THE VEGETABLE PROTEINS

BY

THOMAS B. OSBORNE, Ph.D.

MONOGRAPHS ON BIOCHEMISTRY

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Dedicated

TO

SAMUEL W. JOHNSON

UNDER WHOSE DIRECTION THE AUTHOR

FIRST UNDERTOOK HIS INVESTIGATIONS OF

THE VEGETABLE PROTEINS

GENERAL PREFACE.

THE subject of Physiological Chemistry, or Biochemistry, is enlarging its borders to such an extent at the present time, that no single text-book upon the subject, without being cumbrous, can adequately deal with it as a whole, so as to give both a general and a detailed account of its present position. It is, moreover, difficult, in the case of the larger text-books, to keep abreast of so rapidly growing a science by means of new editions, and such volumes are therefore issued when much of their contents has become obsolete.

For this reason, an attempt is being made to place this branch of science in a more accessible position by issuing a series of monographs upon the various chapters of the subject, each independent of and yet dependent upon the others, so that from time to time, as new material and the demand therefor necessitate, a new edition of each monograph can be issued without re-issuing the whole series. In this way, both the expenses of publication and the expense to the purchaser will be diminished, and by a moderate outlay it will be possible to obtain a full account of any particular subject as nearly current as possible.

The editors of these monographs have kept two objects in view: firstly, that each author should be himself working at the subject with which he deals; and, secondly, that a *Bibliography*, as complete as possible, should be included, in order to avoid cross references, which are apt to be wrongly cited, and in order that each monograph may yield full and independent information of the work which has been done upon the subject.

It has been decided as a general scheme that the volumes first issued shall deal with the pure chemistry of physiological products and with certain general aspects of the subject. Subsequent monographs will be devoted to such questions as the chemistry of special tissues and particular aspects of metabolism. So the series, if continued, will proceed from physiological chemistry to what may be now more properly termed chemical physiology. This will depend upon the success which the first series achieves, and upon the divisions of the subject which may be of interest at the time.

R. H. A. P.
F. G. H.

PREFACE.

ALTHOUGH the proteins of plants early claimed the attention of many chemists, our present knowledge of them has not yet advanced beyond what is, in fact, a mere beginning of a serious study. The isolation and purification of vegetable proteins present so many difficulties that for a long time the available methods were too crude to enable those who undertook such work to succeed in their task. The development of the methods used by physiological chemists in their investigations of animal tissues, the great development of organic chemistry, and the no less important knowledge of the use of antiseptics and the action of enzymes has only recently made it possible to prosecute a study of the vegetable proteins with a reasonable prospect of success.

A knowledge of the vegetable proteins is in many ways of importance to the animal physiologist, but the latter has had so many rapidly increasing lines of research opened to him during the past few years that he has had little time to give to the literature of the vegetable proteins. The writer has, therefore, thought it more important to devote the limited space of the present monograph to a discussion of the general chemical and physical properties of the vegetable proteins than to give a descriptive account of the individual proteins at present known. It is hoped that by this method of presentation the opportunities offered by the vegetable proteins for obtaining a more definite knowledge of the properties of protein matter in general will be better appreciated, and that in the future the studies of vegetable and animal proteins will be brought into closer relations than in the past.

The knowledge of the chemistry of the carbohydrates has been chiefly founded on studies made with those of vegetable origin, and it is not at all beyond the range of possibility that further study of the vegetable proteins will result in greatly extending our knowledge of the chemistry of the proteins of both animals and plants.

T. B. O.

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CHAPTER I.

HISTORICAL REVIEW.

In 1747 Beccari (26) published in the *Proceedings* of the Bologna Academy an account of his experiments with wheat flour, in which he described the separation of the flour into two parts, one of which, he says, was similar to "those things that are extracted from vegetable substances," and "the other was such that it did not seem possible to extract it except from animal matter". He states that he had already communicated this fact to the Academy in 1728, but this communication appears to have never been published. After giving a detailed description of the method by which he obtained this peculiar substance, which we now know as wheat gluten, he describes at length the experiments by which he compared its properties with products of animal origin and contrasted its behaviour with that of other known substances of vegetable origin. In making these comparisons he used destructive distillation and pointed out that the distillates from vegetable materials were acid, while those from the wheat gluten were alkaline like the distillates from animal substances. A comparison was also made between the products of putrefaction of gluten with those yielded by animal and vegetable matter under similar conditions. Attempts to obtain a product like gluten from beans, barley and other seeds failed, and wheat appeared to be the only plant whose seeds contained anything resembling substances of animal origin.

Kessel-Meyer (187) in 1759 was the next to call attention to gluten, and gave a brief description of its preparation and of experiments to determine the action of various solvents upon it.

