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OF

THOMAS BURR OSBORNE

1859-1929

BY

HUBERT BRADFORD VICKERY

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Thomas B. Osborne

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A scientific investigation that is continued without interruption or diversion from the main theme for forty years can be conducted only by one who has to an unusual degree the qualities of pertinacity and patience combined with a love of science and of work for its own sake. Few of those who possess such characteristics are so inspired in their initial choice of a problem or so richly rewarded by its outcome as was Thomas Burr Osborne. His death on January 29th, 1929, removed one of the most distinguished pioneers of American biochemistry, a man whose name will always be linked with the subject he made peculiarly his own and upon which he firmly imprinted his forceful personality.

Osborne's life was one of whole-souled devotion to a single purpose, the understanding of the relationships of proteins to each other and to the animal world. He began his researches upon the vegetable proteins in 1888, at a time when little attention was being paid to these substances either by physiologists or chemists. He lived to see the study of proteins become one of the major topics of biochemistry, and to experience the satisfaction of knowing that this broad increase in interest was to no small extent a direct result of his own efforts. The great leaders in this field of scientific endeavor, Fischer, Kossel, and Osborne, have now all passed to their reward leaving a world infinitely the richer for their labors.

Osborne's entire life was spent in New Haven, Connecticut. There he was born on August 5th, 1859, there he received his education and scientific training, and there he did his work. He was seldom absent from his laboratory and his outside interests were few. He cared not at all for organized sports nor for the formal social life of the community, but preferred to take his recreation hunting or fishing or, in later years, in long country walks with a friend. On these occasions his extraordinarily acute powers of observation for the bird life around

him, and his shrewd comments on matters of mutual interest, were never-failing sources of delight to his companion. For many years he was a Director of the Second National Bank of New Haven. At the meetings of the board his keen understanding of business problems and the relentless logic with which he pursued his arguments won him the respect and regard of his fellow members. He led a life of rigid routine, but his adherence to the details of a well-ordered daily life was far from being an index of any lack of imagination. Above all else Osborne lived for the future. His interest in the past was restricted; he desired merely to know what were the ascertained facts, what were the well-supported and most fruitful hypotheses: upon this firm ground he planned his investigations, ever watchful for the obscure signs that point the way to further advances in sound knowledge.

Osborne's heritage is significant. The earliest recorded paternal ancestor in this country was Richard Osborne, born in England in 1612, who settled at Hingham, Massachusetts, in 1635, removed to New Haven in 1639, and later (1650) to Fairfield, Connecticut. His mother was descended from William Blake of Little Baddow, Essex, who came to America in 1630 and settled in Dorchester, Massachusetts; Eli Whitney Blake, the inventor of the stone crusher, was Osborne's grandfather, and Eli Whitney, the inventor of the cotton gin, was a great-great uncle. Through his maternal grandmother he was sixth in descent from the Reverend James Pierpont, a graduate of Harvard in 1681, who became the pastor of the first church in New Haven in 1684 and was one of the prime movers in the formation of Yale College. Osborne was the elder son of Arthur Dimon Osborne and Frances Louisa Blake. From his father, a member of the Connecticut bar, and a banker, he inherited an interest in the daily affairs of men that was developed by informal family discussions into an unusual training in finance. From his mother he inherited the love of trying new things and new ways—the desire to create—that informed his whole life-work.

He received his early education at the Hopkins Grammar School. As a boy he paid little attention to games or other amusements, his chief delight being to ramble through the woods and marshes in pursuit of birds. He rapidly acquired an intimate knowledge of their habits and accumulated a valuable collection of the local species. One of his companions on these expeditions has recently written: "He was intensely interested in all features of Natural History and knew plants and animals quite as well as he did birds, although we devoted more time, perhaps, to collecting birds. I do not know how large a collection of bird skins he had, but it must have been an important one."¹ Many of Osborne's notes and observations of bird life are recorded in the "Review of the Birds of Connecticut" published by C. Hart Merriam in 1877 and some of the skins from his collection are still in existence in the possession of Henry H. Townshend of New Haven.

