorganic constituents to these mixtures. It is not surprising, therefore, that he should have obtained the best results with the tissue feeding, for the comparative failure of the isolated proteins may have been due to other causes than differences in their constitution.

In none of the experiments thus far discussed has consideration been given to the possible influence that bacteria may play in the transformations that are required to convert a protein of wholly different constitution from the tissue proteins into the substances which compose the body of the animal. The capacity of these organisms to effect profound chemical changes is quite sufficient to transform a considerable part of the amino-acids which result from digestion of the food protein into forms of totally different constitution. By this means an excess or deficiency of one or another amino-acid may be compensated and the animal supplied with an entirely different combination of amino-acids from that originally fed to it. To what extent such changes occur in the intestine, or to what degree the substance of the bacteria is digested and assimilated, we do not know. The fact that animals in cages on restricted diets are prone to eat their own fæces indicates that they thereby secure some element of food which they crave, and is suggestive that the bacteria may have more influence in feeding experiments than has heretofore been supposed.

After all that has been said and written concerning the synthesis of the body protein by recombination of the amino-acids from the food proteins, it must not be forgotten that in the process of digestion the amino-acids themselves may be deaminized and converted into wholly different substances, and that from these new products the amino-acids required by the animal may be later reconstructed.

Many facts have indicated that this may happen, but the most important indication has very recently been given by Embden and Schmitz, who have found by perfusing the excised liver with blood to which pyroracemic acid or lactic acid had been added that alanine is formed. They also obtained tyrosine and phenylalanine after adding oxyphenylpyroracemic acid or phenylpyroracemic acid respectively.

In reviewing the literature of feeding experiments made with a view to determine the possible synthesis of protein by the animal, it is evident that these have been much too brief and have been made without sufficient consideration of many important factors. In most of these the ages of the animals have not been given, in others the isolated proteins have been commercial products of doubtful purity or have been prepared in the laboratory by hasty methods which do not yield products of definite character; no sufficient consideration has been given to the requirements of the animal for inorganic constituents, and none whatever, so far as I can find, to changes caused by intestinal bacteria in the constitution of the nitrogenous elements of the food. Each of these factors may have an important influence and must be the subject of special investigation before final conclusions can be reached. Such investigations must be made along many lines before a foundation can be secured from which conclusions of value can be drawn. These problems are among the most complex that have been presented to the physiologist, and it needs but a little reflection to show that a solution can be reached only by long-continued and patient work.

A beginning in this direction was made about a year and a half ago by Mendel and myself with the hope that in time we may secure some data which may ultimately be of help in solving some of these important questions of nutrition. Our experiments, which have thus far been conducted with rats, have already yielded some interesting results.[27]

From a large number of experiments with many different proteins, singly and in combination, we have learned that the cause of failure in most of the previous experiments is due to an unsuitable choice of the inorganic constituents of the food. By using an inorganic salt mixture similar to that used by Röhmann, in the only approximately successful artificial feeding experiments heretofore reported, we have succeeded in keeping rats, not only in

[27] Carnegie Inst. Wash. Pub. No. 156 and No. 156, Part II.

positive nitrogen balance, but in full weight and perfect health over long periods of time. Thus in our most successful experiment we have kept a rat for ten months; for the first two months on a mixture containing milk casein and wheat glutenin, and for the succeeding eight months on one containing wheat glutenin as its only protein. Many other rats have been kept in fine nutritive condition for long periods with casein or mixtures of casein with other proteins. When the conditions for such experiments are well established we expect to extend them to the many forms of food proteins with the hope of learning something more definite than is now known of the effect of differences in the constitution of the food protein on nutrition.

One of the most interesting results of our experiments has been the discovery of the fact that while a mature rat can be maintained on a food containing the isolated proteins, a young rat, on the same food, fails to make more than the slightest growth. Three young rats which weighed from 60 to 70 grams each have been fed for more than three months with the same glutenin food, which fully satisfied all of the requirements of the mature rat just mentioned. All of these have remained nearly stationary in weight, although during the entire period the food intake has been fully equal to that of other rats of similar weight which were in full normal growth. Many experiments with other proteins, including milk casein, have given a similar result, and we have also found that such stunted animals when transferred to a normal mixed diet, or to one containing milk powder, at once grew normally. Future investigations must show whether or not this means that a mature animal actually constructs little if any new tissue protein from its food protein, and that a growing animal, which must do so, is unable to utilize proteins of widely different constitution from its tissue proteins for this purpose. The possibility that this may be so is suggested by the interesting experiments by Aron,[28] who finds that dogs which are kept on such a restricted quantity of mixed food that they do not increase in weight during long periods grow in size at the expense of their muscular tissues, so that their skeletons are equal in weight to those of normally nourished dogs which have doubled their weight during the same time. Although we have not yet measured the different parts of our stunted rats it is evident from their appearance that their skeletons have not developed to any marked extent. They look exactly as if tissue growth had entirely ceased from the beginning of feeding with the single protein, and, if this is so, it is possible that they cannot make new tissue from the pure protein of their food. The slight growth made by some animals when thus fed can easily be attributed to the activity of intestinal

[28] Aron: *Biochem. Z.*, **30**, 207 (1910).

bacteria which convert a small part of the food protein into other forms from which new tissues could be constructed. This possible participation of bacteria immensely complicates the conduct of such feeding experiments, and renders the interpretation of the results difficult, for even if the fæces are in some way collected so that the animal cannot eat them we have no assurance that dead bacteria may not be digested within the intestine and thereby supply the animal with substances which have been carefully excluded from the food.

WHAT AND HOW MUCH SHOULD WE EAT?[1]

I

Under normal conditions of supply and normal conditions of health, little attention is given by the great mass of mankind to the question what or how much should be eaten. They simply eat what they want and as much as they want, and then stop and go about other business. They know nothing of the dietary elements which the nutrition expert tells them are so essential for their well-being, and even for their very existence.

How can they long survive in such ignorance? Why does the community allow them to endanger, not only their own lives, but those of posterity? The only possible answer is that they are endowed with instincts which guide them so well, that under normal conditions of life they escape the many dangers that until recently they were unconscious of.

In view of the successful part played by instinct in dealing with the problems of nutrition,—which modern science is beginning to show are among the most complex that the human mind has ever yet undertaken to investigate,—perhaps it might be well to pay a little more respect to instinct than has lately been the fashion, and at the same time see if by observation some useful hint may be obtained which will help in interpreting the results of investigations in the laboratory.

Even the pig knows how to protect himself against dangers arising from indiscretions in eating, not only as to quantity, but as to the proportion of the various food-constituents. This is shown by Evvard's experiments. He allowed pigs to feed themselves *ad libitum* with corn, meat-meal, oil-meal, salts, and the like, from separate hoppers. During early growth, when new tissues were being made rapidly, these pigs ate much larger proportions of protein than when growth became slower. Later, when smaller amounts of corn were eaten, the protein deficiency

[1] Reprinted from *The Atlantic Monthly* for September, 1918, by permission of the editor.

thus caused was met by an increase in the amount of meat-meal eaten. Under these conditions of free-choice feeding the pigs grew faster than any previously recorded which had been fed on mixtures made for them by the combined talent of agricultural experts, trained both in the science of nutrition and in the practice of the art of feeding.

Similar experiments made in my laboratory with albino rats gave much the same results. These animals were given their choice between two food-mixtures, one adequate for growth, the other inadequate, owing to the deficiency, or absence, of some one factor essential for growth. Although these foods were alike in physical properties, and so nearly alike in their constituents that it was difficult to believe that the rats could distinguish between them by any of their senses, nevertheless, all but one of the several rats so chose their food as to make practically normal growth. How they did this is one of the wonders of nature.

Considered solely from the standpoint of a supply of energy,—that is, of fuel for the maintenance of the body as a running machine,—the food-problem has long been the subject of very carefully and accurately controlled experiments. These have shown that, for the expenditure of a given amount of energy in the performance of physical work, a corresponding amount of potential energy in the form of food is required. In other words, the law of conservation of energy applies to the animal machine as strictly as it does to the machine in the factory.

The practical conclusion to be drawn from this is that the animal body must be supplied with enough energy, not only to keep it running, but to perform the work done by it. Recently we had an illustration of what happens to the machinery of our industries when the supply of energy in the form of coal runs short; and we may soon have an illustration of what will happen to the labor employed in these industries if the supply of energy in the form of food suffers similarly.

Let us first consider the question how much energy is really needed; or, to put it the other way, how little food can we get along on and still do the work necessary for the successful conduct of the war? As already stated, the relation of food eaten to the energy expended has been very carefully established by exact experiments which, under the conditions studied, are beyond criticism. How can these studies be applied to the needs of daily life? It is obviously impossible to determine the energy expended by a blacksmith working on a battleship, or an engineer running a locomotive, or a horse ploughing a field. None of these can be put to work in a calorimeter and the heat value of their work measured, nor can any imitation of such working conditions be reproduced whereby even an approximate estimate might be made. Nevertheless, authorities on nutrition furnish us with tables showing how much energy must be supplied in the form of food for those who are engaged in a very wide variety of occupations, and these tables are largely used in determining suitable rations under different conditions.

It may fairly be asked, if it is impossible to measure the energy expended, how have such tables been made? They have been made by carefully studying the amount of food actually eaten by large numbers of people engaged in all sorts of occupations, and determining the calorific value of these foods. The energy expended in the various occupations was not measured directly by scientific methods, but indirectly, on the assumption that it is the habit of people, as well as of animals, to eat according to their calorific requirements. If men and animals were not endowed with instincts that enable them to adjust their food intake to the energy expended in maintaining their bodies, as well as in doing their work, they would be constantly suffering from the ills of over-eating or of under-nutrition.

That nature provides protection against many misfortunes which may befall an individual in the course of life, has been pointed out most interestingly by Dr. Meltzer in a paper on "The Factors of Safety in Animal Structure and Animal Economy." From the numerous examples set forth by Meltzer it seems probable that the ills following over- or under-eating are, in some way, also provided against. It has long been recognized that underfeeding is temporarily guarded against by a conversion of sugar into a substance similar to starch,—glycogen,—and storage of this in the liver and muscles. The potential energy thus husbanded is readily drawn on or replenished according to the minor fluctuations in demands for more, or for less, energy, which may be made necessary by the daily variations in physical activity, or the daily changes in external temperature. Larger demands, extending over longer times, are met by the reserve of fat and muscle-tissue, which in every normally nourished individual is sufficient to supply enough energy for a not inconsiderable time.

Are these the only means of dealing with inequalities in energy output, or food-supply? It is conceivable that, in addition, the speed at which chemical changes go on within the body may vary, to adjust consumption to requirements. Allen and DuBois state that the profound effect of confinement and under-nourishment on heat-production has never received the attention it deserves. If reducing the body weight, by lowering the food-intake below the amount which instinct prompts, reduces the rate of metabolism,—that is the sum of the chemical changes which are taking place within the body,—we should expect the converse to be true, and to find that increasing the food-intake above the amount that can be met by storing glycogen and fat is further met by an increase in the rate of metabolism. If it should turn out that a change in the rate of metabolism thus provides a hitherto unrecognized factor of safety, the whole question of over-eating will have to be considered from a new angle.

It has been generally held that over-eating, except within narrow bounds, is impossible, for the subject will either grow fat, which of course has its limits, or will feel badly and cease to eat in excess until a normal condition is reëstablished, or will dispose of the surplus food by exercise. According to this view, those who cannot live in comfort without a game of golf or some other agreeable form of activity are habitually over-eating, in so far as fuel needs are concerned. There are other factors, however, involved in the exercise problem, which we will consider later.

If surplus food above that needed for the daily tasks of life can be disposed of by an increased rate of metabolism, we ought to know more about it than we now do if we are to deal with the problem of the most efficient use of our food-supply. Can any important amount of food be wasted in this way? A certain rate of metabolism is required to support the body functions and temperature, and a corresponding quantity of food is necessary to continue that metabolism, if body tissues are to be maintained. If more than this amount of food is eaten, it is wasted, if it serves no other purpose than to produce useless heat which must be gotten rid of in some way.

In my own case it has seemed that an unaccustomed plethora of food has been followed by a continued sensation of heat, and efforts to dispose of this extra heat by reducing my clothing below that habitually worn. If subjective sensations of this kind are to be trusted, it would seem that under such conditions surplus heat is being eliminated by radiation in consequence of dilation of the capillary blood-vessels. This agency is provided to rid us of the excess of heat incident to physical work; and it would seem not improbable that it might be called on to dispose of surplus heat produced by increased metabolism caused by an excess of food.

The extra heat eliminated after eating protein, which Lusk properly regards as a result of stimulated metabolism, is an example of wasted energy of the same kind that may result from a plethora of other kinds of food. Another example is the increased rate of metabolism caused by caffein, which may explain the extensive use of coffee and tea. So long as carbohydrates or fats are assimilated only in amounts in excess of the maintenance and energy requirements which can be met by storage in the form of glycogen or fat, no evolution of heat can be expected; but when the amount is greater than can be thus cared for, the plethora must be burned, if bodily health is to be maintained.

To what extent a surplus of food can be disposed of by such an increase in the rate of metabolism, or whether such a stimulation of the metabolism can be frequently endured without sensations of discomfort, are questions which have been so little studied that definite answers cannot be given to them. My own observations have led me to suspect that there may be a wider difference in the capacities of individuals thus to meet the dangers involved in occasional over-eating than has heretofore been supposed. Possibly those who are said to have "good digestions" are those whose metabolism is easily stimulated, so that they are able to oxidize promptly whatever surplus food they may happen to eat. If such should prove to be the case, the ills commonly attributed to indigestion may in many cases not be due to a failure to digest food, but may, on the contrary, be the result of assimilating food which has already been digested in greater quantity than the body-cells are capable of oxidizing promptly.

Waste of food, if in fact there is any, from this source is doubtless small, and quite likely is fully compensated for, because a large proportion of the "good feeders" are among the most efficient in every community. While many seem to think that high thinking and plain living are essential to good living, it does not by any means follow that a high plane of metabolism does not imply a high plane of both mental and bodily efficiency. Certainly, among cold-blooded animals the increased rate of metabolism which results from raising the temperature of their environment leads to marked evidences of increase in physical efficiency.

II

Leaving this question for future investigations to settle, let us consider whether we have at present any better means of determining how much food—how much energy—is needed under given conditions than our present one founded on observations of what people actually eat when guided solely by their instincts?

It is very generally assumed that those who are in a position to do so eat too much, probably because all of us are tempted to eat when confronted with an abundance of attractive food. Although many do yield to this temptation, few fail to eat less at subsequent meals, and soon reduce their consumption, even if enticing food is continually put before them. A millionaire could not possibly eat as much in a week as a coal-heaver, unless he engaged in exercise more severe than would be agreeable. How much more than is necessary can be eaten without discomfort? Does over-eating cause a waste of food sufficient to justify the efforts necessary to control it? Can a man over-eat habitually, without either growing very fat, or becoming a dyspeptic? Does not this evil usually cure itself? Here are questions which are difficult to answer positively. Plenty of people will answer them with assurance; but have they good reasons for their answers?

It is difficult to fatten animals beyond a certain limited degree, and even then it takes a long time. If too much tempting food is supplied, they "go off their feed." Even pigs, as has already been stated, can successfully feed themselves from hoppers with concentrated foods. They apparently do not eat too much. Occasionally cattle or horses which by chance get access to the feedbin will eat so much that they die; but such cases are probably nutritional accidents, where fermentations cause decomposition of the food before it can be digested. During parts of the year almost all animals in a state of nature have the opportunity to eat too much, but we have no reason to believe that they do so. In a long experience, gained by feeding many hundreds of albino rats whose food-intake was limited only by their instincts, I have never suspected that any one of them ate too much. Successful stockmen make their animals eat all they will, in order to obtain maximum production and profit.

Excess of food results in accumulations of fat, but these form comparatively slowly. Chickens or Strasburg geese are fattened more rapidly by force-feeding than in the natural way, because thus they can be made to consume more food than their instincts will permit. Pigs can easily be made very fat; but these animals have been bred for generations with the purpose of developing a breed having a capacity for accumulating fat beyond the normal. Taking the country over, fat men are not very numerous, and most fat women have spent years in becoming so. There is probably far less over-eating, as measured by accumulations of fat above the normal, than is popularly supposed; but that there is some is evidenced by the not inconsiderable number of fat people, especially women, seen in our large cities.