Rouelle (440) in 1773 announced that the glutinous matter, which up to that time was known to exist only in the seeds of wheat, was present also in other parts of various plants. This he considered to be the nutritive substance from which the caseous part of milk was derived. He stated that it was insoluble in water and gave rise to the same products as the gluten of flour, and also that it can be changed into a body having the same odour as cheese, as Kessel-Meyer had found to be the case with wheat gluten.

Rouelle (441) separated this glutinous substance from the juice of hemlock by heating to a moderate temperature and filtering out the coagulum, which had a bright green colour. He also obtained by fractional coagulation a part which contained nearly all of the colouring matter and, at a higher temperature, a part which was nearly free from colouring matter. The colouring matter could also be extracted by digesting the coagulum with alcohol. The protein nature of this substance was proved by the products of destructive distillation. Rouelle was, therefore, the first to obtain evidence of the wide distribution of protein substances in the different parts of plants.

During the same year Parmentier (369) described his extensive study of various vegetable substances used for food, and among other questions to which he devoted his attention was the gluten of wheat. He found that this substance, while insoluble in mineral acids, was soluble in vinegar, and that by neutralising its solution with sodium carbonate it was precipitated with apparently unchanged properties. Its solution in vinegar when evaporated to dryness left a horny and yellow residue which was not hygroscopic. When the gluten was extracted with spirits of wine some was dissolved, and the yellow solution when evaporated left a transparent residue which burned with a strong odour of burning animal matter. Nevertheless he appears to have regarded this residue as a resin. When the gluten was boiled with water it lost its tenacity and coherence and had evidently suffered a decided physical change. When the gluten was exposed to dry air at a low temperature it left a residue which, when treated with water, regained its original moist weight; from this he concluded that it occurred in the seed in the dry form and on contact with water became hydrated, hence the necessity for kneading dough.

Parmentier (370) in another paper stated that on drying, gluten lost two-thirds of its weight, and the dried product thus obtained, when triturated with water, was restored to its original glutinous and elastic condition. He also found that the quantity of gluten obtained from different flours was proportional to their colour, the darkest flour yielding nearly twice as much as the whitest.

In 1776 Parmentier (371) further stated that wheat flour must be very much altered in order to give no gluten, and that this substance completely disappears only when the seed has germinated.

At about this time Berthollet (32) reported that when gluten was treated with nitric acid nitrogen was evolved and the residue became yellow.

In 1789 Fourcroy (122) gave an extensive account of the occurrence

of coagulable protein in the juices of various parts of many plants, and described the method by which he obtained preparations of what he supposed to be pure plant albumin. This he found to have all the properties of animal albumin, and he gave an account of a comparison of these two substances. This observation of Fourcroy was the first to demonstrate the presence of two kinds of protein in plants.

From 1799 to 1805 albumin was found in the juices of many plants and in the sap of trees by a number of different investigators, who, however, added but little to the information which had been furnished by Fourcroy (*cf.* 91, 559, 182, 554, 62, 123, 385, 386, 555, 63, 556).

In 1805 Einhof (105) discovered that a part of the gluten of wheat was soluble in alcohol, and described the existence of similar proteins in rye (105) and barley (106). He, however, assumed that all of the gluten of wheat was soluble in alcohol, and considered this to be a characteristic property of all vegetable proteins except the albumin, which occurred dissolved in the juices of plants. He also undertook an extensive investigation of the constituents of the potato (104), barley (106), peas and beans (107) and lentils (108), and found that the leguminous seeds contained a form of protein which was not soluble in alcohol or in water. He assumed that this belonged to a distinctly different group of substances, although he recognised that it was related to the gluten which he had found in the seeds of the cereals. His discoveries showed the presence of two new forms of protein matter in plants and laid the foundation for a more extended knowledge of these substances.

In 1809 Gren (141) in his *Grundriss der Chemie*, in reviewing the literature of vegetable proteins, stated that gluten contains carbon, hydrogen, nitrogen, phosphorus and calcium, for by distillation it yields products which contain these elements. He further stated that plant albumin contains hydrogen, nitrogen, carbon, sulphur, oxygen and probably calcium phosphate, and cites as his authority the analyses by Fourcroy and a then recent paper by Jordan. He does not mention, however, the nature of the evidence given by these latter investigators from which this composition of plant albumin was deduced, and as he makes no reference to the original publications by either of these authors, it has not been possible to determine the basis for his statement, which appears to be the first publication in regard to the ultimate composition of vegetable proteins.

During the next ten years little advance was made beyond an addition to the number of seeds in which such substances were found (*cf.* 53, 180, 511, 557, 227, 558, 50, 387, 565).

In 1819 Taddei (537) separated wheat gluten into two well-characterised parts, one of which, soluble in alcohol, he called gliadin, the other, insoluble therein, zymom. By this observation three distinct proteins were shown to be present in wheat flour.