He was encouraged in scientific activities by his uncle, Eli Whitney Blake, Jr., professor of physics at Brown University. Letters from the uncle refer to the microscopic examination of diatomaceous earth, to the dissection of clams and to other scientific subjects. Perhaps the most entertaining of these letters was written March 5, 1877, and contains a full description of Alexander Graham Bell's then recently invented telephone. Osborne used this information in the preparation of a school essay in which the telephone was described and illustrated by means of a diagram and a working model. He referred, in the essay, to the fact that he had been present when Dr. Bell demonstrated in Providence that music and the human voice could be transmitted over wires from Boston.

Osborne planned to continue his education at Cornell University where facilities for the study of science in the academic course were offered. He was, however, dissuaded and in 1877 took up his studies at Yale, thereby following the traditions of a family of Yale graduates that extended in almost unbroken line from a great-great-great grandfather, Ebenezer Dimon, who was graduated in 1728. The required study of moral philosophy

¹ Personal communication from Dr. Robert T. Morris, New York.

and the classics at Yale held little attraction for him and it is not surprising that his extracurricular activities at this time occupied much of his attention. Nevertheless, he received first colloquy appointments in both junior and senior years and was made a member of Psi Upsilon and of Skull and Bones; rewards that imply conspicuous attainments. He was president of the Yale Society of Natural History during the last three years of his course and surviving copies of the papers read at some of the meetings indicate that this was a group of serious minded young men who welcomed an opportunity for the discussion of scientific topics. It is interesting to note that a number of them were later eminent in various fields and, besides Osborne, at least five² became members of the National Academy.

The most important of Osborne's activities during his college career was entirely extramural. Through a school friend whose father was a prominent miller, he became familiar with a problem that had arisen in the flour milling industry. The existing method for the purification of middlings was in some respects unsatisfactory and millers were eager to secure a cheaper process. The adherence of tobacco ash to a gutta-percha pencil used to ram down the contents of his pipe suggested to Osborne that the difference in the electrical properties of the particles in the ground wheat might be used as the basis of a method of separation. A machine was devised in which the middlings passed under rolls constructed of rubber upon the surfaces of which an electrostatic charge was induced by causing the rolls to rotate in contact with sheep's wool pads. Devices were added to agitate the middlings and to remove and collect the lighter particles of bran which flew up and adhered to the electrified rolls. An American patent No. 224719 was granted to Osborne for this machine on February 17, 1880, and shortly afterwards American and foreign patents covering the original invention and certain improvements were granted to him and to his associates. The electric middlings purifier was used with success

² Edmund B. Wilson, C. Hart Merriam, Joseph P. Iddings, Louis V. Pirsson, and Samuel W. Williston.

for several years in a number of mills but was eventually superseded by other machines.

Osborne took his bachelor's degree in 1881. At that time the most promising career for a man with scientific leanings appeared to be medicine and he therefore enrolled in the medical school where he studied for one year. As this did not prove to his liking, in 1882 he entered the graduate school and took up the study of chemistry under Professor W. G. Mixer; the following year he was made laboratory assistant in analytical chemistry and obtained his first opportunity to embark upon original chemical investigation. He prepared two papers on the analytical separation of zinc which were published under his name alone in 1884, and in 1885 presented a dissertation on "The Quantitative Determination of Niobium," for which he was awarded the doctor's degree. He remained at Yale another year, occupied in research and teaching; in May, 1886, at the invitation of S. W. Johnson, professor of agricultural chemistry in the Sheffield Scientific School at Yale, and director of the Connecticut Agricultural Experiment Station, he became a member of the station scientific staff. In the same year he married Elizabeth Annah Johnson, Professor Johnson's daughter.

During the years which followed Osborne gradually took up the life of a scientific investigator. An influence which had the most far-reaching effects upon his mature life was exerted upon him by his distinguished father-in-law. The two men were united by bonds of the closest nature. Johnson was his mentor and teacher, the director of his early researches and intimate associate; Osborne became Johnson's most eminent pupil, the exemplar of the principles, in the training of investigators in agricultural science, to which Johnson devoted his life. They were in daily contact for nearly thirty years, Johnson the scholar, the master of the literature, the administrator; Osborne the clear and logical thinker, the determined investigator, the careful experimenter.