Since the records of what people on the average actually do eat when left entirely to their instincts have been demonstrated to be on the average very nearly what they should eat for the proper maintenance of their bodies, it appears that in general there is not much, if any, over-eating. Such as may occur can be controlled by the scales; for if one is not obviously fat or gaining weight, he is presumably not over-eating. There is evidently little food saving to be expected from efforts directed to suppressing over-eating.

If the food supply is to be conserved by reducing the amount of food below that now eaten under the direction of instinct, what will be the result? The first effect will obviously be a loss of weight and consequently a reduction in the amount of food needed to move the body, as in walking, getting out of bed, or rising from a chair—a very small fraction of the total needed for maintaining the bodily machine and performing the tasks of daily life. It will not reduce materially the amount of food needed to do the work of daily life; for, as Anderson and Lusk have recently shown, the energy requirement for work done is exactly the same whether the animal is well fed or starved. All that is saved by reducing weight is merely the fuel needed to do the mechanical work involved in moving the smaller load imposed by body weight. Experiments to show the reduction of energy resulting from reduction in weight have been made chiefly on men or animals whose work consisted in lifting the body, as in walking, or hill-climbing. Under such conditions a diminution of energy expenditure is involved which is almost proportional to the reduction in body weight. Under the conditions of activity of the great mass of our population, no corresponding saving can be expected, for few are engaged in occupations where lifting the body comprises more than a very small part of the mechanical work which they do.

Loss of weight involves loss of the factor of safety which nature provides in the form of fat; for even those who are not commonly regarded as "fat" have a very considerable amount of fat in the various tissues of their bodies. It may also involve a loss of substance from the muscle-tissues, if the reduction in weight is carried far, or if the subject was at the outset supplied with fat below the normal. Just what effect it has on the easily mobilized supply of glycogen which is needed to maintain uniformity in daily metabolism, I do not know. It would seem as if this too might be reduced to a minimum inconsistent with efficiency. There is no doubt that a certain amount of reduction in weight can be endured by the vigorous for a considerable time, but not without serious loss in efficiency, if long continued. In every community there are many men below the normal weight, and these are always looked upon with suspicion by insurance companies and enlistment officers, even though no pathological cause can be found for their underweight.

Restriction of the food-intake means the loss of a factor of safety other than that furnished by body fat—one that is in the food itself. Food furnishes more than fuel for the body: it supplies, in addition, the materials needed to renew the wear and tear incident to life, and also those mysterious substances called vitamines, the absence of which in a food renders it incapable of supporting life. No one knows what vitamines really are, for as yet they have not been isolated. Their presence is revealed only by the effect they produce upon nutrition. They are not uniformly distributed in the various parts of the plants and animals we use as foods; and in rejecting a part of an animal, or by over-refinement in milling, we may throw away these indispensable substances. The germs of wheat, rice, and other seeds, the liver and kidney of animals,—all of which are composed of highly active cells,—and the cells of yeast, contain a far larger proportion of vitamines than do the endosperm or berry of wheat and rice, or the muscle-tissue of the animal. Addition of a very small quantity of the germ of the wheat-kernel to a vitamine-free but otherwise adequate mixture of nutrients, renders it capable of sustaining life; whereas a very large addition of white flour scarcely suffices.

Whenever the food-intake is cut down, the supply of vitamines is reduced, with how serious an effect no one as yet knows. That the need for vitamines is quantitative has been demonstrated within the last few months. The weight and health of animals fed on a diet free from vitamines, but otherwise fully adequate, can be maintained so long as they are supplied each day with a small but definite amount of yeast or wheat-germ or some other substance rich in vitamines. If the daily dosage is gradually reduced, a point is reached at which body weight begins to fall and the health of the animal is impaired. Further reductions in the amount of vitamines are followed more rapidly by these evidences of malnutrition. Body weight and health can be restored at once by increasing the daily supply. While in general, for the animals of a given species, the necessary amount of vitamine-containing material is nearly the same, there are individuals who require a larger or a smaller quantity. Vitamines seem to act as if they were stimulants to the metabolism, and individuals seem sensitive to this stimulus in different degrees. Do not vitamines play a part worthy of consideration in connection with restricted food-supplies?

An apparent example of the mysterious way in which instinct guides human beings to secure a supply of vitamines is shown by those tribes of Eskimos who eat the contents of reindeer stomachs as a delicacy. Doubtless the lack of this necessary element in the Eskimo dietary, which is largely made up of meat and fat, is the reason why the vegetable tissues gathered in their roamings by reindeer, and collected in their stomachs in an easily obtainable form, are regarded by the Eskimos as tidbits.

It is not at all improbable that many delicate people of sedentary habits, who eat but little, suffer chiefly from a deficient supply of vitamines, enough of which in the diet appears to impart physical vigor. Here we may have a clue to the reason for the benefit which exercise seems to confer upon people who otherwise lead physically inactive lives. The more these exercise, the more they eat; hence, the more vitamines they get, the better they feel. Those who never take exercise, but are always well, are perhaps persons so constituted that they react readily to a relatively small proportion of these life-giving substances.

III

How much protein should be included in the daily diet, is a question which has been the subject of contention among physiologists and nutrition experts for a long time, and as yet no agreement appears to be in sight. That those who can afford to buy the expensive foods which supply this element customarily eat more protein than they actually need to maintain their bodies in seemingly good condition, has been demonstrated by the well-known experiments of Chittenden, who showed that men can live for several months without apparent detriment on diets containing about one-half the amount of protein usually eaten. That similar low-protein diets can be used continuously, is shown by the fact that many eastern races habitually live on such.

The low-protein diets of the masses in Japan are unquestionably the result of necessity, for the more prosperous classes in that country provide themselves with foods very similar to those common in America. This change in habits is more likely to be the result of instinct than of a desire to imitate Europeans. It is a matter of common experience that dietary habits which satisfy the promptings of instinct are among the most difficult to change; whereas those which do not satisfy instinct are very easily changed. That more protein should instinctively be eaten than is absolutely necessary, is in accord with the plan of nature of averting danger by providing a factor of safety. Too little protein leads to inevitable disaster, too much (within reasonable limits) can be disposed of without apparent harm.

Physical well-being can be maintained within very wide limits of protein intake. Just where the minimum, and where the maximum lies, is not certain, but that these limits are avoided by normal persons is certain. I have known a number of individuals who lived with enthusiasm for quite a time on low-protein diets, and who thought that their health was thus improved. All but one of these are now eating the normal amount.

There is no denying the fact that mankind in general instinctively eats more protein than the physiologist tells us is needed for actual maintenance. Why should this be so? One reason has been discovered since the experiments were made on which this dictum was founded, and this is, that all proteins do not have the same nutritive value. A quantity which fully suffices for all the bodily needs when one kind of protein is eaten, may be insufficient if another is eaten in its stead. To guard against this danger, we all instinctively eat a variety of foods, hence a variety of proteins; and it is curious how the selection thus made agrees with what our new knowledge shows to be desirable. Experiments have demonstrated that combinations of the cereal proteins with those of milk, meat, or eggs are much more efficient for promoting the growth of young animals, and for renewing the tissues of adults, than are the cereal proteins alone; and these are the very combinations which mankind eats whenever opportunity makes it possible.

Protein is decomposed in the process of digestion into fragments called amino-acids. Nearly all proteins yield in varying proportion eighteen different amino-acids. In some proteins one or more of these may be absent. When new protein is required by the body, for the growth of the young or for the replacement of broken-down tissue in the adult, amino-acids derived from food are recombined into the protein of the new tissue. As the proteins of our food do not contain the same proportion or amount of the different amino-acids needed to construct the new-tissue proteins, there easily may be available too much or too little of any one of them. If any one amino-acid is furnished in too small quantity, then growth or repair will be retarded. The greater the quantity of protein eaten, and the greater the variety, the less danger there is of running short of the necessary quantity of any one essential amino-acid. Whatever surplus may remain is easily disposed of; so that the danger lies on the side of too little protein rather than too much. We must avoid too near an approach to the protein minimum in our diet until we know more about the chemistry of proteins and their true value in nutrition. Our instinct assures us of a margin of safety which is doubtless wider than is necessary, but how much wider, no one knows.

It is not at all improbable that another feature is involved in the question of the protein minimum, for it may well be that the greater efficiency of the meat-eating nations, which has often been used as an argument against a low-protein regimen, may be thus explained. It has long been known that an increase in the amount of protein consumed above that needed to protect the body-tissues from loss of nitrogen is accompanied by an increase in the amount of heat given off by the animal. This occurs only when the protein eaten is greater in quantity than can readily be stored in the body cells. A similar increase in heat-output does not take place when carbohydrates or fats are eaten in quantities above those needed for maintenance. Rubner considered this extra production of heat to be peculiar to proteins, and called it their "specific dynamic action." He assumed that the activities of the body-cells as a whole were constant, and consequently required a constant supply of energy from the food to maintain their normal functions; and that any quantity of protein above what was needed for these normal functions was simply burned up with evolution of heat, but with no effect on the cellular metabolism.

Amino-acids resulting from the digestion of protein cause an extra evolution of heat when fed to animals. This has been interpreted by Lusk as due to a stimulation of metabolism, for the heat developed is greater than could be caused by combustion of the amino-acids supplied.

If protein stimulates metabolism, its effect on the well-being of an organism, especially of one so highly developed and sensitive as man, may well be very considerable. Under the influence of this stimulus the output of work, both physical and mental, may easily be increased. Certainly the known relative efficiency of the meat-eating nations compared with the seed-eating nations of the Orient is not inconsistent with such a possibility. The efficiency frequently shown by men on experimental low-protein diets, which might be cited as evidence against this view, has often been attributed to psychological causes; for the enthusiasm of converts to new cults often leads them to most remarkable accomplishments.

Whatever the truth may be, the instinct of the great majority leads them away from a low-protein diet; and, in view of the many wonderful ways in which instinct saves us from nutritional disasters of other kinds, attention certainly ought to be given to the amount of protein which man instinctively eats when not restricted by available supplies, or by poverty.

IV

Reviewing our recently gained knowledge from the standpoint of one seeking information by which to regulate his own dietary habits, we find that the chemical requirements of nutrition can be met only by the use of a variety of food-products, and that instinct, which impels man to crave this variety, saves him under normal conditions from the dangers involved in a too-restricted choice.

Those of us who habitually eat an unduly large or unduly small proportion of any particular kind of food will do well to alter our habits in this respect, and conform more nearly to the practice of the average American, whose daily ration consists of about three and a half ounces of sugar, four and a half ounces of fat, eight and a half ounces of flour, and three and a half ounces of protein.

The widely different sources that may be drawn on for the protein in this ration permit the needed variety. Protein is furnished by milk, eggs, meat (including poultry and all kinds of sea-food), and, to a limited extent, by vegetables and fruits. Proteins from these different sources do not have equal value in nutrition, but instinct leads the normal man to eat the very combinations which science proves to be the best. Young rats in my laboratory grew very slowly when wheat-flour furnished all the protein of their diet; but when meat, milk, or eggs supplied one third and flour two thirds of the protein, they grew rapidly. Bread and milk, bread and meat (sandwiches), and eggs on toast are combinations evolved by human instinct long before science discovered a chemical explanation of their efficiency. Man's natural desire for a varied diet thus takes account of even the fine points of the chemistry of the proteins.

Lusk has recently published a long list of foods, natural and manufactured, with their retail prices, calculated on the basis of the amount of fuel they furnish to the body for the performance of its daily work. It is curious to see how uniform these prices are for the foods which are eaten chiefly for their fuel-value. A higher, but fairly uniform, price is paid when protein is the chief factor furnished by the food. Far more costly than either of these are the vegetables and fruits which furnish very little that formerly was considered essential for nutrition. This is an impressive demonstration of the accuracy of man's instinctive judgment as to the relative values of the food-products he buys; and when we see how he has learned through instinct to combine the things he eats, and realize the underlying necessity that prompts his apparent extravagance, we cannot fail to be impressed by the very high price that he is willing to pay for vegetables and fruit.

Flour and meat contain relatively little, and sugar and fat contain none, of the vitamines which must be in every ration in sufficient amount, if life is to be sustained. The amount of vitamines contained in milk and eggs is too small to render it probable that they alone will supply enough when consumed in the amounts ordinarily eaten. That man does live and, in general, flourish on the kind of food he instinctively eats, demonstrates beyond question that the supply of vitamines in his usual diet is sufficient for his needs. The only conclusion to be drawn from this is that vegetables or fruits, probably both, supply this most important food-factor, and that for this vital need man is ready to pay a good round price.

At the present moment science can add very little definite information on this most important aspect of our food problem. Until investigations now in progress are completed, we can give only general advice. In the meantime, I believe that instinct is a safe guide, that it is prompting us to eat the kinds of food we should.

In general, we eat very nearly the amount of food that we really need. He who does hard physical work needs to eat more than does the sedentary brain-worker whose labor involves no expenditure of energy that must be supplied by extra food; and so he who works with his brain instinctively eats less than he who works with his muscles. The old belief that different foods were of widely different digestibility has yielded place to the knowledge that what was formerly called indigestion really arises from a failure to completely assimilate the full amount that has been digested. Some foods—sugar, for instance—are so concentrated and so readily digested that it is easy to overload our metabolic processes with the products of their digestion. The muscle-worker can more easily oxidize and dispose of a surplus of food than can the brain-worker. Both need, however, the same kind of food in differing proportions. The sedentary man needs proportionately less sugar, fat, and cereal products than does the muscle-worker.

We are now confronted by restricted supplies, and nearly all of us have been compelled to modify our dietary habits so that we are no longer protected by instinct. While the war lasts, we shall have to adjust our habits to conditions more and more. Already, what and how much we shall eat has become a very practical problem.

Science can help much in meeting this emergency; but, like every other agent which is being employed to win the war, it has its limitations. Unless dietitians fully realize the limits imposed by our present imperfect knowledge, and heed the lessons to be learned from instinct, we shall encounter, not only nutritional difficulties, but serious social discontent.

Fortunately the United States has a Food Administrator, surrounded by a body of expert advisers who are not only alive to all that science can do to aid them in dealing with their serious problems, but are also awake to the necessity of carefully considering the part played by instinct in the food-habits of the individual. Hard times are ahead of us, but we may be sure that such advice as the Food Administrator gives will be the best that any nation has had. No one will suffer in health or efficiency by following his directions. During the war, we must trust him. After the war, we must learn more about this important subject.

MIGRATIONS OF BIRDS[1]

Nature provides many ways by which the rigor of winter may be met by different living beings, few of which follow during this season the course of life pursued in summer.

Most of the quadrupeds hibernate in holes in the ground or in trees from which they rarely come for food until spring, spending the winter in sleep and slowly assimilating the substance of their bodies. The reptiles bury themselves in the ground or in mud beneath water, where they remain out of reach of frost in a state of helpless torpor. Many of the insects which live over the winter present a problem that was brought to my attention one day this winter. Although it is not connected with the subject of bird migration it may be of sufficient interest to consider for a moment.

One cold day in January, a wasp which had in some way become warmed up, crawled on to my laboratory table, having come probably from under the roof of the building. I at once wondered how, under ordinary circumstances, such an insect could find a place where it could pass the winter without freezing, and as I

[1]A paper read before the Friday Night Club, New Haven. This and the following paper are posthumous manuscripts published here to illustrate Dr. Osborne's interest in biological science.

could not believe this possible, I put the wasp into a small bottle with a thermometer and put it out of doors, where the thermometer, whose bulb touched the wasp, soon marked below 20° F. After leaving the wasp at this temperature for a couple of hours, much more than long enough to have frozen the entire bottle full of water, I took it out and, though at first apparently dead, in a few minutes it was flying about the room.

Now the question is, if its fluids were frozen and circulation thereby stopped, why was not vitality entirely suspended, and how, if this was so, could life be resumed on thawing out, unless vitality is nothing more than a chemical phenomenon? That is, given the proper chemical conditions, does not life follow as a consequence? But to return to our subject.