Gorham (135) described, in 1821, a protein soluble in alcohol which he obtained from the seeds of maize and to which he gave the name zein. A year later Bizio (37, 38) described the results of his investigation of this seed, and stated that zein was a mixture of gliadin and zymom, which Taddei had then recently found in wheat gluten, together with fat, and he regarded this mixture as similar to the gluten of wheat.

Braconnot (54) in 1827 described the protein constituents of some leguminous seeds. He named the protein which he obtained from them legumin and showed that it formed salt-like combinations with acids.

During the next few years little further progress was made in the study of plant proteins (*cf.* 256, 34, 35, 584, 55, 56, 57, 158, 44, 133). Up to this time the knowledge of vegetable proteins had extended only to a recognition of their general occurrence in plants and to a more or less crude description of their physical properties and solubility.

In 1836 Boussingault (51) published elementary analyses of several plant proteins which marked a new epoch in the development of their study, for these analyses were soon followed by those made in 1839 by Mulder (283) and by those made by Liebig and his pupils in 1841 and the years immediately following (*cf.* 223, 551, 451, 181, 155, 433, 434, 224). Apparently largely on the ground of these analyses, which agreed closely with those obtained with animal proteins, Liebig asserted in 1841 (223) that the different forms of plant proteins known at that time were identical with the proteins of animal origin which bore similar names. He recognised four such substances, namely, vegetable albumin, plant gelatin, legumin or casein, and plant fibrin.

Throughout the previous history of the development of knowledge of plant proteins and up to the time of Liebig, the idea of their identity with the animal proteins appears to have been universally accepted, and every effort had evidently been made by those who studied them to discover similarities between the proteins from these two sources. In the year following, however, Dumas and Cahours (97) presented the results of an elaborate study of the elementary composition of a considerable number of animal and vegetable proteins, which formed the foundation of a new advance in the knowledge of protein substances in general and contributed especially to the future studies of the pro-

teins of plants. By means of their then newly developed method for determining nitrogen, they were able to clearly establish differences in the elementary composition between many of the proteins, and they showed that these differences were particularly great in the case of some of the vegetable proteins. The identity which Liebig (223) assumed to exist between the vegetable and animal proteins was thus disproved and the further accurate study of the proteins of vegetable origin became a matter of importance.

Little was done during the next ten years to materially advance the knowledge of vegetable proteins, but in 1855 Hartig (148, 149) published the results of his elaborate investigations of seeds, in which he showed that a large part of the reserve protein was present in the cells in the form of crystals and grains of more or less definite structure. This discovery was followed three years later by Maschke's (267, 268) announcement that he had succeeded in artificially crystallising the protein of the Para or Brazil-nut, which Hartig had shown to be present in this seed in the form of rhombohedral crystals.

In 1859 Denis (88) showed that many protein substances of both animal and vegetable origin were soluble in neutral saline solutions, and this presented to chemists an entirely new means for isolating and purifying these substances. Although Denis' discovery has since been of fundamental importance in the modern study of proteins, especially those of seeds, its importance was not appreciated for several years.

In 1860 Ritthausen (396) began the first serious study of the vegetable proteins, and devoted himself for many succeeding years to the production of preparations of the highest attainable purity, and to accurate determinations of their composition. As a result of these investigations the prevailing knowledge was greatly extended, and it became plain that these substances occurred in many diverse forms in the different seeds. Ritthausen's work, therefore, furnished the first broad foundation for a knowledge of the vegetable proteins, and the service which he rendered in developing this field of knowledge deserves far more recognition than it received during his lifetime.

In 1876 Weyl (569, 570) applied to seeds the method of extraction by solutions of neutral salts which Denis (88) had proposed in 1858, and showed that a large number of different seeds contained protein soluble in saline solutions, and that this protein had properties similar to the globulins of animal origin. These vegetable globulins he divided into two groups, the myosins and vitellins, according as they were insoluble or soluble in saturated solutions of sodium chloride. The views which he advanced in respect to the general character of

protein substances in seeds received immediate and extended recognition on the part of physiologists and of those familiar with proteins of animal origin; and as he claimed that the preparations which Ritthausen had obtained by the aid of weak alkaline solutions were altered products of the original protein constituents of the seeds, the work of Ritthausen soon fell into disrepute and was discredited as not showing the real protein constituents of the seeds which he had studied. Although Ritthausen (415, 416, 417, 418, 421, 423, 425) soon after showed that many of his preparations were largely or wholly soluble in saline solutions and had for the most part retained their original solubility unaltered, and also that the products obtained by direct extraction of seeds with neutral salt solutions agreed in many cases entirely with those which he had previously described, physiologists continued to overlook and disregard his assertions. Since Ritthausen ceased his work little has been done in this field outside of the work carried on by the writer for the past eighteen years. During this time papers have appeared which dealt with special questions in the chemistry of these substances, but no connected and extensive investigation of the vegetable proteins has been undertaken by any one else. 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, the writer has directed his attention largely to a review and extension of Ritthausen's work with the hope of clearing up some of the existing uncertainties. For these reasons the questions which are discussed in this monograph are largely based on the writer's own work and are chiefly a review of subjects which he has studied.