When Osborne first went to the experiment station laboratory, Johnson had been engaged for some time upon a study of the sugars obtained by the hydrolysis of various plant gums.

He had prepared a number of these substances but had been unable to obtain the services of an analyst trained to conduct combustions for the determination of carbon and hydrogen. The first assignment given the new assistant was the analysis of these preparations. Their nature as pentose sugars was soon established, but, in view of the publication of Kiliani's discovery that arabinose was a five-carbon sugar, and of the extensive investigations of similar substances being undertaken in Germany, Johnson did not publish the results. For several years Osborne was occupied with various problems of agricultural chemistry. He developed the so-called beaker method of mechanical soil analysis and was active in the general analytical work of the station. He also refitted the small home laboratory formerly used by his wife's father and for a number of years thereafter spent many evenings in private commercial work.

The passage of the Hatch Act in 1887 made federal funds available for scientific investigations at state experiment stations. This addition to the very limited resources of the Connecticut station permitted an enlargement of its activities, and one of the first projects undertaken was the study of vegetable proteins.

In order to understand how this came about it is necessary to go back to the year 1853 when Johnson was a student for a few months under Erdmann in Leipzig. One of Erdmann's assistants was Heinrich Ritthausen, a young man who later became interested in vegetable proteins and, in 1872, published a book, *Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Ölsamen*, in which was described an astonishing amount of original research upon the proteins of seeds. Ritthausen's work was continued until the early 90's but was little appreciated either in Germany or in this country. Among the few who grasped its significance was Johnson, who recalled his acquaintance of student days as an earnest and trustworthy investigator, but was convinced that even more thorough studies were necessary before the relationships of the seed proteins to each other and their value in human and in animal nutrition could

be understood. Finally, in 1888, the opportunity to take up this work was presented. In Osborne, Johnson had a highly trained and capable assistant who had already demonstrated his capacity for independent research. The necessary funds had become available. One vacation day in August of that year, while walking up the hill to his summer home at Holderness, New Hampshire, with his son-in-law Johnson proposed that an investigation of the proteins of seeds should be undertaken. The problem was to be Osborne's own and, under the new conditions, he would be able to devote his whole time to it. The younger man eagerly assented and, a few weeks later, began the studies that were to continue throughout his life. Many years before (1845, 1849) J. P. Norton, who had been Johnson's teacher at Yale, had published two papers on the proteins of the oat kernel. This grain had also been investigated by Kreuzler in 1869 but it had not been studied by Ritthausen. In view of this and of its great economic importance the oat kernel was selected for the initial investigation.

Osborne's work on the vegetable proteins falls naturally into three phases. From 1889 to 1901 the chief interest was in the preparation of pure specimens of the proteins of plant seeds. The investigation of the oat kernel (13, 14), published in 1891, was followed by a series of papers in which the proteins from no less than thirty-two³ different seeds were described. At least five of these, flax seed, adzuki bean, cow pea, soy bean and cotton seed were studied for the first time by him. Every seed was found to yield several different proteins and each of these was prepared, where possible, by a number of different methods. All available methods were employed to ensure that the preparations should represent homogeneous material as nearly as possible identical with the protein as it occurred in the seed.

³ Osborne studied in detail the proteins of wheat, rye, barley, maize, oat, rice, kidney bean, adzuki bean, pea, vetch, lentil, horse bean, soy bean, cow pea, yellow lupine seed, blue lupine seed, squash seed, castor bean, hemp seed, flax seed, cotton seed, almond, peach kernel, Brazil nut, hazel nut, English walnut, American black walnut, butternut, sunflower seed, and potato tuber. He also prepared the proteins of the lima bean and coconut but published no detailed paper on them.

The only criterion of the purity and individuality of protein preparations that was known at this time was the ultimate analysis of the material for carbon, hydrogen, nitrogen, and sulphur. That this criterion alone was hopelessly inadequate became apparent later and, since the recognition of this inadequacy was largely due to Osborne and amounts really to a turning-point in the history of protein chemistry, it may be worth while to discuss it more fully.