As you know, nearly all of the species of birds breeding in temperate regions go to the south in winter to escape the cold. Only forty-three species are recorded as resident throughout the year in this state and most of these are rare. Of the common species thus resident, by far the greater number of individuals go south in winter or north in summer. For instance, a very few robins and blue birds sometimes stay with us during the winter and a very few chickadees and nuthatches remain during the summer to breed.

What causes the birds that have gone south in winter, where they find plenty of food and comfortable weather, to start north at the approach of spring is a question that has caused much speculation on the part of ornithologists, but has never been satisfactorily explained. It cannot be that the conditions of life, at the south, become unsuitable, for individuals of a majority of the migratory species remain to breed far south of the northern limits to which others of the same species wander in their spring migration.

Many believe that sentiment is the main cause which prompts the bird to undertake the long journey,—in many cases more than a thousand miles—that the bird is impelled by an overpowering love for the home in which it was reared to return each year and renew the pleasant associations of the past. This seems to be an absurd explanation for, so far as I have observed, birds are absolutely devoid of sentiment and affection, their actions being wholly controlled by blind instinct. No birds seem to have more affection for their home, their mates or their young than domestic pigeons, but any one who has studied their habits can manage them in such a way as to make them do many things that an affection of this sort would render impossible.

The true motive leading to migration is an instinct which cannot be explained any more than those instincts which lead a human being to cry with pain or grief, and laugh with joy. It is simply a fact of nature that must be accepted as such. The most striking feature of bird migration is the regularity with which it takes place, a regularity far greater than most people who have not kept annual records of the arrival of birds would realize.

The date at which the first individual of most species appears in the spring varies very little from year to year and is independent of the condition of the season, that is, whether the spring is early or late. Thus, I have noted the arrival of the first oriole for many years and have never known him to come before the fourth of May nor later than the ninth, while in the great majority of years I have seen the first one on the seventh. During these years I have kept a record of the first blossom on a particular cherry tree, which shows that the oriole arrives with a far greater regularity than the flower, the bird varying but five days while the flowers varied fourteen.

It seems at first thought surprising that birds should be so exceedingly regular in their movements, but I think this can be easily explained on purely physiological grounds. The period of gestation and of menstruation among all animals, even when domesticated, are extremely regular, recurring at perfectly definite periods for almost every individual of each species. With birds, definite periods of sexual activity exist for males as well as females, and it seems almost certain that, with the return of the sexual activity, the impulse to migrate develops and incites the bird to undertake the journey. We can thus understand how it happens that they arrive in the spring with such regularity, although we have no explanation of why this sexual activity should cause them to move north. As to the southward migration, this theory, of course, offers no aid, for there is no apparent reason why the bird should be impelled at a certain fixed time to start south when sexual activity ceases.

That each species leaves at nearly the same time in the fall is well established, but that they move with the same regularity as in the spring is not demonstrated. It is far easier to recognize the arrival of the first individual of each species in the spring than the departure of the last, or even of the majority, of a species in the fall. Further, as there must necessarily be many weak individuals remaining, who are unable to take long journeys, their presence would always cause uncertainty in regard to the actual date of fall migration.

Such evidence as we have, however, indicates pretty plainly that the fall migration is more leisurely than the spring, though the great bulk of it seems to take place within a few days. Every sportsman recognizes the fall flight of woodcock, which takes place about the first of November, during which a place that has been hunted thoroughly the night before and found destitute of woodcock may be full of them the next morning and almost any other favorable spot will also abound with them. These flights last but three or four days, and often do not occur, the birds doubtless passing rapidly by and not stopping at all. Such was the case in this vicinity last fall, when almost no woodcock, except those raised about here, were shot by anyone.

In accord with the idea that the birds are started north by a return of sexual activity, we find in many species that the males precede the females by a week or more. Thus, while the meadows may be filled with red-winged blackbirds, one may search in vain for many days before a single female can be found.

During migration most species move at night, though in no sense nocturnal in their habits. Such include most of our smaller birds, as sparrows, warblers, thrushes, vireos, blackbirds and many others, while hawks, swallows, robins, ducks and others, go by day and some go indifferently by night or by day. That birds migrate at night has been proved in a variety of ways. Anyone interested in birds can hardly have failed to hear their calls at night while passing overhead during the season for migration. In this way many species can be recognized by their notes. I have frequently interested myself, when trying to get to sleep, in listening to their calls and trying to identify them.

It is well to know that during migration large numbers of birds are attracted to and killed by flying against lighthouses. In this way much has been learned of their nocturnal movements. From the dead birds found about the lights it is evident that most, perhaps all, the species migrate in flocks of their own kind. Even those species which are never seen in flocks during the day associate in large numbers when they migrate. It is only in cloudy and thick weather that the migrating birds come to the lights, for on clear nights they evidently fly high.

Several interesting observations have been made regarding their mode of travel by watching them with a telescope as they pass across the face of the moon. At Princeton, Mr. Scott (October, 1880) saw a large number of birds through an astronomical telescope fly across the face of the moon at a height estimated to be from one to three miles. These birds passed at the rate of from four to five a minute. Among them many were recognized as warblers, woodpeckers and blackbirds.

Later, F. M. Chapman, also a well-known ornithologist, in three hours saw 262 thus fly across the face of the moon, at heights of from 1,500 to 15,000 feet. Five of these birds were Carolina rails, that were flying at a height of more than 2,000 feet above the earth. To one acquainted with these birds upon the marshes this seems a most extraordinary statement, for Carolina rails are rarely seen to fly at all unless driven from the grass at high tide by pushing a boat almost on top of them. When thus forced to fly, they spring up, fly heavily for a few rods and drop again into the grass. Often it seems as if even this exertion were too much for them, and they so quickly drop upon the marsh as to make it nearly impossible to shoot them.

An interesting account of similar observations was published in the *Auk* by Libby, who watched the migration for three successive nights at Washburn Observatory, September 11, 12 and 13, 1897. During these observations, 583 birds were counted, 358 being seen on one night, and 45 passed during one period of 15 minutes. These observations indicated that the greatest number passed before midnight, and up to this time nearly all the birds were flying directly south. After this hour the direction varied much; birds passed in all directions, though two-thirds still flew south.

If one considers how small a portion of the air is under observation when looking in this manner at the moon, the number of birds that pass over the whole heavens above the United States will far exceed the number of wild pigeons about which you are so skeptical. That birds so small as were a very large proportion of those recognized, should thus fly thousands and thousands of feet up in the air when migrating, is most extraordinary, especially as most of the species noted fly but short distances by day, and seem incapable of sustained flight. It is possible that they find it easier to fly at first nearly perpendicularly until they have reached a great height and then, as it were, slide down hill to their destination. This is indicated by the apparent fact that their migrations high in the air take place early in the night, and that later they are seen flying in directions other than south as though looking for a suitable resting place. Such observations as we have upon the rate at which the migrating flocks advance also make this probable, for it would appear that the daily advance is much less than would be expected, if the birds ascended so high in order, afterwards, to follow a horizontal path.

Brewster has observed that small birds preparing to cross from Point Lepraux to Campobello, about twenty miles away, would mount upwards in a spiral until about 500 feet high and then take a direct course for their destination. This, however, may have been simply for the sake of getting to a better position from which they could see to follow their course. .

In 1884 and 1885 an extended series of observations upon bird migration in the Mississippi valley were undertaken by the division of economic ornithology of the United States Department of Agriculture, a report of which was published by W. W. Cooke. A large number of observers were found who volunteered to keep uniform records of the arrival and departure of birds throughout the valley. At the same time meteorological records were carefully kept and weather maps made upon which the progress of the migrations and the condition of the weather were recorded. The results of this study were not so valuable as might have been expected, apparently largely because many of the observers were either incompetent or had not sufficient time to watch the birds as closely as they should have done. One interesting point, however, seems to have been established, and that is that most of the smaller birds during the spring migration advanced only about 25 to 30 miles a day, a distance much smaller than I had supposed. It also appeared that the bulk of the migration took place when the barometer was falling and the wind was south. As the south wind usually rises when the barometer falls, the former condition was doubtless the chief factor. It was not demonstrated whether the direction of the wind or the warmer weather which usually accompanies a south wind was the cause of the northward movement. The bird migration weather maps showed that northern points were sometimes reached before other points to the south of them, since the birds followed the area of warmer weather with south winds, as it pushed its way north in a sort of peninsula with colder weather lying on both sides of it. These places to the east and west were afterwards filled by the birds coming into them from all sides.

How it is that birds find their way over such great distances is a matter of dispute among ornithologists. The only theory that has many adherents is that they are directed by sight, being guided by rivers, mountain chains and coast lines, which they have learned to follow by having passed over the country before, the young and inexperienced being guided by the older ones. In this country many of the well-known ornithologists hold this view, but in England and on the Continent it is not accepted. This disagreement is due to the fact that, in this country, observers believe that they have proved that most of the older birds go south first, but that enough remain behind to act as guides for the young, while in Europe the contrary is considered to be demonstrated. Of course if the young go first they cannot find the way by recognizing landmarks with which they are not familiar. It would be remarkable if European birds found their way south in a different manner from the American.

There is much in favor of the theory that birds are guided by the sight of natural objects. In the first place, carrier pigeons are believed by all familiar with them to be so guided, and it is necessary to train them for long flights by taking them gradually greater distances from home, and flying them over a course until they become familiar with it. In the second place, it is believed on what is considered good authority that river valleys, mountain ranges and coast lines form the main paths along which most of the migration takes place.

That migrating birds follow the coast, can be seen by anyone here in New Haven who takes the trouble to watch the migrating birds day by day, especially in the fall. In October large numbers of hawks and swallows may frequently be seen passing, all moving west. It is a curious thing that large flocks, especially of hawks, are most often seen when the wind is strong from the northwest. At such times they force their way up into the wind and make evident efforts to avoid being blown to sea. It is supposed that they work up into the wind and fly along the coast well inland in order to avoid being blown out to sea. With a south wind, which might be supposed to blow them inland, they feel no danger and fly freely over the water.

Some think that in migrating birds are governed simply by a sense of direction and that, as savages have a more fully developed sense of direction than civilized men, birds may be still keener. As an illustration, which I have not seen given, but which seems to me a good one, the bee line made by the honey bee flying to its nest, might be mentioned. It does not seem probable that a bee finds its way by observing surrounding objects, but it gets there all the same.

It has been suggested that the magnetic pole in some unexplained way attracts them north, but those who have advanced this theory seem to have forgotten that they go south in the fall. They ought to have extended this theory by supposing that when in a condition of sexual activity they were magnetically attractive, but at other times repulsive.

THOUGHTS ON BIOCHEMISTRY[1]

A few weeks ago the Connecticut Agricultural Experiment Station celebrated the fiftieth anniversary of its founding and in the evening a dinner was given in honor of Dr. Jenkins, who had been Director of the Station for many years and a member of its staff from the beginning. At that dinner I sat next to Professor Johnston and had a very pleasant evening, probably chiefly because he let me do most of the talking. A few days later he came into my office and invited me to attend the exercises in this laboratory to-day. Why he did this to me I don't know, unless, on the basis that misery loves company, he wanted you to suffer as he had done. In reply to his invitation I read him an answer I had just that moment written to Professor H. C. Sherman of Columbia, who had invited me to deliver a lecture on proteins in a course of lectures to be given during the coming summer on "Modern Chemistry."

Even this letter did not get me out of my scrape because Professor Johnston insisted that it was my duty as a member of

[1] An unpublished paper read before the chemistry seminar at the Sterling Chemistry Laboratory of Yale University, New Haven, in November, 1925.

the faculty to take part in at least one university function before I retired. As he told me I might talk on any subject I might choose, I am going to say something about biochemistry, not because I know much about it, but because the experience of a lifetime in the laboratory has given me points of view which I find some chemists do not yet appreciate and which I want you as chemists to think about.

In the first place there ought to be no such thing as "biochemistry," for the problems which the biochemist deals with are in fact *chemical* problems, pure and simple. When I first began my work with proteins, there were no biochemists, those working in this field being called physiological chemists. This was due to the fact that practically all investigators of the chemical aspects of physiology were trained as physiologists primarily and as chemists incidentally. Most of them were not trained to think as chemists and had little appreciation of what the chemist regarded as proof of the chemical identity of the substances they isolated from plant or animal tissues. As a consequence the literature is filled with much that is worthless and much that is of doubtful value.

I remember that, early in my career, my father-in-law, Professor S. W. Johnson, who was the Director of our Experiment Station here, and one of the first professors of chemistry in the Sheffield Scientific School of Yale University, said to me that progress in physiological chemistry would not be made until *chemists* entered this field. This opinion was soon confirmed by Emil Fischer, who solved the problems of the purines, which up to that time had been in a state of confusion. Soon after, he did the same for the sugars and later did more than all others who had been working with proteins to give us an insight into the structure of these complicated molecules. Without question Emil Fischer was the greatest biochemist who ever lived and he was this because he was a great chemist. It is impossible to measure the extent of his contribution to biochemistry. Certainly not the least was the psychological effect produced among organic chemists. Before this time most organic chemists regarded the physiological chemist almost, if not quite, with contempt. They were poor dubs working with messes which rarely yielded crystalline products, and when they did, these poor fellows did not know enough chemistry to identify them. There was much to justify this attitude, but the fault did not lie so much with the physiological chemist as with the organic chemist who was too proud, or too lazy, to help solve the important problems of the chemistry of life.

I can well remember the superior attitude of most of my chemist friends during the early years of my work with proteins. It was not until Emil Fischer began his work in that field that they seemed to regard me as quite fit to associate with. Now what I have said of biochemistry applies to all the other kinds of chemistry, organic, physical, inorganic, agricultural, immuno and the other various branches of chemistry. These are all chemistry, pure and simple, and none is wholly independent of the other; each needs contact with and help from the other, but none more so than biochemistry. In this field we have problems of every kind and of the most extraordinary complexity. Satisfactory progress cannot be made until all kinds of chemists unite in the attack.

The organic chemist can shut himself up in his laboratory and study single problems of structure, or synthesis, and succeed without the help of others. The physical chemist or the inorganic chemist can do likewise, but not so the biochemist; at every turn he meets new problems in the other fellow's field. He must be an intellectual phenomenon who can do much alone. The variety and profundity of the knowledge needed to cope properly with most of even the simple problems is greater than one man can have. Even keeping up with the literature of a limited field takes so much time that there is little left for the laboratory, to say nothing of golf or tennis.

The only escape from these difficulties, as I see it, lies in coöperation, not between individual biochemists, but between groups of real chemists. This is an age of specialists who are rapidly becoming more and more circumscribed in their fields. There is great danger in this, because contacts are becoming fewer and fewer when they ought to be becoming more frequent.

Now what I am coming to is this, that here at Yale we have an opportunity for coöperation in chemistry which could be utilized to great advantage in promoting biochemistry although this would not in any way impede the progress of the several departments in their own fields.

It is quite logical that an effective biochemical group be developed at Yale, for a beginning was made here before the Sheffield Scientific School was founded. As early as 1847, J. P. Norton, who came here to be a professor of chemistry in Benjamin Silliman's laboratory, was engaged in studying chemical problems of agricultural interest. Norton was a man of no mean ability and, had he not died as a young man, would have filled an important place in the history of biochemistry. He was followed by Professor S. W. Johnson who was trained by Erdmann, von Kobel, Liebig and other leading European chemists. Johnson's interest in chemistry was chiefly in its relation to agriculture and it was largely through his efforts that the first American agricultural experiment station was established in Connecticut. For several years the laboratories of this station were furnished by the Scientific School in South Sheffield Hall. Although Johnson would not be called an agricultural chemist, he was a true chemist in every sense of the term. Chittenden, one of Johnson's pupils, for many years brought fame to Yale through his many researches in physiological chemistry. More recently Wheeler, Johnson (T. B.), Mendel, Underhill and Henderson have done their full share in maintaining the traditions of Yale in this field of science. This work is now being continued by the younger associates of these men with every promise that the high standards of the past will be maintained in the future.