CHAPTER II.

OCCURRENCE OF PROTEINS IN DIFFERENT PARTS OF PLANTS, AND THEIR GENERAL CHARACTERISTICS.

PROTEINS are found in the living parts of all plants. They occur in the dissolved state in the circulating fluids and in the solutions of the cell vacuoles, that is in the cell sap. In a semi-dissolved state they occur in the protoplasm, and in the undissolved state as reserve protein in the cells of seeds, tubers, bulbs, buds and roots.

In many of the cells of these parts of the plant the undissolved protein is found in the form of well-developed crystals of various forms, formerly called crystalloids; in irregular, semi-crystalline forms with faces and angles on a part of their surface, and as regular or distorted spheres, all of which several forms are found in aleurone grains; and in an amorphous, finely granular form, generally designated aleurone. The reserve protein occurs in the cells together with the non-nitrogenous reserve food materials, starch, oil, etc., which several substances fill the cells, leaving a thin layer of dried protoplasm between them and the cell wall [*cf.* Hartig (148, 149); Radlkofer (388); Nägeli (286); Schimper (453); Schulz (462)].

In most monocotyledonous plants the cells of the endosperm and embryo occupy distinct parts of the seed. The tissues of the endosperm of such seeds when fully ripe are, therefore, made up of cells which are almost entirely filled with the reserve food substances, since the thin layer of protoplasm next to the cell wall forms a very small part of the contents of the cell.

The tissues of the embryo contain protein associated with a greater variety of substances than are present in the cells of the endosperm, and are also rich in nucleated cells, in which much of the protein apparently exists in the chromatin substance of the nuclei in special forms of combination with nucleic acid which are generally known as nucleoproteins and nuclein. In this part of the seed the chemical conditions are therefore more complicated than in the cells of the endosperm, since the metabolic processes of the embryo apparently require a greater variety of substances than exist in the cells of the endosperm of the fully ripe seed, whose chief office is to supply food to the subsequently

developing embryo. That this variety in the nature of the constituent substances is shared by the proteins of the embryo tissues has been shown by studies of the protein constituents of the wheat embryo which will be later considered more in detail.

In most dicotyledonous seeds the cells containing the reserve protein are distributed among those of the embryo tissues.

In roots, bulbs and tubers the undissolved reserve protein occurs suspended in the cell sap, frequently in the form of crystals.

Little that is definite is known concerning the chemical properties of any of the plant proteins except those of seeds, for the proteins occurring in the physiologically active cells and fluids of plants have been but little studied, owing to the relatively small quantities in which they occur and the difficulty of separating them from each other.

We have just seen that the total protein is contained in several different parts of the seed, namely, in the endosperm cells as reserve protein, in the protoplasm of these cells, and in the cells composing the tissues of the embryo, both in the cytoplasm and in the nuclei. As it is not possible, in most cases, to separate these different parts of the seed by mechanical means, in sufficient quantity to permit a study of the proteins of each, extracts of the entire seed will consequently contain a mixture of proteins from all the different parts of the seed, and may be expected to contain a number of different proteins. Experience has shown this to be the case, as a careful examination of the extracts of all seeds thus far studied has shown the presence of a number of different types of protein. The whole of the protein contained in seeds is, therefore, not reserve protein. Extracts of seeds always contain in addition to a relatively large amount of one or two types of protein, which are manifestly the reserve protein of the seed, a certain small proportion having distinctly different properties from those of the latter. It is probable that most of the former protein is yielded by the cells of the embryo as well as by the protoplasm of the endosperm cells. With most seeds definite evidence of this has not yet been obtained, but in the case of wheat the embryos are separated by the commercial process of milling in a nearly pure condition, and a study of this product has shown that those proteins which are obtained only in small quantity from the entire seed are present in relatively large amounts in this embryo meal. The proteins of this embryo both in chemical and physical character differ from those of the endosperm and resemble more nearly the physiologically active proteins of animal tissues. These embryo proteins are globulin, albumin and proteose, and associated with them is a large quantity of nucleic acid, so that products

similar to the nucleoproteins and nucleins are also obtained from this embryo meal.