The early pioneer work of Beccari, Rouelle, Fourcroy, Einhof and Gorham suggested that there are four chief types of vegetable proteins, albumins, which are soluble in water and are coagulated by heat, plant caseins, plant fibrins, and alcohol-soluble proteins that have some properties in common with gelatin. The resemblances between these types of vegetable proteins and the more generally known proteins of blood and of milk gave rise to the idea, clearly expressed by Liebig and upheld by later writers such as Gerhardt and Kolbe, that there are in fact only four kinds of protein in nature, albumins, caseins, fibrins, and gelatins, and that these occur both in plants and in animals. According to this view it was easy to account for the value of vegetable food in the nutrition of animals and, moreover, the notion was philosophically satisfying since it tended towards the simplification of nature. As time went on, certain doubts arose that the relationships between the vegetable and animal proteins were quite as simple as this, but, when Osborne began his studies, most scientists were persuaded that the total number of different proteins in nature was very limited. Moreover, it was almost universally held that proteins from different sources had equal nutritive values. Only a few years earlier Johnson had explicitly stated in an official publication of the station that, according to general belief, vegetable albuminoids do not greatly differ from each other in nutritive effect.

Ritthausen's extensive investigations had been planned to demonstrate that proteins of similar type from different seeds are identical with each other. He had found, however, that there are at least two different kinds of plant fibrins; these he designated as legumin and conglutin, proteins that are classified today

as globulins. The alcohol-soluble proteins also failed to fall neatly into the scheme of general identity and Ritthausen had become convinced that the gliadin of wheat is a mixture of several constituent proteins which differ in their solubility in different concentrations of alcohol. The alcohol-soluble proteins of barley and maize were regarded as mixtures of these same constituents but in different relative proportions.

This was the situation when Osborne's investigations began. As seed after seed was studied and the number of carefully prepared and highly purified proteins increased, it became clear that many of these could no longer be grouped under a common name since they were in fact distinct substances. Specific designations were, therefore, coined and the older names were reserved for 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. Gradually the principle was evolved that differences between proteins are more important than similarities and the search for criteria whereby these substances could be differentiated and characterized was pursued with greater and greater energy.

In 1892 Osborne (18) described crystallized globulins obtained from six different seeds and concluded that the globulins from the Brazil nut and the oat are distinctly different substances. The globulins from hemp seed, castor bean, squash seed and flax seed were, however, closely alike. He said, "It is at present impossible to assert that these four globulins are the same, but since differences exist between different preparations of globulin from the same seed as great as those found among the globulins of these different seeds, the writer is disposed to consider these four globulins as identical." Later, in 1894, the amorphous globulins of wheat, maize and cotton seed were shown to have the same ultimate composition as these four crystalline globulins and he wrote (23), "as the properties of the preparations obtained from all these sources are substantially alike, there can be little doubt that one and the same proteid exists in them all. For this body we adopt the name *Edestin*

from the Greek ἔδεστος signifying edible, in view of its occurrence in so many important food-stuffs."

In 1896 Osborne pointed out (31) that the globulins from the peach kernel and almond are so closely alike that little doubt of their identity was entertained, further that the globulins of the walnut and the filbert are apparently identical with each other but that these differ from the edestin present in the seven seeds mentioned above. The differences he described had been overlooked by previous investigators and all of these proteins had been classed together as vegetable vitellin, a term which, in view of this newer investigation, Osborne proposed to abandon, since it was "associated with many erroneous statements as to its occurrence, composition, and characters." At this time he had prepared "six perfectly distinct proteids which have been confounded together under the name vitellin or conglutin." These six were *edestin* derived from hemp seed, castor bean, squash, flax and cotton seeds, wheat, rye, barley, maize and the coconut, *amandin* from the peach kernel and almond, *corylin* from the walnut and filbert, *excelsin* from the Brazil nut, *avenalin* from the oat, and *conglutin* from lupine seed.