You thus see that we have here at Yale a biochemical background continuing without a break for more than 75 years. A long time; for remember we have not yet celebrated the 100th anniversary of the first organic synthesis, Wöhler's production of urea being the first demonstration that a natural organic substance could be produced in the laboratory.

As an old man, I might be content with this recital of past achievements, but I am not yet too old to look forward to the possibilities of the future with more satisfaction than I do to the achievements of the past.

It seems to me that we have here in New Haven exceptional opportunities which have not yet been developed. We have a fine chemical laboratory equipped for work in all branches of pure chemistry, we have another laboratory in the Sterling Hall of Medicine equipped for work in biochemistry and we have a small laboratory at the Experiment Station with special equipment for work in protein chemistry. In these various laboratories we have men who are masters in their various fields, capable of conducting research of a high order of excellence. Heretofore all these laboratories have coöperated in a friendly spirit, but I do not think this coöperation has reached the limits of its efficiency. I recognize that each laboratory has its own problems in which it is especially interested and I do not suggest that anything should be considered which would interfere with work now in progress.

It has occurred to me that it might be possible for us all to get together some time and see what might be done to help each other. A beginning might be made in arranging the subjects for theses of some of the graduate students so that their work might fit into a scheme which, as it developed, would contribute to the solution of some of the problems which are already the subject of continuing investigations. Thus in my own field there are many lines which we are unable to deal with, both through lack of knowledge and experience, as well as through lack of facilities and personnel.

For example, we greatly need to know the physical constants of the amino-acids. We can make reasonable quantities of several of these, but we are not in a position to study them properly. We cannot expect the department of physical chemistry to undertake such investigations unless a suitable supply of amino-acids is available. Might it not be possible to give one or more graduate students a problem in this field and let us supply him with material and help him in any way we can with such knowledge and experience as we may have? I believe that if a student had a problem of this kind which he knew might yield results of definite use to those working in protein chemistry, he would be more interested in it than if he had a problem of only general interest. Furthermore it would bring him in contact with those working along lines quite different from those he encounters in the laboratory of physical chemistry, and so give him a fuller realization of the importance of his work. In this way some good physical chemists might get interested in the biological field, in which there are a multitude of problems for them to study.

In a similar way we need to know much that the organic chemist can find out for us. Very little work has been done with most of the amino-acids and here is a large field in which the organic chemist can do most useful work. In our attempts to separate quantitatively the products of hydrolysis of proteins we are constantly blocked by a lack of knowledge of properties of the various amino-acids. Now that most of these products are known qualitatively, it ought to be possible to make derivatives which would enable us to estimate quantitatively some of them more accurately than is now possible. As an example, if we could find a way to separate leucine and phenylalanine, or to estimate phenylalanine in a mixture of these two amino-acids, the whole process of analysis could be greatly simplified. Also the oxyamino-acids present difficulties which I feel sure a better knowledge of their properties would do much to overcome. Serine, oxyproline and oxyglutaminic acid are important constituents of many proteins, but these have been so little studied that at present we have no means for determining, even in the crudest way, the amount of any one of these yielded by a protein. From this you can easily appreciate that there is a great deal of purely chemical work to be done by the organic chemist before the biochemist is in a position to do what he is now attempting to.

Beside the chemical questions which group themselves about the proteins, there are multitudes of others which concern the environment in which the protein exists within the living cell. Although physiologists have long been interested in this subject, almost nothing supported by chemical evidence has been learned. After the necessity in nutrition of those substances now called vitamines was recognized, it became important to know more about the soluble constituents of living cells, because some of these are the so-called vitamines. Having been attracted to this subject by the need of preparations for feeding which would introduce into the diet as little as possible of constituents of unknown nature, I was soon brought face to face with the problem of cell chemistry. This same problem was presented from another side when we attempted to isolate protein from green leaves. In this work we found it possible to obtain the juice of the leaves free from all of the formed elements which seemingly exist in a colloidal state, presumably for the most part protoplasm. Since, for several reasons, we wanted to know the nature of the substances present in this juice, our laboratory undertook to apply available methods for isolating definite substances from it. While an enormous number of well-defined substances have been isolated from one plant or another, apparently no one has ever attempted to get even an approximate idea of the relative proportions of the various groups of compounds which might be present in a single plant. The same applies equally to animal tissues. In other words we have at present almost no knowledge of cell chemistry, the very foundation of both plant and animal physiology.

For the past three or four years we have been working at this subject, applying all the available methods with astonishingly little success; a result we foresaw, because we looked at the problem from a purely chemical standpoint and realized the difficulties to be encountered. One immediate aim, however, was to demonstrate to those of our fellow workers, especially in the experiment stations, how futile it usually is to continue making so-called analyses by indirect methods supposed to show the chemical nature of the materials analyzed, when in fact they furnish no evidence whatever that a real chemist would consider for a moment. I call our work from this aspect a fool-killing research, from which, if nothing else results than the saving of time and money at present going into the accumulation of comparatively worthless data, I feel that our work will be worth all it costs. However, I am much more hopeful than this, because I have a firm faith that good work in a field of such importance to science cannot help but prove to be of ultimate value. We also hoped that when our results demonstrated how appallingly little is known of the constituents of cells, other chemists might be stimulated to help us. I think that Professor T. B. Johnson appreciates this point of view, because in his work with the chemistry of the tubercle bacillus he has encountered the same difficulties.

Until a beginning has been made to explore such fields, the nature of the problems in organic and physical chemistry which must be studied will never be appreciated. The kind of work needed requires men of special training, each in his own field, but with contacts with each other that will not only make their efforts effective, but interesting and inspiring.

The biochemist, that is, the chemist who does this pioneer work of exploration, seldom has the technical experience to deal as successfully with his many problems as those who are trained along narrower lines. He has so many kinds of questions to struggle with that he is usually a Jack-of-all-trades and master of none. He needs the sympathetic help of specialists, not their contempt, which in the past has so frequently been accorded him.

In concluding my appeal for help I want you to understand that I am not addressing it to the heads of the departments, or to the professors and instructors only, but to the students as well. Unless the student is interested, little in the way of coöperation can be expected, for those participating must have a real interest in the work. If the student is really interested he can force a reluctant professor to aid him in attaining his ambition far more easily than the professor can force a reluctant student.

Just how coöperation can be secured remains to be determined. My idea is that at first one or more students might be given some of the simpler problems for their thesis work. The experience which the student gains will inevitably be shared by his instructor who, another year, may find himself in a better position to direct the work of other students. In this way it seems to me that a mutual interest in each other's work will develop between the different professors and instructors and that these will cause a bond of union in their work which will do much not only to stimulate research in the department of chemistry, but to contribute to the application of chemistry to biology.

There are two aspects of engaging in coöperative work of this kind that deserve consideration. First, the relation of such research work to the future career of the student. For the organic chemist and doubtless also for the physical chemist some of the simpler problems may be just as good as any other as far as giving him experience is concerned. If his ambition is to specialize later along biochemical lines, either as a teacher of premedical courses or in medical schools, such a subject is eminently suitable. There are, however, relatively few positions in this field which offer inviting possibilities of reward, hence the student who specializes too narrowly in such work runs some risk of disappointment. There is an increasing demand in research institutes and medical schools for men who are capable of doing good work and perhaps the chance of success for such men as are really competent is as good as in any other field of scientific research.

This brings us to the second consideration, namely, what provision can be made for the post Ph.D. student who attempts such work? Good research work on many of the most important biochemical problems will not allow much, if any, time for teaching or the other duties for which the new fledged Ph.D. is usually paid. I believe that if a start can be made with graduate students working on biochemical subjects which contribute to the progress of some major continuing investigation, it will be possible to secure funds sufficient properly to endow fellowships and to support their work. If we here at Yale are in a position to demonstrate that the appointees to such fellowships can make good, I do not believe it will be hard to secure the necessary funds. My own experience has been that financial support followed accomplishments about as fast as I was in a proper position to receive it.

NOTES ON THE NEST BUILDING HABITS OF THE PIPE ORGAN WASP[1]

The nest consisting of three tubes cemented together is a part of one of originally five tubes, two of which were broken when removed from a partition in a stable at Washington, Conn., about August 22, 1916. This was fastened to a smooth board in a nearly vertical position with the opening downwards, as indicated in the drawing. About three feet away another nest was being built. When first noticed, this was about one inch long, at 11 A. M. Two wasps were engaged in constructing this nest. One, smaller than the other, and apparently browner in color, was inside; the other, black with a blue lustre, worked on the outside, collecting clay and putting each portion in place. The clay was brought moulded into a strip about three-fourths inch long and one-sixteenth inch in diameter. This strip, as near as I could see, was carried on the front leg and held by one end with the jaws. This end was applied to the crotch at the median line of the nest and then rapidly attached to the edge of the piece which had previously been put in place. Apparently it was moulded by the jaws and pressed into position until the end of the strip reached the end of the preceding portion and there came into contact with the board to which the nest was attached. The end was then flattened out against the board, the jaws and fore feet being used to make it adhere firmly. During this process, the wasp inside made a buzzing sound and seemed to be engaged in maintaining a proper sized bore for the tube under construction. When the clay was attached the larger wasp went inside the tube and the two set up a loud buzzing for a few seconds. It then came out and flew away to return after two or three minutes with another strip of

[1] During the summer of 1916, Dr. Osborne, head of the Department of Biochemistry of this Station, spent his vacation in Washington, Conn., and became interested in watching some mud wasps construct their nest on a board of the barn. On returning to New Haven he brought me one of the wasps, the nests, and the notes and said that I might use them in any way I saw fit. The wasp was identified by Dr. H. T. Fernald, Amherst, Mass., as *Trypoxylon albitarse* Fabr. (male). The nests were provisioned with spiders which were sent to Mr. J. H. Emerton, Boston, Mass., who reported as follows: "The spiders from wasps nests are all *Epeira trivittata* Keyserling, all females, and half of them adults. They make round webs and live in the tall grass and bushes all over the country." W. E. Britton.

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clay which was put in place on the opposite edge of the opening of the nest. This process was continued. Each time the outside wasp returned it struck the wall about three or four feet from the nest, but located the nest after a few seconds by flying about on one side or the other, coming gradually nearer each time as though attracted to it by some invisible force. After every four or five trips by the outside wasp the inside wasp would come out and fly away. When it returned it struck the wall much further away than did the outside wasp and had more difficulty in finding the nest. Whether or not it brought anything with it I could not see. At any rate, whatever it carried was very small. On returning, this wasp went directly into the nest and stayed there until the outside wasp had put three or four more strips of clay in place. I did not see the first tube finished or the second tube begun for I had to go away when the former was about two inches long. About 9 A. M. the next morning the first tube was approximately three inches long and a second tube had been made which was about one and a half inches long when I first saw it. I watched the construction of the second tube until 11 A. M. when it was as long as the first. When it reached this length the inside wasp set up a very loud buzzing when the outside wasp came back with more clay and apparently made such a fuss that it did not dare attempt to put the clay in place. After attempting several times to do so the outside wasp flew off with the clay and came back again, after a few minutes, apparently without any clay and went inside the nest. It then made regular trips coming back with such small quantities of clay that I was unable to see that it carried anything. When it entered the nest, clay must have been brought because after several trips a partition had been built across the lower end of the tube. I did not see any spiders put into the tube before the partition was completed, but I did not watch them continuously and this may have been done while I was away or it may have been done during the trips of the inside wasp, but I think I would have seen the spider when it came back to the nest if it brought any. A heavy thunder shower put an end to the work for the day. The next day, no more work seemed to be done and the outside wasp was not seen. One, or both, were in the nest in the afternoon as evidenced by the buzzing which followed on putting a straw into the tube. The tube first made had a partition about one-half inch from its mouth. It probably had two others lower down. Both tubes were attached to the wall in a vertical position with the mouth opening downwards.

HONORS AND MEMBERSHIPS

THE GOLD MEDAL OF THE PARIS EXPOSITION, 1900

In 1900 Dr. Osborne sent a number of preparations of seed proteins to the Paris Exposition. He received a gold medal with the following diploma.

RÉPUBLIQUE FRANÇAISE

MINISTÈRE DU COMMERCE, DE L'INDUSTRIE, DES POSTES ET DES TÉLÉGRAPHES

EXPOSITION UNIVERSELLE DE 1900

LE JURY INTERNATIONAL DES RÉCOMPENSES DÉCERNE UN DIPLÔME DE

MÉDAILLE D'OR

à Monsieur *T. B. OSBORNE,* STATION AGRONOMIQUE DE CONNECTICUT, À NEWHAVEN

GROUPE VII.—CLASSE 38. ETATS-UNIS.

LE COMMISSAIRE GÉNÉRAL
A. Picard

LE MINISTRE DU COMMERCE, DE L'INDUSTRIE, DES POSTES ET DES TÉLÉGRAPHES
A. Millerand

PARIS, LE 18 AOÛT 1900

HONORARY DEGREE FROM YALE UNIVERSITY

The degree of Doctor of Science *honoris causa* was awarded to Dr. Osborne on June 22, 1910. The presentation by the Public Orator was made in the following words:

"It is wholly a New Haven life which is honored in Dr. Osborne. Here he was born, here he was educated, and here for twenty-four years, as chemist at the Connecticut Agricultural Station, he has patiently carried on those laborious and minutely exacting studies on the albuminous constituents of plant forms and the chemical structure of the proteins which have received the support of the Carnegie Institution and found wide recognition both in America and Europe. He sacrificed the certainty of large financial gains for a secluded life spent with rare devotion in the solution of the most intricate and difficult scientific problems. His success has brought no pecuniary rewards and little public heralding, but is all the more genuine. An honorary degree cannot restore to him the library, the laboratory equipment, or the invaluable preparations which perished in the recent fire at the Station; but it can assure him that his University, his teachers and his townsmen are proud of him."

HONORARY FELLOWSHIP OF THE CHEMICAL SOCIETY

In March, 1912, Dr. Osborne received the following communication:

CHEMICAL SOCIETY

BURLINGTON HOUSE,
Piccadilly, London W.,
March 7th, 1912.

SIR,

I have the honour to inform you that at a meeting of the Chemical Society held this day you were elected an Honorary and Foreign Member of that Body.

I have the honour to be, Sir,

Your Obedient Servant,

ARTHUR W. CROSSLEY,
Honorary Secretary

To
Dr. Thomas Burr Osborne
Newhaven (Conn.)

THE JOHN SCOTT MEDAL

At a meeting of the Board of Directors of City Trusts (Philadelphia) held March 8, 1922, the following resolution was adopted:

Resolved—Upon the recommendation of the Advisory Committee, that the John Scott Medal and Certificate, with Premium of $800, be awarded to Thomas B. Osborne, for "his researches on the constitution of the vegetable proteins."

HISTORY OF THE MEDAL

The John Scott Medal Fund was established under the will of John Scott of Edinburgh, Scotland, and is administered under a

Power of Attorney dated April 2, 1816. The will contains the provision that ". . . . the interests and dividends . . . . be laid out in premiums to be distributed among ingenious men and women who make useful inventions; but no one of such premiums to exceed twenty dollars, and along with which shall be given a copper medal with this inscription 'To the most deserving.' "

A decree of the Court of Common Pleas of Philadelphia states: "And now, this nineteenth day of February, A.D. 1919, the Report of the Master having been duly filed and no exceptions having been taken thereto, it is adjudged and decreed that the same be confirmed, and that the Board of Directors of City Trusts, having in charge the Trust created under the will of John Scott, deceased, be authorized and directed in the administration of said Fund to distribute the income arising from the Fund as it stands with its accumulations as of the date of this Decree, in premiums to be distributed among ingenious men and women who make useful inventions, but no one of such premiums to exceed Eight Hundred Dollars ($800.00) in value; and along with such premium shall be given a copper medal with this inscription 'To the most deserving' conformably to the tenor of the Will of the said Testator.

"It is further ordered and decreed that in the selection of the recipients, the said Trustees shall be at liberty to make such rules and regulations for enabling them to make a wise selection of beneficiaries either by the selection of an advisory board or otherwise, as they may deem best. The premiums shall be awarded for useful inventions which shall include any inventions that will be useful to mankind in the advancement of chemical, medical or any other science or in the development of industry in any form; the test being that the invention is, in the judgment of the Trustees, definitely accomplished, and that it may add to the comfort, welfare, and happiness of mankind."