Thus Osborne and Voorhees (366) [*cf.* also Frankfurt (125) and O'Brien (297)] found that the flour of the entire kernel of samples of spring and winter wheat yielded the quantities of the proteins enumerated in the following table:—

	Spring Wheat per cent.	Winter Wheat per cent.
Glutenin	4·68	4·17
Gliadin	3·96	3·90
Globulin	0·62	0·63
Albumin	0·39	0·36
Proteose	0·21	0·43

Osborne and Campbell (336) obtained from the wheat embryo meal no gliadin or glutenin, 10 per cent. of albumin, 5 per cent. of globulin and about 3 per cent. of proteose. The embryo meal contains a relatively large proportion of nucleated cells and therefore a large amount of nucleic acid, which was extracted in combination with the proteins, though much remained in the meal residue in insoluble combination with the remaining protein. In view of these facts there can be little doubt that much of the globulin, albumin and proteose obtained from the entire seed was originally present in the tissues of the embryo, although a part, possibly, was yielded by the very small amount of protoplasm in the endosperm cells. Similar conditions must certainly exist in other seeds, and the proteins that are found only in very small quantity in the extracts of many seeds may be derived from the embryo and protoplasm and are not to be regarded as a part of the reserve protein of the seed. Thus, for instance, the hemp-seed, which yields a large part of its protein in the form of crystalline edestin, also yields a very small quantity of one or two other proteins coagulated by heating the extracts to about 80°, which latter are probably not a part of the reserve protein of this seed.

The proteins of seeds have been the subject of extensive investigation, and we now know much concerning the chemical and physical properties of a number of different proteins from several species of seeds. Most of these, which are unquestionably the reserve proteins of these seeds, are products of the metabolism of the plant, and, in the fully ripe seed, no longer take part in its physiological processes. They may, therefore, be regarded as in a sense analogous to excretory products, for, as Pfeffer has said (603), "All protoplasmic secretions which appear externally and are lost to the plant or which can take no further part in metabolism are to be regarded as excretory substances".

In many ways the reserve seed proteins bear a relation to the

physiologically active tissues of the parent plant similar to that which the animal albuminoids bear to the physiologically active tissues of the animal, for the reserve protein of ripe seeds has even less connection with the living tissues of the plant which produced it than the albuminoids of hair, horn and hoof have with the living tissues of the animal.

This physiological analogy extends, within certain limits, to the chemical characteristics of these substances. Like the animal albuminoids, the reserve seed proteins are more stable toward chemical and physical agents than are the proteins which form parts of the living substance of animals, and also, like most of the albuminoids, they differ more widely in the proportion of some one or more of the amino-acids which they yield when hydrolysed than do the more physiologically active proteins of either vegetable or animal origin, the protamines excepted. Thus gliadin of wheat and hordein of barley yield approximately 37 per cent. of glutaminic acid, while silk fibroin yields 36 per cent. of glycocoll and 21 per cent. of alanine. The alcohol-soluble seed proteins yield the basic amino-acids in very small amount as do also some of the albuminoids, *e.g.*, elastin and keratin. No such wide differences have been found among the physiologically active proteins of plants or animals, and in this respect many seed proteins and albuminoids present a marked structural difference from the tissue proteins. The seed proteins, moreover, offer an advantage for chemical study which most of the albuminoids do not, as, unlike most of the latter, they can be obtained in a soluble form and very frequently in a well-crystallised state which makes their purification much easier than that of the insoluble albuminoids.

Owing to their relatively great stability most of the seed proteins can be readily subjected to extensive fractional precipitation, and the chemical and physical properties of successive fractions can be easily compared. These proteins, therefore, yield more definite and well-characterised preparations than do most of the known proteins of animal origin, and it is expected that a study of them will result in more definite knowledge of the chemistry of protein matter than can be obtained by a study of proteins from any other source.

The majority of seed proteins thus far studied are globulins, that is, they are soluble in neutral saline solutions from which they are precipitated by dilution or by dialysis. Those seeds, as yet studied, in which the protein occurs within the cell in the form of crystals or spheroids or of partly crystalline masses yield large quantities of globulin precipitable by dialysis in forms similar to, or identical with, those which exist in the seed. Seeds of the cereals which contain the

protein in a finely granular condition and without definite form yield relatively small quantities of globulin, for the great mass of the reserve protein isolated from these seeds is insoluble in saline solutions. The proteins isolated from many seeds are apparently obtained with unchanged properties. The crystalline and spheroidal forms exhibited by the proteins within the cells of those seeds which yield large quantities of globulin suggest that this protein was deposited within the cell by a process similar to dialysis, by which such forms can be artificially reproduced.

In those cells of the endosperm in which protein is most frequently found in the form of crystals or of small spheres there is usually observed a globule, composed largely of insoluble mineral substance, commonly called a globoid. This globoid was formed from soluble mineral salts contained in the fluid of the cell, and it is possible that, as its constituents passed out of solution, through the formation of an insoluble combination, the concentration of the salts in the cell fluid was correspondingly reduced and the globulin thereby precipitated, in much the same way as occurs when salts are removed by dialysis.

As already stated, the proteins extracted from seeds are obtained in various forms which represent distinctly different protein substances. The solubility of the protein matter in different seeds varies greatly, but in general it is found that a part is soluble in water, a part is soluble in neutral saline solutions and a part is insoluble in either of these solutions but soluble in dilute solutions of acids or alkalies, while in the seeds of the cereals a part is also soluble in alcohol of from 70 to 90 per cent. The proteins extracted from seeds by these several solvents will be considered in greater detail later.