In 1898 a paper on the pea, lentil, horse bean and vetch (44) described the proteins legumin, vicilin and legumelin. These four seeds were all found to yield preparations of the globulin legumin between which no essential differences were detectable, and the preparations of the albumin-like legumelin from them were also closely similar to each other. A protein that was designated vicilin was found in the first three named. Legumelin appeared to be likewise present in the adzuki and soy beans and in the cow pea.

The papers that describe these investigations are technical and are frequently long; they possess, however, a property that is almost unique in the early protein literature. The operations are so minutely and carefully outlined that it is possible to repeat Osborne's work to its last detail and secure preparations that correspond exactly to those he described.

The chemical and physical properties of many of these proteins were such as clearly to show the advantages for scientific

investigation of the reserve proteins of seeds over the proteins of animal origin. Efforts of others to isolate proteins of definite properties from the complex mixtures in animal tissues had been for the most part unsuccessful, and even as late as 1911 not more than two or three animal proteins had been clearly characterized as definite chemical substances. On the other hand, many seed proteins were early shown by Osborne to be chemically distinct and, furthermore, the preparations were reproducible at any time. He had crystallized a number of the 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.

Up to 1899 Osborne had been interested almost wholly in the preparation of proteins from as many different sources as possible. In this year he began to subject his wealth of material to more critical and detailed chemical examination. This marks the beginning of a second phase of his labors, a period in which the properties of the proteins became the matter of chief importance and which culminated in the elaborate amino acid analyses that laid the foundation for the nutrition studies of his later years.

The first paper (47) in which this change in interest is apparent showed that the crystalline protein edestin from hemp seed forms two distinct compounds with hydrochloric acid, that the solubility of edestin in acid increases in direct ratio with the amount of acid present and that this and a number of other crystallized vegetable globulins neutralize 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 one of the ends towards which the careful descriptive studies had been directed, a demonstration that some proteins, at least, have many of the properties of definite chemical individuals. The position here taken was strengthened by other papers in which it was shown that proteins exhibit many evidences of a capacity to undergo electrolytic dissociation and enter into ionic reactions.

These investigations clearly demonstrated the necessity for more complete chemical characterization of the different proteins. The older view, which narrowly limited the total number of vegetable proteins, was manifestly inadequate since a relatively short period of intensive investigation had greatly increased the number of kinds of protein that could be prepared from seeds. But what should be the next step? There was plenty of widely diversified material at hand. How should it be attacked? It is interesting to note how, during the period from 1899 to 1903 Osborne tested first one lead and then another, each time obtaining results important in themselves but not contributing much to the central problem. He worked on egg proteins, on the nucleic acid of wheat embryo and on the sulphur content of proteins. The data of this last paper (54) were used in a discussion of the possible molecular weight of proteins and are still employed for this purpose. Finally the required suggestion was obtained from the extraordinary results of Drechsel, Hedin, and of Kossel and their simple application to the proximate analysis of proteins by Hausmann in Germany. When proteins are boiled with strong acid they are slowly decomposed into relatively simple crystalline substances, the amino acids. At least twenty-one different substances of this type have been secured from proteins and most proteins yield fifteen or more of these. Kossel had attacked the problem from the amino acid point of view and had established the principle that protein analysis could most effectively be accomplished by the quantitative separation and estimation of these simpler derivatives. In particular he had developed a method whereby the three basic amino acids arginine, histidine, and lysine could be determined. With two exceptions all of the known amino acids derived from proteins contain an α -amino group; the three basic amino acids contain additional nitrogen in other parts of the molecule combined in basic structures that have properties different from those of the amino group. It is clear, therefore, that there are several different forms of combination of nitrogen in the protein molecule. This idea had been grasped by Hausmann who in 1899 proposed a simple scheme of protein analysis

whereby the proportion of the total nitrogen that was present in each of three well-marked forms might be determined. Hausmann's method met with considerable adverse criticism but it appealed to Osborne as a means by which additional knowledge of proteins might be readily secured and as a useful preliminary to the far more elaborate analysis of the basic amino acids according to Kossel's procedure. It soon became apparent that, although the method might not yield accurate absolute results, it did yield valuable comparative results when employed under suitable conditions.