A resolution adopted by the Board of Directors of City Trusts states:

Resolved—That the award of medals under the John Scott Medal Fund be made hereafter upon the recommendation of an Advisory Board, to consist of five persons, to be appointed by the Board of Directors of City Trusts; three to be nominated by the National Academy of Sciences, one by the University of Pennsylvania, and one by the American Philosophical Society.

This Advisory Committee is constituted as follows:

National Academy of Sciences—H. H. Donaldson, Theobald Smith, W. B. Scott;

University of Pennsylvania—Arthur W. Goodspeed;

American Philosophical Society—Samuel M. Vauclain.

THE THOMAS BURR OSBORNE MEDAL

ADDRESS OF THE PRESIDENT OF THE AMERICAN ASSOCIATION OF CEREAL CHEMISTS AT THE PRESENTATION OF THE THOMAS BURR OSBORNE MEDAL

JUNE 7, 1928

It has long been the desire of many of the members of the American Association of Cereal Chemists to honor those scientists who have contributed signally to the advancement of our knowledge in this field of specialization. This desire took definite form at the time of the 1926 convention in Denver when President Clark proposed in his presidential address that provision be made for the presentation of a medal to be periodically awarded to those who contribute unusual papers based upon cereal chemistry research. The association referred this recommendation to the executive committee which was empowered to outline a plan and make the other necessary arrangements for the award of such a medal. The executive committee gave careful attention to this new and important project and it was decided that rather than award such a medal at regular stated intervals, the award be made only at such times as were justified by unusually meritorious contribution. The president of the association was requested to appoint a jury of awards whose duty it should be to scrutinize the development in cereal chemistry and select the medalists. The promulgation of rules governing the basis on which awards are to be made was delegated to this jury, and in all matters respecting the medal their decision was to be final.

Basing his action upon the recommendations of the executive committee, President Clark appointed the following jury of awards:

Dr. Carl L. Alsberg, Chairman, The Food Research Institute, Stanford University, California,

A. W. Alcock, Western Canada Flour Mills Co., Ltd., Winnipeg, Canada,

Dr. R. A. Gortner, University of Minnesota, University Farm, St. Paul, Minnesota,

Paul Logue, The Provident Chemical Works, St. Louis, Missouri,

Washington Platt, The Merrell-Soule Co., Syracuse, New York.

The executive committee also decided that it would be appropriate to name this medal in honor of some well-known American chemist who had made notable contributions to cereal chemistry. The jury of awards took this recommendation under advisement and later requested of Dr. Thomas Burr Osborne the privilege of naming the medal in his honor. Dr. Osborne very graciously acceded to this request with the result that this medal will be known for all time as the Thomas Burr Osborne medal.

Later the jury selected Dr. Osborne to be the first recipient of the medal which has been named in his honor. This happy selection has very evidently proved acceptable to the members of the Association.

Dr. Alsberg, chairman of the jury of awards, engaged an artist to prepare the design of the medal. From this design the necessary dies were manufactured under Dr. Alsberg's direction. The medal bears the medallion portrait of Dr. Osborne in low relief on the obverse, and a suitable inscription on the reverse. In notifying Dr. Osborne that this medal had been awarded to him, he was cordially invited to attend this convention at Minneapolis as a guest of our Association, but to our regret he was forced to decline the invitation because of ill health. He designated as a proxy to represent him on this occasion, Dr. Carl L. Alsberg, who accordingly has come here to-day as the personal representative of Dr. Osborne.

Dr. Alsberg, will you please come forward. In behalf of The American Association of Cereal Chemists I take great pleasure in handing you this gold medal which you in turn will present to Dr. Osborne with our best wishes.

LESLIE R. OLSEN.

ACCEPTANCE OF THE MEDAL

Mr. President and Members of the American Association of Cereal Chemists.

I greatly regret that conditions are such that I must delegate to another the acceptance of the medal with which you have honored me. This recognition from your Association is particularly gratifying to me in view of the almost total lack of recognition my work received during the many years in which I was chiefly occupied in studying the proteins of the cereals. If it had not been for the warm support of Professor S. W. Johnson, then director of the Connecticut Agricultural Experiment Station, and that of the later director, E. H. Jenkins, as well as my own firm conviction that anything that could be learned about the chemistry of cereals was important, I am quite sure I should have been discouraged. During those years none of my fellow chemists seemed to take any interest in my work; in fact many of them intimated pretty plainly that I was wasting my time working in a hopeless field. Consequently you can realize that I am greatly pleased to find that, as my active life is closing, your Association thinks that what I have done is so well worth while as not only to deserve a gold medal, but one named after me, and of which I am the first recipient. Gentlemen, I thank you.

As I contrast the conditions prevailing in agricultural chemistry today with those prevailing at the time I first began to work with cereals, and realize that now the workers in cereal chemistry alone are sufficiently numerous to form your relatively large Association, I can hardly believe that this has all come to pass. When my first papers on the vegetable proteins were written, we did not dare print them in the reports of the Connecticut Agricultural Experiment Station lest, when the question of our appropriation should come up, some witty member of the legislature should ridicule the papers and their author on the ground that there was no apparently practical application of the results that would be useful to the farmer. Those first papers were published in the *American Chemical Journal,* edited by Ira Remsen. After he had received three or four of them he plainly intimated that more would not be received with joy. Then I sent the next to the *Journal of the American Chemical Society,* which accepted it with some signs of appreciation. This encouraged me and I continued to publish there until the *Journal of Biological Chemistry* was founded. Since then my papers have appeared in this publication.

It is interesting to recall that the first real signs of appreciation of this line of work came from Germany, where all of the papers which I had printed during several years were gathered together, translated into German and published in a volume. After that it was much easier to carry on, and more and more chemists became interested in this work. I must confess that I had not found it easy to be working in such an isolated field and the reception of some recognition was decidedly helpful. Looking back to this beginning, you can readily see how gratifying it is to me to receive this medal today.

I have no recently completed research to offer you, according to the custom on occasions like this. Instead, I wish to call your attention to some present-day problems in the work you are engaged in. Under conditions prevailing today it is far easier to devote oneself to research in a field that offers little promise of immediate practical application than it was when I began, and I therefore hope that what I have said of my experience will inspire other workers to take up new lines of work with the confident expectation that whatever may be learned and definitely established in regard to the chemistry of cereals cannot fail sooner or later to be of use and to be worth all that it costs in time and money. When I see in how many different directions the results of the work which I started have been applied, I am astonished. Many of these applications have been in fields in which these results could not have been expected to be of any use. This is particularly true in their application to problems in medical science. In fact for many years men engaged in research in the biological sciences were the first to make real use of my work, and I found myself better known among biologists and physicians than among agricultural chemists.

In the field of cereal chemistry there are still many problems awaiting investigation. With modern facilities in experiment stations, research institutions and universities, and improved apparatus and available funds, it ought to be possible to learn much that will be useful and of broad application. We do not yet know by any means as much about the proteins of the cereals as we ought to know. This is particularly true of wheat gluten.

The nitrogen content of thoroughly washed gluten indicates a larger proportion of non-protein substance than has as yet been accounted for. It is possible that a part of this may be combined with protein as a compound neither basic or acid, and hence not soluble in either dilute acids or alkalies. That such a protein complex may occur in the seeds of cereals is indicated by the relatively small proportion of the nitrogen which can be brought into a clear solution with alkalies. We have heretofore assumed that the protein precipitated by neutralizing such a solution made from wheat gluten represents the whole of the protein insoluble in alcohol which has been designated wheat glutelin, but that such is the case has, by no means, been proved.

My former colleague, Dr. D. B. Jones, has recently made a study of the glutelins in several of the cereals and has obtained such small yields of purified preparations that it seems hardly credible that these represent all of the residual nitrogen, unless a very considerable part of the protein of the cereals exists in some such combination as I have suggested.

It ought to be possible to get some evidence as to the approximate proportion of the residual protein nitrogen by properly conducted hydrolyses. Heretofore, when studying proteins we have all tried, as far as possible, to avoid causing any changes in them through the action of reagents. Here we seem to have a case where perhaps we could learn something by the opposite procedure. Now that we know so much about the products of hydrolysis of proteins it is possible that we might get some good indirect evidence as to how much of the relatively insoluble nitrogen may belong to protein by studying these products. Through such a procedure we might learn something more about those proteins of which we now know so little.

The first cereal with which I worked was the oat kernel and I have long wished that someone would explain the unusual behavior of the proteins of this seed. In all my experience I have never found a seed containing proteins behaving towards solvents as these do.

Proteins are by no means the only constituents of cereals that are important. Now that we know the part played in nutrition by insignificant quantities of the so-called vitamines, we must isolate and chemically define every possible constituent of the cereals. Those of you connected with the industries may have exceptionally good facilities to procure material for special studies that is better than any heretofore obtainable.

The embryos of wheat and corn are full of all kinds of things the nature of which is little known. These two products afford opportunity for chemical research of the highest order and both scientific and practical importance. Your Association of Cereal Chemists is in a position to promote such investigations successfully. In these days of modern science coöperation is essential. It is no longer possible in such a field as yours for one man to work alone effectively. Cereal chemistry involves far more than one would at first suppose, for not only are carbohydrates, proteins, fats and inorganic salts included in this field but also many other groups of substances which occur in smaller proportions.

The importance of a knowledge of the chemistry of these minor constituents of our food has only recently been appreciated. What is it in the wheat germ that renders an animal fertile which would otherwise be sterile? Why is the commercial wheat germ meal rich in the so-called vitamine B, while the pure germ appears to be practically destitute of this essential food factor?

Here are only two of the many problems that the cereal chemist has before him and these have not only scientific, but practical importance. To solve these problems requires the coöperation of many among you who have exceptional opportunities both in chemical training and mechanical facilities.

Chemists have done much for the industries and it is now time that the industries should appreciate this and be generous in paying their debt.

Gentlemen, I again thank you for the honor you have done me and congratulate you on the opportunities that lie before you for important contributions to science and industry.

T. B. Osborne.

MEMBERSHIPS

Dr. Osborne was a member or a fellow of the following learned societies:

American Chemical Society,
American Physiological Society,
American Society of Biological Chemists,
Society for Experimental Biology and Medicine,
National Academy of Sciences,
The Chemical Society,
American Academy of Arts and Sciences,
American Association for the Advancement of Science,
Société Royale des Sciences Médicale et Naturelles de Bruxelles,
American Philosophical Society,
Die kaiserlich deutsche Akademie der Naturforscher zu Halle,
Sigma Xi.

RETIREMENT

LETTER FROM THE STATION STAFF

Dr. Osborne retired from the active direction of the Biochemical Laboratory of the Connecticut Agricultural Experiment Station on June 30, 1928. The following action was taken by the Station staff:

Dr. Thomas B. Osborne,
Connecticut Agricultural Experiment Station,
New Haven, Conn.

Dear Doctor Osborne:

We have recently learned of your expressed desire to be relieved of active direction of the Department of Biochemistry and to continue service in the capacity of Consulting Biochemist. This we understand has now been arranged.

Since this arrangement does not remove you from our midst, but merely marks a change from one form of activity to another, it does not greatly disturb us. But it does give us an opportunity to express our appreciation of your brilliant career in your chosen field of endeavor. We are proud of your long record of outstanding scientific achievement and of your fine example of devoted public service.

But we are moved by feelings far deeper than those of mere pride in your success. Above all we cherish that friendship which has grown up between us and endured so long; and we rejoice in the prospect of a long continuance of those personal contacts which have been so uniformly pleasant in the past. We hold you in our admiration for what you have done, and in our esteem for what you are.

You have our best wishes for complete enjoyment of that leisure which you richly deserve and which lightened responsibility will bring.

Very sincerely yours,

YOUR FRIENDS AND COLLEAGUES ON
THE STATION STAFF

October, 1928.

MINUTE OF THE BOARD OF CONTROL

In the retirement of Thomas Burr Osborne from active charge of the Biochemical Laboratory, the Connecticut Agricultural Experiment Station loses one of the ablest and most valued members of its Staff.

In the forty-two years which he has served on the Staff, he has won distinction for himself and the Station, and he is today one of the acknowledged leaders in his chosen fields of study, the structure of proteins and the newer aspects of nutrition. His mind has always been raising questions which he was able to define with rare precision and then with equal discernment he has devised means for their experimental investigation and solution.

The members of the Board, in testimony of their recognition of his valued services, of their respect for his abilities, and of their high personal esteem, enter on their records this minute of their hearty appreciation. The members of the Board further rejoice that from time to time the Station may still have the benefit of his personal suggestions and advice.

G. A. Hopson,
Secretary.

Adopted by the Board
October 25, 1928.

OBITUARIES

MINUTE ADOPTED BY THE STATION COUNCIL

The death of Dr. Thomas Burr Osborne on January 29 brought deep sorrow to his friends and colleagues on the Station staff. Identified with this Station during all of his long and productive career, his conspicuous achievements in a difficult field have reflected great credit upon this institution which he has served so well.

In 1889 at the suggestion of Professor Samuel W. Johnson, then Director of the Station, he undertook a study of the nitrogenous substances contained in the kernels of oats. This marked the beginning of an intensive and sustained study of vegetable proteins which was to become the major project of his life work. For almost forty years he devoted his creative genius, his analytical skill and his powers of critical interpretation to the intricate problems of protein chemistry and of animal nutrition. Two hundred and forty-nine published papers and monographs, many of them of a pioneer character, represent the fruits of his labours. This vast and important work from the time of its humble beginning to its brilliant conclusion was pursued in all modesty and in keeping with the best traditions of unselfish public service.

Abundant recognition of his substantial contributions to the advancement of scientific knowledge has come to him and to the Station at the hands of colleagues in his own field of endeavor, both at home and abroad; and it is most gratifying to us all that these testimonials came while he could enjoy them as he deserved to do.

We who have been associated with him in his active service do not fail to appreciate his scientific worth; but above that comes our deeper sense of personal loss born of years of pleasant associations and of our esteem for the many admirable qualities of the man.

February 4, 1929.

CARNEGIE INSTITUTION OF WASHINGTON

REPORT OF THE PRESIDENT[1]

Through the death of Thomas B. Osborne on January 29, 1929, the Institution loses one of its most distinguished associates in research. Dr. Osborne was connected with work of the Institution from May 1902 until his retirement from active investigation

[1] Reprinted from Year Book No. 28, 2 (1929) by permission.

in July 1928. He was a pioneer in the development of our knowledge of the chemistry of the cell, and the nature and nutritive properties of vegetable proteins.

The thoroughness with which Dr. Osborne and his associates carried on their investigations brought results of exceptional scientific value. His accomplishments greatly advanced the scope and improved the methods of biochemistry and laid foundations for the important work now being continued by Dr. Lafayette Mendel and Dr. Hubert Bradford Vickery.

It is interesting to note that the studies through which Dr. Osborne and his colleagues have so ably advanced physiological chemistry have now direct relation to the biochemical investigations included in the program of the newly organized Division of Plant Biology.

JOURNAL OF BIOLOGICAL CHEMISTRY[1]

We regret to record the death, on January 29th, 1929, of Thomas Burr Osborne who was associated in an advisory editorial relationship with *The Journal of Biological Chemistry* since its foundation in 1906 and became a member of the Editorial Committee when the latter assumed responsibility for the general conduct of the Journal.

Dr. Osborne was born in New Haven, Connecticut, on August 5th, 1859, the son of Arthur D. Osborne (B.A., Yale 1848) and Frances Louisa Blake Osborne, daughter of Eli Whitney Blake, of New Haven. Thomas B. Osborne was prepared for college in the Hopkins Grammar School, and was graduated from Yale College with the degree of B.A. in 1881. He received the degree of Ph.D. from Yale in 1885, after having pursued postgraduate studies in chemistry there. The honorary degree of Doctor of Science was conferred upon him by his Alma Mater in 1910.