The possibility of making preparations of proteins which may fairly be considered as chemical individuals deserves special attention. These substances present unusual difficulties in this respect, for they have none of those physical and chemical characters which the chemist usually depends upon to show the individuality of an organic chemical compound; in other words, the proteins have no single property by means of which any judgment can be formed in regard to the strict chemical individuality of any one of them. The best that can be done at present is to establish a constancy of the ultimate composition of successive fractional precipitations of the protein under consideration, and to show the constancy of the physical properties and products of hydrolysis of these fractions so far as this is possible. There can be no question, where differences are found in composition or properties between successive fractions of a protein preparation, that the substance

in question is a mixture. We can, therefore, for the present, treat as individual proteins only those products whose extensive fractionation has given no evidence that a mixture is being dealt with, and we must await new methods of study before any one of these proteins can be definitely accepted as a true chemical entity. Many, however, of the proteins with which we are at present familiar have shown such constancy of composition and properties that we feel justified in now considering them as substances of reasonably definite character.

In this connection it is worth while to consider the evidence now available which indicates the possibility of separating these proteins from one another, as well as from associated substances, for it has been generally assumed that the difficulties presented in making such separations are so great that there is little hope for success. Most of the seed proteins now known are globulins which are precipitated by dialysis in the form of crystals or spheroids which are relatively large when compared with the minute particles of an amorphous precipitate. These separate from solution very slowly, and are deposited as dense precipitates which adsorb associated substances to a far less extent than do the amorphous, bulky precipitates of the proteins of animal origin. The best evidence of this is the fact that the globulin of the castor bean, as Osborne, Mendel and Harris (365) have found, could be freed by a single reprecipitation by dialysis from all but minute traces of any toxic substance, though the solution from which it separated on the first dialysis contained a large quantity of extremely active ricin. Ricin, if not a protein, is, at least, so intimately associated with the albumin that it is practically quantitatively precipitated therewith. The conclusion is, therefore, justified that the separation of the castor-bean globulin from the water-soluble albumin by a single reprecipitation by dialysis is in a high degree complete. This fact is good ground for considering it possible to separate proteins very completely from one another under such conditions.

As to the separation of globulins from the non-protein substances with which they are associated in the seed extracts, the fact that many of these globulins can easily be obtained so free from carbohydrates that their preparations give no trace of reaction with the Molisch test, is strong evidence that they can be obtained entirely free from non-protein contaminations. Although such evidence is indirect and does not prove that preparations of these proteins can be obtained in a state of purity and unmixed with other proteins, nevertheless, it strongly indicates that these can be obtained in a higher state of purity and of more definite character than is commonly supposed to be possible in preparing proteins of animal origin.

CHAPTER III.

ISOLATION AND PREPARATION OF SEED PROTEINS.

VARIOUS methods have been employed to isolate the different protein constituents of the several seeds which have been examined, but as essential modifications of the necessary procedure are required for almost every one of these seeds it is impossible to give here an account of the methods in sufficient detail to enable the reader to make practical use of them in isolating and purifying preparations of the individual proteins. He is therefore referred to the original papers in which the proteins are described or to special publications dealing with this subject. An outline of these methods and a consideration of the results which can be expected from them deserves some discussion and will be dealt with in this chapter.

The solvents used to extract the proteins of seeds are water, neutral saline solutions, 70 to 80 per cent. alcohol, and very dilute acids and alkalies. By the successive application of these solvents the greater part of the protein can be extracted from most seeds when finely ground, but the residue, even after extracting as completely as possible, usually contains more or less nitrogen. Although not definitely proved it is probable that this nitrogen belongs largely to protein enclosed in cells of the seed tissue which have escaped disintegration in grinding. In most cases the amount of this undissolved nitrogen is so small that it does not require further consideration.

A. Extraction with Water.

Water may dissolve several types of protein from the ground seeds, namely, proteoses, albumins, globulins, soluble in very dilute saline solutions, and such soluble compounds of any other proteins with acids as may be present in the seed, or formed during extraction. It may, as frequently happens with many of the leguminous seeds, also dissolve a relatively large proportion of protein soluble in water when uncombined with acid but insoluble in the form of its salts, that is when the protein is combined with a definite but small proportion of acid. (See Chapter IV., p. 28.)