Osborne's application of the Hausmann method to his preparations marks the beginning of a new era in the problem of protein characterization. He wrote (56), "we have found by its use that some of our preparations from different seeds which were so nearly alike in composition and reaction that no difference could be detected between them sufficient to warrant the conclusion that they were not the same chemical individual, yield such different proportions of nitrogen in the several forms of binding that there can be no longer any doubt that they are distinctly different substances. On the other hand, many preparations of different origin, which we have heretofore considered to be identical, have yielded the same proportion of the different forms of nitrogen and consequently our former opinion respecting the identity of these protein preparations is very greatly strengthened."

Judged by the new criterion the edestins derived from hemp and cotton seeds and the castor bean still appeared closely alike but differed from those from other sources. These others likewise differed among themselves and, consequently, instead of a single edestin derived from ten different seeds there were now at least seven different proteins that had previously been referred to under this one name. Cotton seed globulin had been found to react positively towards the Molisch reagent while hemp seed and castor bean globulins did not. The name edestin was therefore restricted to these two globulins with the further cautious statement (56): "Whether the globulins from these two seeds are in fact alike is rendered doubtful by the other

results of this investigation, for only those proteins appear to be identical that originate from seeds which are closely related botanically." The legumins obtained from pea, lentil, horse bean and vetch gave closely agreeing results and were therefore still held to be identical with each other as were the legumelins from the same seeds. The corylin of the filbert differed from the similar protein from the English walnut with which it had previously been identified. The gliadins from wheat and rye still seemed identical, but hordein from barley was slightly different and zein from maize was widely different from these. Thus in one clean-cut investigation Osborne showed that most of the vegetable proteins with which he was familiar differed more or less among themselves. Although the notion of absolute specificity could not come until a method of a finer power of discrimination, the later developed anaphylaxis test, was used, it was clear that purely chemical methods were capable of yielding highly important results.

Studies were undertaken of the tryptophane reaction, the Molisch reaction, the solubility limits in salt solutions and of the specific rotation of different proteins. Beginning in 1906, with the aid of a number of collaborators, Osborne carried out a series of analyses of the amino acid composition of proteins employing Kossel's method for the basic amino acids and Fischer's ester distillation method for the mono amino acids. 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.

By 1908, when the paper on "The Different Forms of Nitrogen in Proteins" (96) appeared, data had been accumulated clearly indicating that most of the known proteins could be satisfactorily characterized by the methods of amino acid analysis, coupled with a study of the physical properties. In a review

of his own work (94) published at this time Osborne stated: "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."

At the present day there is probably no qualified protein investigator who would be prepared to assert that any two proteins from different sources are in all respects identical. To be sure there are a few pairs or small groups of proteins, notably those from different varieties of maize or the gliadins from wheat and from rye, the differences between which are no more notable than the differences between two preparations of the same protein. Even the extremely delicate biological tests for

differences sometimes fail to discriminate between them; but to argue from this that no difference at all is present implies that more is known of these proteins than is really the case. Largely as a result of Osborne's careful characterizations of the seed proteins it is now generally held that each kind of plant and animal cell has its own equipment of specific proteins. While similarities occur in many cases and, in fact, are sometimes very conspicuous, rigid identification between proteins of unlike origin is not at present possible.

The demonstration that proteins differ widely in their amino acid composition and the definite knowledge of this composition that had been obtained, turned Osborne's attention in 1908 back to a problem that had been foreshadowed in some of his early papers and which he had had in mind almost from the beginning. In 1902 (56) he had written, "The animal can . . . synthesize protein from a mixture of the crystallizable products produced by the decomposition of proteins. Since such a wide difference exists between the proportions in which the several groups of products are yielded by the different food proteins, this synthesis must consist in something more than a recombination of the several fractions of the molecule of the food protein; it must involve a more or less extensive alteration of these fractions and conversion of one into another before the requisite number of groups of proper nature are at hand from which the new molecule can be constructed." He had realized, however, 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.