Dr. Osborne's earliest activities in the academic field were in the domain of analytical chemistry. He became a member of the Research Staff of the Connecticut Agricultural Experiment Station at New Haven in May, 1886, continuing active association therewith until his retirement last year. Throughout his long scientific career Dr. Osborne retained his affiliation with the Connecticut Station, during most of this period being in charge of a laboratory specially devoted to his own research interests.

Dr. Osborne's name will long remain associated with his pioneer investigations of proteins of plant origin. The first contribution (1890), dealing with the albuminous components of the oat kernel, was followed in rapid succession by a series of related studies which were summarized in his classic monograph on

[1] Reprinted from **81** (1929) by permission of the editor.

The Vegetable Proteins, in 1909. A revised edition appeared in 1924. The descriptive consideration of a large group of plant proteins was followed by investigations of the amino-acid derivatives of various purified proteins. Presently Dr. Osborne enlarged the scope of his research interests to include the biological properties of these substances. In collaboration with Lafayette B. Mendel and with continued generous support of the Carnegie Institution of Washington, of which he was a Research Associate since 1904, he directed some of his energies to the problems of nutrition, from 1910 until the time of his death.

During his scientific career Dr. Osborne published 253 papers and monographs, most of which deal with proteins and their derivatives. He was appointed a Research Associate of Yale University with professorial rank in 1923.

Recognition came to him from many sources through election to learned societies and through the award of medals for scientific distinction. The soundness of his chemical work was recognized by his election as an Honorary Fellow of the Chemical Society of London. Within the past year the American Association of Cereal Chemists instituted the periodical award of the Thomas Burr Osborne medal for distinction in the field of cereal chemistry research, Dr. Osborne himself being the first recipient.

Those who were privileged to enjoy a personal acquaintance with Dr. Osborne soon learned to appreciate his many sterling qualities as a man. An unusual enthusiasm for research which exhibited itself in more than one field of human inquiry; neverfailing patience in the execution of experimental work; rigorous critique applied to his own results as well as the work of others; vigorous opposition to sham in any form; rare appreciation of the best contributions to science and industry; delightful companionship and deep loyalty to his friends—these were some of the characteristics of our respected colleague.

MINUTE ADOPTED BY THE BOARD OF DIRECTORS OF THE SECOND NATIONAL BANK OF NEW HAVEN[1]

It is with great sorrow that we record in our minutes the death of Thomas Burr Osborne, a member of this Board, which occurred at his home in New Haven on January 29th, 1929.

Dr. Osborne's grandfather, Judge Thomas B. Osborne, was a director of this bank from the time of its organization in 1864 until his death in 1869; his father, Arthur D. Osborne, was a director from 1869 to 1916, was its President for seventeen years of that time, and Dr. Osborne himself succeeded his father as director December 28th, 1916. Both grandfather and father were lawyers.

[1] Printed with the permission of the president of the Second National Bank.

Dr. Osborne soon after his graduation from Yale engaged in research work in chemistry and devoted himself especially to study of the chemistry of nutrition and growth—biochemistry, in which he won great distinction. Yale gave him the honorary degree of Doctor of Science and appointed him research associate with rank of Professor, he served as member and officer of learned societies, was made honorary member of learned societies abroad, was awarded medals both here and abroad, and assisted in editorship of scientific journals—the story of his discoveries is known the world over. Pausing occasionally to publish his results, he has given his fellow-scientists new ground from which to work and contributed much to the physical well-being of all. Applause, however, he scarcely heard, for by the time it reached his ears he had eagerly resumed his quest for further truth. He was modest but realized the value of his own work—his modesty was that of the earnest man who regards success as a new foothold for further progress.

We, his associates on this Board, however, knew him in the broader relations of life. We respected him for his achievements in science, but we also came to realize and respect his knowledge and understanding of business, especially in its relation to government finance. He brought to the service of the bank a natural and inherited interest in its business, an understanding of its relation to the community and a lively interest also in public affairs and the larger problems of finance, which were considered by him with the relentless searching logic of the scientist, who must be sure of his premises. We have found him a real friend and have shared with him his sympathetic interest in the life of those around him. As directors we have been helped by his sound and kindly counsel. We gratefully acknowledge his service to this bank and the community.

Attest:

E. G. Allyn,
Secretary.

THE YALE JOURNAL OF BIOLOGY AND MEDICINE[1]

The death of Thomas Burr Osborne on January 29, 1929, removes one of the most distinguished figures in American biochemistry. Descended from a long line of Yale men, himself a graduate of the class of 1881, his passing will be lamented by all who were associated with him, as well as by the many scientific investigators who have been influenced by his work.

Dr. Osborne's entire life was devoted to research in what was,

[1] Reprinted from 1, 187 (1929) by permission of the editor.

at the beginning of his labors, an almost unexplored field. The origin of his interest in chemistry may be traced to his friendship and association with Samuel W. Johnson, who was, for many years, Professor of Agricultural Chemistry in the Sheffield Scientific School and Director of the Connecticut Agricultural Experiment Station. After graduation, Osborne continued the study of chemistry and in 1885, received the degree of Ph.D., being the tenth man to obtain this degree in chemistry from Yale. His dissertation was entitled "The Quantitative Determination of Niobium," a subject certainly far enough removed from what later became his life work. This investigation had been preceded by studies on the analytical separation of zinc and was followed by a number of others also dealing with analytical problems. The foundation of his career was thus laid upon a thorough knowledge of analytical chemistry.

In 1886, Dr. Osborne was invited by Professor Johnson to join the staff of the Connecticut Agricultural Experiment Station, and he thus formed a connection which was maintained until his death. Professor Johnson had become interested in the work of Ritthausen upon the proteins of seeds, and, in 1888, he suggested that further study in this field might lead to useful results. The selection of the oat kernel as the first seed for investigation was probably influenced by some early work of J. P. Norton. That the choice was perhaps unfortunate is indicated by a statement made in Dr. Osborne's address on the acceptance of the Thomas Burr Osborne medal on June 7, 1928. "The first cereal with which I worked was the oat kernel, and I have long wished that someone would explain the unusual behavior of the proteins of this seed. In all my experience I have never found a seed containing proteins behaving toward solvents as these do." In spite of the extraordinary difficulties presented by the material chosen, he succeeded in isolating the proteins of the oat in a pure form, one of them, a globulin, in crystalline condition. This paper was followed by a series of investigations extending in almost unbroken sequence for ten years, in which preparations of the proteins from more than thirty different seeds were described. These descriptive studies were followed by several papers dealing with the nucleic acid of the wheat embryo and with the basic character of the protein molecule; papers which are among his most important contributions to science. In them he announced the discovery of a nucleic acid and showed that it contained the purines, guanine and adenine, in molecular proportions. He made it clear that preparations described in the literature as nucleoproteins were in reality salts of proteins with nucleic acid, and that the highly variable proportions of phosphorus in such preparations were to be accounted for by the fact that nucleic acid and proteins may unite with each other in variable proportions since proteins may behave as polyvalent bases, while nucleic acid is a polyvalent acid.

He next showed that edestin from hemp-seed could unite with hydrochloric acid in two different proportions forming definite and reproducible compounds, the compositions of which, with respect to the acid, were in the simplest possible stoichiometric relationship to each other. This was an accomplishment of the highest significance, since it indicated that proteins behave in accordance with the laws which govern the behavior of other and simpler basic substances.

This investigation initiated a second phase of Dr. Osborne's work in which the chemical properties and composition of proteins were primarily considered. Perhaps the best known of these studies is that which deals with the different forms of nitrogen in proteins (1908), in which it was shown that a definite part of the protein nitrogen is found, after hydrolysis, as ammonia, and that the amount of ammonia is so related to the proportions of glutaminic acid and aspartic acid derived from the proteins as to lend great weight to the view that these acids must occur as amides in the protein molecule. A further proportion of the nitrogen of proteins can be precipitated by phosphotungstic acid after hydrolysis and, therefore, probably belongs to basic substances. Dr. Osborne found that this proportion is very close to that to be expected from the sum of the amounts of arginine, histidine and lysine yielded by these proteins. He further demonstrated that the additional quantity of ammonia obtained when proteins were hydrolyzed by means of alkali, over that secured when acid hydrolyzing agents were used, could be closely accounted for by the secondary decomposition of arginine. This paper is one of the most widely quoted of Dr. Osborne's contributions to protein chemistry. In it he allowed himself almost the only prophecy to be found in his work. With respect to the proteins of the cereal grains he says, "they in some ways differ in structure from all the others which have been examined, and . . . . they may possibly contain some other dibasic acid not yet isolated from their decomposition products." This prophecy was fulfilled ten years later by Dakin's brilliant discovery of oxyglutaminic acid in several of the proteins to which Dr. Osborne had referred.

Beginning in 1906, and continuing for about six years, with the aid of a number of collaborators, Dr. Osborne carried out a series of analyses of the amino-acid composition of proteins. These analyses set a standard for such work, surpassed only since the introduction, in recent years, of greatly improved methods, and laid the foundations for the studies of the nutritive properties of proteins begun in collaboration with Prof. Lafayette B. Mendel in 1909, and continued until 1928. He had become greatly interested in the physiological properties of proteins, and a series of papers, in collaboration with Prof. H. G. Wells of Chicago, led to a clear understanding of the anaphylactogenic relationships of vegetable proteins.

The results of the work on nutrition problems are well known. A technique for the study of various diets, using rats as experimental animals, was perfected. Studies on the relation of amino-acid composition to the nutritive value of proteins showed that tryptophane and lysine were essential for growth and maintenance. The presence of an accessory food factor in butter-fat, subsequently designated as vitamin A, was discovered independently and this finding was published in 1913. In addition there were many studies on the nutritive value of isolated proteins and upon the mutual supplementation of various foodstuffs with respect to their protein composition. One of the most useful results of the experiments upon the vitamin requirements of animals was the recognition of the peculiar value of cod liver oil in nutrition, and an at least partial explanation of the high esteem in which this oil has long been held by the medical profession.

Osborne and Mendel have also contributed widely to the knowledge of the distribution of the vitamins in natural foodstuffs, and to the factors influencing the phenomena of growth, in particular to the study of dietary regimens resulting in the suppression of growth or in its acceleration.

One of the results of the study of vitamins was a clear conception, in Dr. Osborne's mind, of the importance of an investigation of the constituents of living cells. This led to a vast amount of labor upon the composition of green leaves, much of which did not reach the stage of publication. He enthusiastically coöperated in the labors of his assistants in this field and the work strikingly demonstrated the complexity of the chemical environment in which the life process takes place. Dr. Osborne's labors in the field of protein chemistry were summarized in his classical monograph "The Vegetable Proteins," first published in 1909, and extensively revised in 1924. His monograph on "The Proteins of the Wheat Kernel" (1907) is a standard text among cereal chemists. His published work includes nearly 250 journal reports as well as public addresses and more popular articles.

Dr. Osborne was one of the fortunate leaders of science in a highly technical field to whom recognition came in his own lifetime. He was awarded the honorary degree of Sc.D. by Yale in 1910, and in the same year was elected a member of the National Academy of Sciences. He was made an honorary fellow of the London Chemical Society in 1912, and a fellow of the American Academy of Arts and Sciences in 1914. He was an associate member of the Société Royale des Sciences Médicales et Naturelles de Bruxelles, a member of the American Philosophical Society, and of many other American and foreign learned societies. In 1923, he was made a Research Associate in Biochemistry of Yale University. In 1900, he was awarded a gold medal by the Paris Exposition, and in 1922, received the John Scott Medal. In 1928, he was the first recipient of the Thomas Burr Osborne Gold

Medal of the American Association of Cereal Chemists; a medal founded in recognition of his outstanding contributions to cereal chemistry.

Dr. Osborne was one of the most distinguished pupils of Prof. S. W. Johnson, and through him traced his intellectual ancestry back to Liebig, the founder of agricultural chemistry. The enormous increase in interest in the chemistry of proteins in this country, particularly in recent years, is to no small degree due to his direct influence. He was a painstaking, careful investigator who spared no effort, time, or expense in the attainment of the truth. He accepted no result until it had been subjected to the test of rigorous and repeated experiment, and all his publications bear the marks of meticulous editing lest a statement should to the slightest extent pass the bounds of ascertained fact.

To those who were privileged to be associated with him in his work he was a rare stimulus, a formidable opponent in argument, and an ever genial but just critic. He frequently closed a discussion with the remark that facts were to be found in the laboratory, not in books. He was naturally shy and retiring; the delivery of a public address or of a paper was a severe trial to which he looked forward with trepidation; but among a small group of friends he showed himself as a gifted conversationalist, who was equally able to discuss the latest achievements of science, the current political situation, the intricacies of the world of finance, or the faults of the modern educational system.

It has been said that the value and significance of a man's work is, in the long run, in no way influenced by what is said of it: ultimately it stands alone upon its intrinsic merit. The work of Thomas Burr Osborne is founded upon the basic principles of accuracy, honesty, and a desire to be of service to his fellow men. No more enduring monument to his memory can be erected than this.

HUBERT BRADFORD VICKERY.

EXPERIMENT STATION RECORD[1]

The career of Dr. Thomas Burr Osborne, ended by his death on January 29, 1929, personifies in an unusual degree the ideals of scientific research at the agricultural experiment stations. Entering the service of the Connecticut State Station in 1886, Dr. Osborne soon began a study of the vegetable proteins which became the major project of his life work and continued for almost 40 years. In this relatively intricate and difficult field he did a notable work and achieved wide recognition as an outstanding authority. As one who followed his studies closely from the

[1] Reprinted from **60**, 701 (1929) by permission of the editor.

beginning has recently written, "he exemplified in the best sense the courage and perseverance of the investigator, coupled with the skill and creative ability so essential to discovery. His contribution was an unusually large one, and has been very far-reaching in its effects on our thinking and understanding."

Dr. Osborne's entire life was spent in the city of New Haven, where he was born on August 5, 1859. He was graduated from Yale University in 1881 and received the degree of doctor of philosophy from the same institution in 1885. His early interests were in the field of analytical chemistry, and for three years he was an assistant in that subject in Yale.

The enactment by the Connecticut Legislature of a law to protect the dairy industry and the general public from the increasing sale of unlabeled imitation butters made possible an increase in the chemical staff of the State Experiment Station in 1886, and it was in connection with this control work that he was given his original appointment. His service as a routine analyst, however, was brief and was supplemented almost immediately by studies of analytical methods. The report of the station for 1886 includes notes from his pen on the filtration of crude fiber and the filtration and weighing of silver chloride, and in the following year he gave much attention to devising methods of mechanical soil analysis.

The Connecticut State Station had been established with the immediate objective of providing a defense against fraud, but its aim, as stated in the act of incorporation, was "to promote agriculture by scientific investigation and experiment," and the station was not permitted to develop as either an exclusively control station or as one content with the simpler and more immediately useful forms of testing. Largely because of the broad vision and high ideals of those in whose charge its destinies had been intrusted, scrupulous care had been exercised so to organize its inspection service that it would not hamper the progress of research, to rest it at all times on the soundest basis which science could provide, and to develop as far as possible the more fundamental inquiries. Accordingly, in 1888, just as the Hatch Act was coming into operation, Director S. W. Johnson proposed an investigation of the vegetable proteins, and the station report for the following year tersely announced that "much time has been given by Dr. Osborne to a study of the nitrogenous matters contained in the kernels of maize and oats."

In this modest fashion began a series of investigations of outstanding importance. The studies were undertaken at a time when work in this field was known to be much needed, but the opportunity was being neglected. The first serious examination of the vegetable proteins had been instituted in 1860 by Ritthausen, who had demonstrated the occurrence of many diverse forms in the different seeds and indicated some of the difficulties and complexities of the subject. His work had been far from exhaustive, however, and with the contributions of Denis and Weyl the matter had been left in much confusion. Because of its intricacy the subject was being regarded by chemists in general with some trepidation.

Dr. Osborne first directed his attention to a reinvestigation of the matters dealt with by Ritthausen. Utilizing and devising improved methods and working with characteristic thoroughness and care, he was soon able to show that the number of distinctive vegetable proteins is far greater than Ritthausen had supposed. Ultimately pure specimens of the proteins from no fewer than 32 different seeds were prepared, usually by several methods, and comprehensive descriptions formulated. Many of the proteins previously grouped together were found to be distinct substances. Specific designations were thereupon given them, and the use of older terms much restricted. As Vickery and Mendel, two of his former colleagues, have pointed out in a recent tribute in *Science,* "this clarification of the nomenclature has been of immense assistance in bringing a semblance of order into an almost hopelessly confused subject."