The usual method employed to isolate the proteins from the aqueous extract is to saturate the solution with ammonium sulphate, filter out the precipitate produced, which contains all the proteins, dissolve this in the dilute solution of ammonium sulphate which results on treating it with water, and, after filtering clear, to dialyse the solution in running water for several days until no more precipitate is produced. If a precipitate results, this contains the globulins, the salts of such proteins as are soluble in water in the uncombined state but have the properties of globulins when combined with the small quantities of acids which usually develop during the process of extraction, and also such insoluble derivatives as frequently result, during dialysis, from changes in the albumins. Such products do not appear to be formed from animal albumins but are not uncommon alteration products of vegetable albumins. After filtering out the precipitate produced by dialysis, the solution should be tested for proteins which can be precipitated as acid compounds from very dilute saline solutions. This is done by adding to a part of the solution about 0·5 per cent. of sodium chloride and passing carbonic acid through it for some time. The small concentration in hydrogen ions thus produced enables such proteins, if present, to unite with hydrochloric acid and thereby form insoluble compounds. [*Cf.* Osborne (315)]. If a precipitate is thus produced the entire solution is treated in the same way, the resulting precipitate filtered out, and the filtrate again dialysed until no more precipitate separates. From the solution, thus freed from globulins, the albumins are separated by fractional coagulation by heating to definite temperatures in a water bath, or by fractional precipitation with ammonium sulphate, at definite concentrations; the procedure, in either case, being determined by preliminary experiments, whereby the proper temperatures or concentrations in ammonium sulphate are determined. When the albumins have been separated from the solution by heat, the filtered solution is dialysed into about twice its volume of alcohol, whereby it is rapidly reduced in volume, and at the same time becomes mixed with a considerable proportion of alcohol. By repeating the dialysis in a fresh quantity of alcohol, or by adding alcohol to the solution, the concentration in alcohol is made sufficiently great to precipitate all the dissolved proteose. If the latter does not separate readily from the solution it can be made to do so by adding a drop or two of strong ammonium acetate solution. The precipitate is digested with absolute alcohol, dissolved in water, filtered from any insoluble residue which may be present if globulins or albumins have not been completely separated

by the previous treatment, and the different proteoses separated from each other by the methods commonly employed for effecting such a separation of proteoses of animal origin.

The complete and quantitative separation of globulins, albumins, and proteoses is a matter of difficulty. By dialysis heteroproteose may be precipitated with the globulins, and, owing to the widespread occurrence of proteins in seeds which in the free state are soluble in water but as salts behave like globulins, more or less of these proteins may be present in the solution after coagulating the albumins, and consequently become mixed with the proteoses. The separation of the above-mentioned proteins soluble in the free state, as well as the globulins, would be comparatively easy if either of these could be completely coagulated by boiling their solutions, as is the case with animal globulins. Unfortunately these proteins are very imperfectly, if at all, coagulated by heat, and therefore cannot thus be separated; in fact many of the so-called seed globulins closely resemble heteroproteoses in their solubility.

The precipitates of globulin obtained by the preceding process are united and dissolved in dilute sodium chloride solution and, after filtering, are reprecipitated by dialysis. If a sufficient quantity of globulin separates it may be further purified by fractional precipitation with ammonium sulphate, according to Hofmeister's well-known method.

By extracting the seeds of the cereals with water only a small proportion of the total proteins are removed and this consists of a little proteose, a few tenths per cent. of albumin, and a very little globulin. Many of the leguminous seeds, such as peas, vetches, beans (except *Phaseolus*), lentils, and soy beans, yield relatively much protein to water which, on adding a little acetic acid, and passing carbonic acid through the extract, or on standing until the extract becomes slightly acid, is largely precipitated. This precipitate, when freshly formed, is soluble in sufficiently concentrated saline solutions, and thenceforth behaves like globulin and for convenience is commonly treated as globulin according to the current arbitrarily employed methods of classifying proteins. From other leguminous seeds, such as the lupines, very little more than traces of protein are extracted by water.

Water extracts much of the protein from many of the oil seeds, when freshly ground, such as the Brazil-nut, the almond, and the hazel-nut, but from many of the others it extracts but little. The aqueous extracts of such of the oil seeds as yield much protein to water give abundant precipitates when treated with carbonic acid, and this method

of separating the dissolved protein was long ago used by many, *e.g.*, Maschke (267), Sachse (445), Drechsel (95), Ritthausen (414), though it was not recognised that this precipitation depends on the combination of the protein with acid until the writer's recent studies (312, 315) of such combinations showed that this is almost certainly the case. (See Chapter IV., p. 22.) The older idea that this solution of the protein was caused by the alkaline phosphates is no longer tenable in view of what is now known of the relations of proteins to bases and acids, and of the small amounts of phosphates which exist in the seed, since recent investigations have shown that nearly all of the phosphorus of seeds is in organic combination.

Water extracts only a small proportion of albumin from most of the seeds yet studied, in regard to the character and occurrence of which the reader is referred to Chapter X., p. 73, where the subject is discussed in further detail.