In 1909 this third phase of the work was begun in collaboration with Professor Lafayette B. Mendel of Yale University; their joint labors continued without interruption until Osborne retired in 1928. The investigation of the nutritive properties of the proteins involved the development of a technique for feeding individual small animals which would permit accurate measure-

ments 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 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 essential for growth other than protein. The preliminary assumption was made that the missing factor might be supplied by the inorganic constituents of the milk, and it was indeed soon 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 at constant weight on hordein, rye and wheat gliadin, but grew well on edestin, wheat glutenin, lactalbumin or casein (114, 115). Further work showed that the failure of animals on a zein diet was due to the lack of the amino acids tryptophane and lysine in this protein; when these were supplied growth occurred (130). Similarly, gliadin could be made adequate for growth by an addition of lysine in which this protein was conspicuously deficient (141).

The use of protein-free milk in diets was attended by certain difficulties. It was 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 that contained 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 eventually failed, complete realimentation occurred.

These results were published in 1913 (134). 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 given. The observations indicated that some 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 (142) 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 Osborne and Mendel and their 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 that empirical experience had shown to be advantageous. The distribution of vitamins in natural food products was studied and considerable success was attained in an effort to prepare a vitamin B 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 of the cell takes place.

It would be incorrect to assume that 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 (132): 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 dilute alcohol (180), the first animal protein that possessed this property to be found. Its anaphylactogenic relationships were worked out in collaboration with Professor H. Gideon Wells in 1921 (224) and it was demonstrated to be distinct from the other three proteins of milk.

The division of the present discussion of Osborne's work into three parts which correspond to the periods when he was chiefly interested in the preparation, the analysis, and in the biological properties of proteins has involved an omission of reference to several lines of investigation that bore less directly on the main theme.

In 1895 an investigation (26, 27, 39) was made of the chemical nature of the amylolytic enzyme diastase from barley malt. This study was undertaken on account of the similarity in the chemical properties of the albumins prepared from wheat, rye, and barley and because of the observation that extracts

from these seeds possess diastatic properties. By the application of the methods employed in protein investigation, specimens of an albumin-like protein were secured from malt with more than six times the amylolytic power of the most active preparations of diastase that had previously been described. It was shown that the enzyme had all of the properties of a protein while, at the same time, the method of preparation excluded the possibility that appreciable amounts of non-protein substances were present. The most active preparation produced ten thousand times its own weight of maltose from starch in seventeen hours at room temperature and it must therefore be reckoned, even today, as an extraordinarily active material. The necessity that chlorides be present in order that diastase may exert its maximum effect was pointed out and many observations were made of the effect of other salts on diastatic activity. These papers were the first that clearly proved the protein nature of an enzyme, they laid the foundation for the subsequent work of others on the enzymes that bring about the digestion of starch to sugar and they have had a far-reaching effect upon the development of the whole problem of the chemical nature of these substances.

In 1900 Osborne made a fundamental contribution to the chemistry of nucleic acids 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. He made it clear that the various nucleoproteins that 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.

A paper on the proteins of the castor bean published in 1905 (69) is of interest since it marked Osborne's first collaboration with Mendel and also illustrates the remarkable refinement of his methods of protein isolation. The castor bean contains an albumin, ricin, and a globulin, together with proteoses of less

clearly defined properties. Ricin is the best known of the toxalbumins. These are proteins and are among the most extraordinarily potent poisonous substances that have ever been described. Osborne made preparations of this material that were fatal to rabbits in doses of 0.0005 mg. per kilo of body weight, that is, one part of the preparation killed two thousand million parts of rabbit. Ricin itself was not secured in a form entirely free from protease, but it was separated from the globulin that accompanies it in the seed so completely that no physiological effect was observed when large doses of the globulin were administered to animals. The hemagglutinin in the castor bean was found to be identical with ricin and the protein nature of this toxalbumin was thoroughly established.

All of the preparation work and much of the chemical investigation of the vegetable proteins were completed before the present day conceptions of acidity had been advanced, nevertheless Osborne had noted the effects of different degrees of acidity on these substances and was alive to the significance of the phenomena. One of his early papers on edestin 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 when adjusting the reaction. 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 observed were fully appreciated. It is this careful attention to detail which gives Osborne's early work a value to the physical chemist and renders it possible to furnish interpretations in terms of modern theory, as has recently been done by Cohn.