This phase of Dr. Osborne's activities continued for about 10 years and led logically to an investigation of the proteins as a group, their structure and their properties. Here again he was delving in a pioneer field, and again he was equal to the occasion. In the words of a resolution of appreciation adopted by the station board of control at his retirement from active service late in 1928, "his mind has always been raising questions, which he was able to define with rare precision, and then with equal discernment he has devised means for their experimental investigation and solution." By analysis and reanalysis he patiently and persistently advanced the boundaries of knowledge of the fundamental chemistry of the proteins and established a basis for the third and culminating stage of his research, a study of their nutritive values.

This study was begun in collaboration with Dr. L. B. Mendel in 1909, and involved among other requirements the development of a technique for feeding individual small animals diets containing the pure isolated proteins which he now had available. Unexpected difficulties arose in obtaining normal growth with young animals on what were supposedly adequate rations, but when these were overcome by the use of a "protein-free milk" prepared by removing the casein and lactalbumin from milk serum and evaporating, wide differences in the efficacy of the various proteins were revealed. For example, the animals grew well on wheat glutenin and edestin, but failed rapidly on zein and gelatin, while maintenance but not growth was possible on hordein and rye and wheat gliadin. Further work showed that by the addition of certain amino-acids the deficiencies could be rectified and growth made possible.

The great value and immediate practical bearing of these findings were generally recognized, but even more important observations were to follow. These observations grew out of the use of the "protein-free milk" employed in the feeding experiments and indicated that there occurs in butter a substance essential for animal growth. Similar discoveries were reported at about the same time by McCollum and Davis, using an ether extract of egg yolk and of butter, and the essential substance was later designated vitamin A. Subsequently, Dr. Osborne and his associates noted the value of cod liver oil as a growth stimulant and particularly as the curative agent for the xerophthalmia regularly encountered in the animals receiving the deficient diets.

In 1911 Drs. Osborne and Mendel showed that the "protein-free milk" was much more efficient in inducing growth than was a corresponding mixture of lactose and pure salts or milk ash, thus implying the presence of some water-soluble organic growth-promoting substance. This early inference was later confirmed, and the studies contributed considerably to the development of our knowledge of the distribution of such a vitamin in foods and its significance in normal nutrition, particularly in connection with growth.

Many other extensive contributions were also made, as indicated in the following summary by Vickery and Mendel: "Much labor was devoted to the study of the nutritive value of the proteins of the commercially important foods, and this work gave a rational explanation of many practices which empirical experience had shown to be advantageous. The distribution of vitamins A and B in natural food products was studied, and considerable success was attained in an effort to prepare a vitamin-rich concentrate from yeast. The phenomena of growth, its suppression and acceleration under various regimens, the effect of the individual inorganic constituents of the diet, these and many other topics received attention at different times."

An idea of the extent of Dr. Osborne's labors may be gathered from the fact that a complete bibliography of his publications is said to reach 253 titles, of which about 200 are contributions to journals reporting his personal scientific work. This large number is the more remarkable in view of his conservatism in announcing his findings and his rigid insistence that no result must be made public that had not been verified by careful, thorough, and repeated experiment. Perhaps because of what has been termed his "meticulous editing," none of his published works are of great length, the most extensive probably being his monograph of 154 pages on The Vegetable Proteins, first issued in 1909 and extensively revised in 1924. This monograph and his report of 119 pages on The Proteins of the Wheat Kernel (1907) have long been regarded as classics in their respective fields.

The success of Dr. Osborne's work may fairly be considered a resultant of his personal efforts, characteristics, and qualifications, and his favorable environment. To an unusual degree he represented the "exceptional investigator," of whom much is heard in discussions of research and research workers. He was well equipped by training and temperament to utilize to the full his advantages and to profit by his congenial surroundings. Assigned as a young man to a highly complex and difficult problem, he grew with his opportunity. Beginning in a small way, devising and adapting his tools for the task, he worked logically, patiently, and sanely. Progress was neither spasmodic nor spectacular, but it was steady and sure, with little lost motion and even less of following of blind trails and retracing of steps. Gradually the mosaic was assembled, and the work little understood or appreciated in its beginnings was impressively revealed.

Without detraction from the achievements of the central figure in the execution of this important project, it may be pointed out that much credit also accrues to his associates, his colleagues and coworkers, and especially to those who have formulated and executed the policies of the institution under whose auspices the work has been done. Outstanding among these have obviously been Director Johnson, at whose instigation and under whose enlightened leadership the investigation was originally conceived and gotten under way, and his successor, Director E. H. Jenkins, who without the incentive of project authorship assumed the responsibility for its continuance and suffered no interference with its progress. Nor should there be overlooked the consistent coöperation and support of successive boards of control, manifested through the years when results came slowly and their applications to practical farming seemed remote and improbable. If it is recalled that even after the passage of the Hatch Act the entire resources of the station did not exceed $20,000 per annum for several years, that the experiment station itself was on trial in Connecticut as elsewhere, with its usefulness as an aid to agriculture still to be fully accepted, and that a host of relatively simple problems of undoubted economic importance and popular appeal were pressing for solution, the courage and the vision of Dr. Osborne's supporters become manifest. When the contention is heard, as happens from time to time, that the agricultural experiment stations are too hard pressed for results of practical value and for immediate application to be looked to for systematic and long-time research along fundamental lines, the maintenance of Dr. Osborne's work year after year may well be included in the many instances which may be cited in rebuttal.

From various aspects, the story of Dr. Osborne intrigues the imagination and supplies a unique inspiration. Fortunately it is not only a story with a moral but a tale with a happy ending. Public recognition came slowly, and first of all, according to Vickery and Mendel, by way of Germany, when Griessmayer in 1897 published a treatise on the vegetable proteins that contained many extracts from Dr. Osborne's papers and stated in his introduction that it was his hope "to bring to light these treasures buried in their American publications." By 1904, however, the fundamental character of the work had been so thoroughly established as to enlist substantial financial support from the Carnegie Institution of Washington, and this support has subsequently been continued without interruption as one of the comparatively few projects carried on at an experiment station which have ever received such aid. In 1908 the work was also accepted by the Office of Experiment Stations as an appropriate project under the recently enacted Adams Act, and this relationship likewise continued without material modification for nearly 20 years.

Personal recognition was ultimately accorded in generous measure. Yale University conferred the honorary degree of doctor of science in 1910, and in 1923 appointed Dr. Osborne a research associate in biochemistry with professorial rank. In 1920 he was elected a member of the National Academy of Sciences, in 1912 an honorary fellow of the Chemical Society of London, and in 1914 a fellow of the American Academy of Arts and Sciences, and he was long a member of numerous other societies at home and abroad. For many years he was associate editor of the *Journal of Biological Chemistry*. Three medals were awarded to him, the gold medal of the Paris Exposition in 1900, the John Scott medal in 1922, and (as the first recipient) the Thomas Burr Osborne gold medal established in his honor in 1926 by the American Association of Cereal Chemists in commemoration of his "notable services to cereal chemistry."

Late in 1928 and at his own request, he was relieved of active charge of the biochemical laboratory, at which time he was given the title of consulting biochemist by the board of control, with appropriate resolutions of appreciation. In the few weeks which followed, the memory of these honors and the many expressions of esteem of his colleagues and others doubtless brought him much pleasure, yet the guess may be hazarded that his highest satisfaction came from his own realization that he had achieved the goal of every true investigator, a lifetime profitably and productively spent in the elucidation of a worth-while problem. As Vickery and Mendel have well said, "his death removes one of the great pioneers of American biochemistry, a man whose name will always be linked with the subject he made peculiarly his own."

AMERICAN JOURNAL OF SCIENCE[1]

By the death of Thomas Burr Osborne on January 29, 1929, at the age of 69, modern biochemistry has lost one of its most fruitful investigators. Few students of science have devoted them-

[1] Reprinted from 17 (April, 1929) by permission of the editor.

selves more wholeheartedly, uninterruptedly and effectively to a few definite research objectives. Dr. Osborne served for a time on the teaching staff of the Chemical Laboratory at Yale. A number of his early contributions dealt with problems of analytical chemistry. In 1886 he became attached to the Connecticut Agricultural Experiment Station, then under the directorship of Prof. S. W. Johnson, and presently began a series of fundamental investigations on the occurrence and properties of proteins in plants, notably in the cereal seeds. These have been summarized in his monograph on *The Vegetable Proteins,* which has become the classic book of reference in its field. Subsequently in collaboration with a number of assistants he devoted himself to analytical studies of the structure of the proteins, whereby he was enabled to make fundamental contributions to our knowledge of the amino-acid makeup of the nitrogenous foodstuffs. The large experience and information thus secured served to awaken Dr. Osborne's interest in some of the biological properties of the proteins and bore fruition in outstanding investigations (in collaboration with Prof. H. G. Wells) on the anaphylactic properties of many plant proteins. Furthermore during a period of nearly 20 years he was engaged, in collaboration with Prof. Lafayette B. Mendel of Yale University, in elaborate investigations of the comparative nutritive value of proteins. Observations made in the course of these studies further led to various discoveries regarding those nutritive properties of foods now designated as vitamins. The physiology of growth likewise became a subject for extensive consideration. Dr. Osborne's various contributions presented in more than 250 scientific papers brought recognition and distinction to him from many sources. Few chemists have been privileged to follow the dictates of their interest so long and successfully without the interruptions or distractions that may retard the progress of the devotees of science.

L. B. M.

JOURNAL OF THE CHEMICAL SOCIETY[1]

Thomas Burr Osborne was elected an honorary Fellow of the Chemical Society in 1912 and his death on January 29th, 1929, removes one of the most distinguished figures in American biochemistry. The Chemical Society has seldom honoured with its honorary Fellowship investigators whose work has been concerned chiefly with the biological side of chemistry, thus Osborne's election was an exceptional tribute to the scientific worth of his labours.

Osborne's scientific life was entirely centered around New

[1] Reprinted from page 2974 (1929) by permission of the editor.

Haven. A student of Yale University, he received the degree of Ph.D. for a thesis on "The Quantitative Determination of Niobium." In the year following graduation he was invited by Professor S. W. Johnson to join the staff of the Connecticut Agricultural Experiment Station, thus forming a connexion which he maintained throughout life. Up to this time Osborne's work had been entirely concerned with analytical chemistry, and the cause of his deflection to biological chemistry may not improbably be referred as much to the heart as to the head. His devotion to Johnson, himself a pupil of Liebig and for long one of the outstanding leaders in agricultural science, and his subsequent marriage to Professor Johnson's daughter combined to make the transition an easy one.

In broad outline Osborne's contributions to biochemistry may be considered in three main groups. First, the preparation of many proteins, especially those found in vegetable seeds, in a state of relative purity quite unattained by previous investigators. Many of these proteins were obtained in crystalline form and characterized in a thoroughly convincing fashion. Having the command of relatively pure proteins in large amounts, Osborne devoted the second phase of his work to the analysis and identification of their products of hydrolysis: the results are of permanent value and are constantly utilized by others, but it may be conceded that the methods made use of were chiefly worked out by Kossel, Fischer, and others and that Osborne's own contributions to methods of protein analysis seem modest in comparison with his other outstanding accomplishments. The third and certainly the most striking part of Osborne's work dealt with the biological properties of his highly purified proteins; first of all in relation to their specific antigenic properties and their ability to produce that extraordinary phenomenon known as the anaphylactic reaction—and subsequently their rôle in animal nutrition. The latter phase of Osborne's work brought into prominence the importance, from the standpoint of nutrition, of minute amounts of substances associated in varying degree with certain foodstuffs which are now grouped under the name of "vitamins." In particular he contributed largely to the discovery of the substance present in butter fat which was later distinguished as vitamin A and is essential for animal existence.

In the following year the important observation was made that the same stimulation of growth could be secured by the addition of cod liver oil to a diet of purified food substances and protein-free milk, a discovery which served to focus attention upon the value of this oil, in particular as a curative agent for the peculiar eye condition known as xerophthalmia, which was regularly encountered in animals on the deficient diets. At the close of the war the sight of many children in Europe was preserved by its use, a remarkable example of the application of scientific results to practical problems.

One of the results of the study of vitamins was a clear conception in Osborne's mind of the importance of an investigation of the constituents of living cells. This led to a vast amount of labour upon the composition of green leaves, much of which did not reach the stage of publication. He enthusiastically coöperated in the labours of his assistants in this field and the work strikingly demonstrated the complexity of the chemical environment in which the life process takes place. Osborne's investigations in the field of protein chemistry were summarized in his well-known monograph "The Vegetable Proteins," first published in 1909, and extensively revised in 1924. His monograph on "The Proteins of the Wheat Kernel" (1907) is a standard work of reference among chemists who are occupied with the problems of cereal foods. His published work includes nearly 250 journal reports as well as public addresses and more popular articles. Shortly before his death Osborne published jointly with his colleague and successor, H. B. Vickery, a monograph in *Physiological Reviews* entitled "A Review of Hypotheses of the Structure of Proteins," which must be regarded not only as a confession of faith but as one of the sanest and most wisely critical essays on a most difficult subject. His last paper on "The Chemistry of the Cell" concludes with the following sentence, which is so typical of Osborne's scientific caution and dislike for premature speculations that it may be quoted: "I fear that for a long time to come much will still remain to be learned about the chemistry of the cell, but if, in the meantime, we can extend our knowledge of this subject it may save us from many erroneous conclusions based on incorrect results obtained without sufficient appreciation of the real nature and complexity of the problem."

Osborne was singularly fortunate in his conditions of work and enjoyed happy associations with many colleagues of distinction, notably Gideon Wells, Mendel, Vickery, and Wakeman. His extensive investigations would have been impossible without generous financial support and encouragement. Throughout the early years, when results came slowly and their application was by no means apparent, the directors of the Connecticut Agricultural Experiment Station allowed no interference or distraction to hinder the progress of the work. Since 1904 a large proportion of financial burden has been borne by the Carnegie Institution of Washington, D. C., of which he was a research associate. Osborne's connexions with both the experiment station and the Carnegie Institution of Washington furnish a striking example of the value to science of a policy of non-interference on the part of those in control of the distribution of funds for research. Except for routine annual reports, he was never asked for statements of progress or for outlines of projects. The relationship was always one of the utmost mutual confidence and esteem.

To those who were privileged to be associated with Osborne in his work he was a rare stimulus, a formidable opponent in argument, and an ever-genial but just critic. He frequently closed a discussion with the remark that facts were to be found in the laboratory, not in books. He was naturally shy and retiring, but among a small group of friends he showed himself as a gifted conversationalist, who was equally able to discuss the latest achievements of science, the current political situation, the intricacies of the world of finance, or the faults of the modern educational system. Few men have been more free from what Bacon has termed "the first distemper of learning, the studying of words and not matter."

H. D. D.[1]

THE YALE ALUMNI WEEKLY[2]

Thomas Burr Osborne died at his home in New Haven on January 29. He had suffered an acute attack of heart disease a few days before. Osborne received his preparation for college at the Hopkins Grammar School. At Yale he was given a first colloquy appointment in both junior and senior years, served as president of the Yale Natural History Society for three years, and belonged to Psi Upsilon and Skull and Bones. He specialized in chemistry in the Yale Graduate School from 1882 to 1885, when he received the degree of Ph.D., and was an assistant in analytical chemistry from 1883 to 1886. Osborne was connected with the Connecticut Agricultural Experiment Station in New Haven as chief in protein research from 1886 until his retirement from active direction of the work of the laboratory last summer and had since held the position of advisory biochemist. He was a research associate of the Carnegie Institution in Washington, and he had been connected with the Yale faculty as a research associate in biochemistry, with the rank of professor, since 1923. Osborne had won preëminent recognition as an authority in the field of vegetable proteins and had served as president of the American Society of Biological Chemists and as an associate editor of the *Journal of Biological Chemistry*. He was the author of *Proteins of the Wheat Kernel* and *The Vegetable Proteins* and had contributed numerous papers to various scientific journals. Yale conferred the honorary degree of Sc.D. upon him in 1910, and he was elected to membership in Sigma Xi in March, 1928. He was awarded the Paris Gold Medal in 1900 and the John Scott

[1] The writer gratefully acknowledges his indebtedness to Dr. Vickery's article on Dr. Osborne's work published in the *Yale Journal of Biology and Medicine*, March ,1929.