The problem of protein differentiation was largely solved when the methods of amino acid analysis were applied to the proteins, but several pairs or small groups of proteins remained that were still indistinguishable by purely chemical means. In 1911 Osborne collaborated with Wells in a study of the anaphylaxis reaction of vegetable proteins that was continued for several years (111, 131, 139, 140, 151, 163). This was the first serious investigation of the biological reactions of such pro-

teins and it led to many valuable conclusions. A number of differentiations were made between proteins that had, up to this time, revealed no chemical differences. Thus the globulins juglansin and corylin from the American black walnut and the filbert, respectively, were found to be distinct as were the flax seed, cotton seed and hemp seed globulins, and the phaseolins of kidney bean and adzuki bean. On the other hand the gliadins of wheat and of rye and the legumins of pea and of vetch reacted strongly with each other. No chemical grounds for the differentiation of these proteins had been found. This result invited the final conclusion that the respective pairs of proteins were really identical. Such a conclusion could not safely be drawn, however, because a number of cases were encountered in which chemically distinct proteins gave powerful anaphylactic reactions with each other. Thus the gliadin and glutenin of wheat reacted positively with each other as did gliadin from wheat and hordein from barley and the vicilin from pea with the legumin from vetch. These reactions were attributed to the existence of common reactive chemical groups in the respective pairs of proteins. The specificity of the anaphylaxis reaction was therefore held to depend on the chemical structure of the protein molecule, but structures sufficiently alike to give rise to this reaction are only observed in proteins derived from plants that are botanically closely related.

The first public recognition of Osborne's exhaustive work came from Germany. V. Griessmayer, in 1897, published a translation of Osborne's papers on vegetable proteins in book form and stated in the introduction that it was his object "to bring to light these treasures buried in the American publications." This encouragement came at a time when few of his associates or scientific friends had any conception of what his work meant. In 1900 he was awarded a gold medal by the Paris Exposition. In 1910 recognition came from Yale University in the form of an honorary degree of Doctor of Science; and in the same year he was elected a member of the National Academy of Sciences and to the presidency of the American Society of Biological Chemists; two years later he was made an honorary

fellow of The Chemical Society (London). In 1914 he was made a fellow of the American Academy of Arts and Sciences and in 1921 was elected to the American Philosophical Society; in 1922 he received the John Scott medal and in the following year was made a research associate in biochemistry of Yale University with full 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.

Osborne's extensive investigations would have been impossible without generous financial support. Throughout the early years, when results came slowly and their application was by no means apparent, the directors of the Connecticut Agricultural Experiment Station, at first Professor S. W. Johnson, and after 1900, Dr. E. H. Jenkins, with the cooperation of an enlightened board of control, allowed no interference or distraction to hinder the progress of the work. After 1904 a large proportion of the financial burden was borne by the Carnegie Institution of Washington, D. C., of which he was a research associate. His 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 nor for outlines of projects. The relationship was always one of the utmost mutual confidence and esteem.

The results of many of his investigations were summarized in a monograph, "The Vegetable Proteins," which first appeared in 1909 (101) and was thoroughly revised in 1924 (243). This small volume has become the classical publication in the field. His extensive studies of wheat proteins were reviewed in 1907 in "The Proteins of the Wheat Kernel" (80), now a standard book of reference among cereal chemists. Including these and a few public addresses and popular articles a complete bibliography of his publications reaches 252 titles, of which about two hundred are journal reports of his personal scientific work.

To those who were privileged to be associated with him Os-

borne 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. 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. His most marked characteristics were, perhaps, the thoroughness with which his problems were investigated and the caution with which his results were presented to the scientific public. 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; casein and the wheat and maize prolamins received extraordinary attention. Time and again he discarded painfully acquired results to make a fresh start, this time to "do it right," as he expressed it. His publications, which cover some four thousand printed pages, are marked by lucidity of style, directness of statement and freedom from self-conscious literary adornment. All that he wrote bears the marks of careful editing lest a statement should to the slightest extent pass the bounds of ascertained fact.

He was more fortunate than most men in that advancing years, distinctions and scientific recognition did not bring with them administrative responsibilities which 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.

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