[2] Reprinted from page 576 (Feb. 8, 1929) by permission of the editor.

Medal in 1922, and in 1928 he was the first recipient of the Thomas Burr Osborne Gold Medal, which was established in his honor by the American Association of Cereal Chemists in 1926. Osborne was an honorary member of the London Chemical Society, an associate member of the Société Royale des Sciences Médicales et Naturelles de Bruxelles, and a fellow of the American Academy of Arts and Sciences. He was married in 1886 to Elizabeth Annah Johnson, of New Haven, who survives him with one of their two sons, Arthur D. Osborne, '08. The younger son died in childhood. Osborne was the son of Arthur D. Osborne, '48, a brother of Arthur S. Osborne, '82, a grandson of Eli W. Blake (B.A. 1816) and Thomas B. Osborne (B.A 1817), a great-grandson of Ebenezer Dimon (B.A. 1783), and a great-great-grandson of Ebenezer Dimon (B.A. 1728). Other Yale relatives are: Eli Whitney (B.A. 1792), David Dimon, a non-graduate member of the Class of 1828, Theodore Dimon (B.A. 1835), E. Whitney Blake (B.A. 1839), Charles T. Blake, '47, Henry T. Blake, '48, William P. Blake, '52 S., George A. Blake, ex-'54, Eli W. Blake, '57, Edward F. Blake, '58, John M. Blake, '58 S., James P. Blake, '62, Frank W. Blake, '72, George A. Bushnell, '76, Francis H. Blake, '82 S., Edward Blake, '84 S., Joseph A. Blake, '85, Henry W. Blake, '86 S., Donald M. Barstow, '89, T. Whitney Blake, '90 S., James K. Blake, '91, Robbins B. Anderson and Howard C. Robbins, both '99, and H. Kingsley Blake and Joseph A. Blake, Jr., both '16.

NATURE[1]

Thomas Burr Osborne, who died on January 29, was the last of the small band of pioneers who laid the foundation-stones of modern protein chemistry. Born in New Haven, Connecticut, on August 5, 1859, of old New England stock, he graduated after the usual course in arts at Yale College in 1881. Turning his attention to analytical chemistry, he took the degree of Ph.D. in 1885, and a year later joined the staff of the Connecticut Agricultural Experiment Station in New Haven. Professor S. W. Johnson, director of the station and professor of agricultural chemistry at Yale, suggested that Osborne should extend Ritthausen's early work on vegetable proteins, and in 1888 he started investigations which continued without interruption until his retirement in 1928.

From 1890 until 1901 Osborne's chief interest was in the preparation of pure specimens of the seed proteins, and his initial investigation of the oat kernel, published in 1891, was the forerunner of a series of papers in which the proteins of thirty-two different

[1] Reprinted from **123**, 613 (1929) by permission of the editor.

seeds were described. These researches demonstrated that proteins could be regarded as definite chemical individuals, and that many substances formerly grouped together under such terms as "legumin," "conglutin," and "vitellin" differed in chemical composition as well as in physical properties. His conception of the protein molecule as a definite chemical entity was strengthened by his work on the acid-binding power of edestin, published in 1899, and by later papers in which it was shown that proteins in general could form salts with both acids and bases, and that they were capable of electrolytic dissociation.

Working as he did in close contact with agriculture, Osborne early realized the need of a chemical characterization of proteins which would give some index of nutritive value, but characteristically deferred any such research until he was convinced that he could first obtain proteins in the highest state of purity. Taking full advantage of the developments in analysis due to Kossel and Fischer, he commenced in 1906 a series of protein analyses which demonstrated that wide differences existed in the amino-acid composition of many proteins of economic importance. These analyses were made with Osborne's usual extreme care and were the basis of his future work on the nutritive value of the proteins, begun in collaboration with Professor Lafayette B. Mendel of Yale, in 1909, and continued with the generous support of the Carnegie Institution of Washington until the time of his death.

The results of Osborne's protein investigations were summarized in a monograph, "The Vegetable Proteins," which was published in 1909, and extensively revised in 1924. His life was devoted almost entirely to his research, and, unlike most investigations, increasing years and fame brought no increase in administrative responsibility, consequently until the last his working hours were spent in the laboratory, and those who were privileged to work with him and gain his confidence found in him not only a genial friend and stimulating critic, but also a man with an unsurpassed wealth of practical experience in his own particular field of science.

Osborne was a member of the National Academy of Sciences, an honorary Sc.D. of Yale, and an honorary fellow of the London Chemical Society. Last year the American Association of Cereal Chemists instituted the periodic award of the Thomas Burr Osborne medal for distinguished research in cereal chemistry, and he was himself the first recipient.

A. C. C.

Obituary notices were also published in the *Bulletin de la société scientifique d'hygiène alimentaire,* 17, 401-404 (1929) and the *Ware-ra no Kwa-gaku,* a scientific journal edited by Prof. R. Nakaseko of the Imperial University of Kyoto, 2, 283-284 (1929).

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[1] The greater part of this paper was translated and reprinted by V. Griessmayer in "Die Proteide der Getreidearten, Hülsenfrüchte und Ölsamen sowie einiger Steinfrüchte," Heidelberg, 1897.

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Also *J. Am. Chem. Soc.,* **18,** 575-582 (1896).[1]

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Thomas B. Osborne and George F. Campbell
Rept. Conn. Agr. Expt. Sta. for 1895, 262-287 (1896).
Also *J. Am. Chem. Soc.,* **18,** 583-609 (1896).[1]

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Rept. Conn. Agr. Expt. Sta. for 1895, 288-301 (1896).
Also *J. Am. Chem. Soc.,* **18,** 609-623 (1896).[1]

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Thomas B. Osborne
Am. Chem. J., **19,** 236-237 (1897).

The Proteids of Lupin Seeds.
Thomas B. Osborne and George F. Campbell
Rept. Conn. Agr. Expt. Sta. for 1896, 342-368 (1897).
Also *J. Am. Chem. Soc.,* **19,** 454-482 (1897).
Translation: Die Proteide der Lupinesamen.
Z. landw. Versuchsw., **2,** 357-384 (1899).

The Effect of Minute Quantities of Acid on the Solubility of Globulin in Salt Solutions.
Thomas B. Osborne and George F. Campbell
Rept. Conn. Agr. Expt. Sta. for 1896, 369-373 (1897).
Also *J. Am. Chem. Soc.,* **19,** 482-487 (1897).
Translation: Wirkung winziger Säuremengen auf die Löslichkeit des Globulins in Salzlösungen.
Z. landw. Versuchsw., **2,** 65-70 (1899).

The Proteids of the Sunflower Seed.
Thomas B. Osborne and George F. Campbell
Rept. Conn. Agr. Expt. Sta. for 1896, 374-379 (1897).
Also *J. Am. Chem. Soc.,* **19,** 487-494 (1897).
Translation: Die Proteide des Sonnenblumensamens.
Z. landw. Versuchsw., **2,** 57-64 (1899).

The Proteids of the Cow Pea (Vigna catjang).
Thomas B. Osborne and George F. Campbell
Rept. Conn. Agr. Expt. Sta. for 1896, 380-386 (1897).
Also *J. Am. Chem. Soc.,* **19,** 494-500 (1897).
Translation: Die Proteide der Kuherbse (Vigna catjang).
Z. landw. Versuchsw., **1,** 450-456 (1898).

Proteid of the White Podded Adzuki Bean (Phaseolus radiatus).
Thomas B. Osborne and George F. Campbell
Rept. Conn. Agr. Expt. Sta. for 1896, 387-390 (1897).
Also *J. Am. Chem. Soc.,* **19,** 509-513 (1897).
Translation: Das Proteid der weissschaligen Adzukibohne (Phaseolus radiatus).
Z. landw. Versuchsw., **1,** 457-461 (1898).

The Amount and Properties of the Proteids of the Maize Kernel.
Thomas B. Osborne
Rept. Conn. Agr. Expt. Sta. for 1896, 391-397 (1897).
Also *J. Am. Chem. Soc.,* **19,** 525-532 (1897).
Translation: Eigenschaften und Zusammensetzung der Proteide des Maiskornes.
Z. landw. Versuchsw., **1,** 441-449 (1898).

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Thomas B. Osborne
Ber., **31,** 254-259 (1898).

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Thomas B. Osborne and George F. Campbell
Rept. Conn. Agr. Expt. Sta. for 1897, 324-337 (1898).
Also *J. Am. Chem. Soc.,* **20,** 348-362 (1898).
Translation: Die Proteide der Erbse.
Z. landw. Versuchsw., **2,** 160-173 (1899).

Proteids of the Lentil.
Thomas B. Osborne and George F. Campbell
Rept. Conn. Agr. Expt. Sta. for 1897, 337-349 (1898).
Also *J. Am. Chem. Soc.,* **20,** 362-375 (1898).
Translation: Die Proteide der Linse.
Z. landw. Versuchsw., **2,** 450-461 (1899).

Proteids of the Horse Bean (Vicia faba).
Thomas B. Osborne and George F. Campbell
Rept. Conn. Agr. Expt. Sta. for 1897, 349-361 (1898).
Also *J. Am. Chem. Soc.,* **20,** 393-405 (1898).
Translation: Die Proteide der Saubohne (Vicia faba).
Z. landw. Versuchsw., **2,** 584-596 (1899).

Proteids of the Vetch.
Thomas B. Osborne and George F. Campbell
Rept. Conn. Agr. Expt. Sta. for 1897, 361-365 (1898).
Also *J. Am. Chem. Soc.,* **20,** 406-410 (1898).
Translation: Die Proteide der Wicke.
Z. landw. Versuchsw., **3,** 63-67 (1900).

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Thomas B. Osborne and George F. Campbell
Rept. Conn. Agr. Expt. Sta. for 1897, 365-373 (1898).
Also *J. Am. Chem. Soc.,* **20,** 410-419 (1898).
Translation: Die Proteide der Erbse, Linse, Saubohne und Wicke.
Z. landw. Versuchsw., **3,** 68-76 (1900).

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Thomas B. Osborne and George F. Campbell
Rept. Conn. Agr. Expt. Sta. for 1897, 374-382 (1898).
Also *J. Am. Chem. Soc.,* **20,** 419-428 (1898).
Translation: Die Proteide der Sojabohne (Glycine hispida).
Z. landw. Versuchsw., **2,** 597-605 (1899).

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Rept. Conn. Agr. Expt. Sta. for 1898, 317-325 (1899).
Also *J. Am. Chem. Soc.,* **21,** 477-485 (1899).

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Thomas B. Osborne
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Rept. Conn. Agr. Expt. Sta. for 1899, 305-339 (1900).
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Also *J. Am. Chem. Soc.*, **24**, 28-39 (1902).

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Z. physiol. Chem., **33**, 240-292 (1901).
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Rept. Conn. Agr. Expt. Sta. for 1900, 443-471 (1901).
Also *J. Am. Chem. Soc.*, **24**, 140-167 (1902).
Translation: Bestimmung des Schwefels in den Proteinkörpern.
Z. anal. Chem., **41**, 25-35 (1902).

The Nucleic Acid of the Embryo of Wheat.
Thomas B. Osborne and Isaac F. Harris
Rept. Conn. Agr. Expt. Sta. for 1901, 365-430 (1902).
Translation: Die Nucleinsäure des Weizenembryos.
Z. physiol. Chem., **36**, 85-133 (1902).

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J. Am. Chem. Soc., **25**, 323-353 (1903).
Translation: Bestimmung der Stickstoffbindung in den Proteinkörpern.
Z. anal. Chem., **43**, 286-298 (1904).

The Specific Rotation of the Nucleic Acid of the Wheat Embryo.
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Am. J. Physiol., **9**, 69-71 (1903).

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J. Am. Chem. Soc., **25**, 474-478 (1903).
Translation: Anwendung von Molisch's Reaktion auf vegetabilische Proteine.
Z. anal Chem., **43**, 299-301 (1904).

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Thomas B. Osborne and Isaac F. Harris
J. Am. Chem. Soc., **25**, 837-842 (1903).
Translation: Über die Grenzen der Fällung mit Ammonsulfat bei einigen vegetabilischen Proteinen.
Z. anal. Chem., **43**, 378-382 (1904).

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J. Am. Chem. Soc., **25**, 842-848 (1903).
Translation: Spezifische Drehung einiger vegetabilischen Proteine.
Z. anal. Chem., **43**, 372-376 (1904).

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J. Am. Chem. Soc., **25**, 848-853 (1903).

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Translation: Über die Tryptophanreaktion verschiedener Proteine.
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Translation: Über die Proteinkörper des Weizenkornes. I. Das in Alkohol lösliche Protein und sein Glutaminsäuregehalt.
Z. anal. Chem., **44**, 516-525 (1905).

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Translation: Über die Grenzen der Fällung mit Ammonsulfat bei einigen vegetabilischen Proteinen. II.
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Am. Med., **9**, 1028 (1905).

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Am. J. Physiol., **14**, 151-171 (1905).
Translation: Über die Löslichkeit des Globulins in Salzlösungen.
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A Study of the Proteins of the Castor-Bean with Special Reference to the Isolation of Ricin.
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Translation: Über die Proteine der Rizinusbohne mit spezieller Berücksichtigung des Rizins.
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Translation: Die Chemie der Proteinkörper des Weizenkornes. II. Darstellung der Proteine in genügender Menge für die Hydrolyse.
Z. anal. Chem., **46**, 749-756 (1907).

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Translation: Die Chemie der Proteinkörper des Weizenkornes. III. Hydrolyze der Weizen-Proteine.
Z. anal. Chem., **47**, 81-105 (1908).

A New Decomposition Product of Gliadin.
Thomas B. Osborne and S. H. Clapp
Am. J. Physiol., **18**, 123-128 (1907).
Translation: Ein neues Zersetzungsprodukt des Gliadins.
Z. anal. Chem., **48**, 429-433 (1909).

Hydrolysis of Phaseolin.
Thomas B. Osborne and S. H. Clapp
Am. J. Physiol., **18**, 295-308 (1907).
Translation: Hydrolyse des Phaseolins.
Z. anal. Chem., **48**, 98-108 (1909).

The Heat of Combustion of Vegetable Proteins.
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J. Biol. Chem., **3**, 119-133 (1907).
Translation: Die Verbrennungswärme der vegetabilischen Proteine.
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Z. anal. Chem., **49**, 270-283 (1910).

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Am. J. Physiol., **19**, 53-60 (1907).
Translation: Hydrolyse des Exzelsins.
Z. anal. Chem., **48**, 616-622 (1909).

Hydrolysis of Hordein.
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Translation: Hydrolyse des Hordeins.
Z. anal. Chem., **47**, 590-597 (1908).

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Translation: Die Proteine der Erbse.
Z. anal. Chem., **49**, 142-146 (1910).

Hydrolysis of Legumin from the Pea.
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J. Biol. Chem., **3**, 219-225 (1907).
Translation: Hydrolyse des Erbsenlegumins.
Z. anal. Chem., **48**, 692-698 (1909).

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Carnegie Inst. Wash. Pub. No. 84, pp. 119 (1907).

Hydrolysis of Glycinin from the Soy Bean.
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Translation: Hydrolyse des Glyzinins aus der Sojabohne.
Z. anal. Chem., **48**, 623-628 (1909).

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Am. J. Physiol., **19**, 475-481 (1907).
Translation: Hydrolyse des kristallinischen Globulins des Kürbissamens (Cucurbita maxima).
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Am. J. Physiol., **22**, 423-432 (1908).

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Am. J. Physiol., **22**, 433-439 (1908).

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Am. J. Physiol., **23,** 81-89 (1908).

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Am. J. Physiol., **24,** 153-160 (1909).

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