B. Extraction with Solutions of Neutral Salts.

Ten per cent. sodium chloride solution is the solvent usually employed for these extractions. This may be used either after the ground seed has been extracted with water, in which case only those proteins soluble in neutral saline solutions are obtained in the extract or it may be applied directly to the meal, in which case the extract will also contain nearly or quite all of the proteins which are extracted by water alone. The latter method is the one which is usually adopted, for it simplifies the process of isolating the proteins and reduces the time necessary for making the extractions, which is an important consideration, as changes appear to take place in the seed extracts, due probably to enzyme action, which may lead to more or less alteration of the dissolved proteins. The amount of protein extracted by sodium chloride solution rarely represents all the protein which remains after extraction with water, but in most seeds it forms a large part of the total protein. The amount of protein thus extracted from different seeds differs greatly. The cereals yield but a small proportion of their total protein to neutral salt solutions while many of the oil seeds yield a very large proportion.

The general plan followed in conducting an extraction with sodium chloride solution is to treat the ground seed with a sufficient proportion of the solvent so that it will subsequently yield an extract of which so much can be filtered clear as to be equal to about three-fourths of the volume of the solvent applied to the meal. The proportion necessary to attain this end depends not only on the amount of the insoluble re-

sidue but also on the proportion of water with which it combines. It also depends to a large extent on the character of the solution which the soluble constituents of the seed yield, for some seeds contain substances which produce extremely gummy, viscid solutions which render filtration extremely difficult. No general statement can be made as to the proper proportion of solvent, or the methods to be employed in filtering the extracts, as each seed requires special treatment. The filtered extract may be subjected at once to dialysis and the dissolved globulin thus separated ; or the total proteins which it contains may be precipitated with ammonium sulphate, redissolved in saline solutions and the globulin precipitated by dialysis ; or it may be first subjected to fractional precipitation from ammonium sulphate solutions of definite concentration and solutions of the resulting fractions separately dialysed.

Extracts of seeds which yield a large proportion of globulin are usually best subjected to direct dialysis, and the globulin which separates fractionally precipitated from saline solutions or fractionated with ammonium sulphate. Those which yield but little globulin are best saturated at once with ammonium sulphate in order that the precipitate may be redissolved in a small volume of water. The proteins soluble in water may be obtained from the filtrates from the precipitates produced by dialysis according to the general plan above indicated for separating them from aqueous extracts.

The globulins may also be obtained by sufficiently diluting the sodium chloride extract with pure water and passing carbonic acid through it. The degree to which it should be diluted and the completeness with which globulin can be thus separated from the solution depends on the solubility of the globulin in question and should be determined in each case by special experiments. The separation is rarely as complete by dilution as by direct dialysis of the extract, and the amorphous precipitates and voluminous solutions are not so easily dealt with afterwards.

The extraction may also be made with warm dilute saline solutions and the globulin precipitated by cooling the filtered extract as, in most cases, the globulins are much less soluble in cold solutions than in warm. This method of treatment often results in the production of crystalline preparations which can be afterwards recrystallised from warm dilute saline solutions and brought to a high state of purity. This method of crystallisation was first extensively employed by Grübler (143), and later by many others, for the production of crystalline preparations from the squash seed. Globulins which sepa-

rate under these conditions in a crystalline form can usually be obtained as a crystalline deposit by dialysing their saline solutions into water, and this method, which was first employed by the writer (301) for the production of crystalline preparations of proteins, is one which presents many advantages and yields products of great purity. Other salts than sodium chloride may be employed for extracting the globulins but these have not been frequently used. Thus Grübler (143) obtained crystalline preparations from solutions of the chlorides of sodium, ammonium, barium, and calcium, of the bromides and iodides of potassium, of magnesium sulphate, ammonium oxalate, and potassium ferrocyanide.

Preparations of seed globulins frequently contain more or less of an alteration product which is insoluble in neutral saline solutions. (Compare Chapter VII., p. 38.) This product, which forms in acid solutions, greatly interferes with the subsequent purification of the globulin, as, owing to the fact that it cannot be restored to its original condition, it is lost for further purification and study. The proportion of such insoluble derivatives of the globulin can usually be greatly diminished if the amount of free acid in the extract is kept at a minimum. It is, therefore, usually advisable to add to the saline solution, used in making the extract, a sufficient quantity of baryta to make the extract neutral to litmus. A larger proportion of baryta should be avoided, for if more than enough is added to make the extract neutral to sensitive litmus paper, the yield on dialysis may be greatly diminished.

C. Extraction with Alkaline Solutions.

Alkaline solutions were extensively used by Ritthausen and, during the earlier years of his work, were almost exclusively employed by him for isolating the proteins which he studied. Owing to the danger of alteration in the protein which might result from the action on it of the alkali, the results which Ritthausen obtained were regarded with suspicion by most physiological chemists. Consequently the use of alkalies has for many years past been abandoned except in those cases where it is not possible to isolate the protein by any other means. It is probable that the alterations which result in the protein during extraction with sufficiently diluted alkalies have been greatly exaggerated, and that such solvents often yield preparations of the unchanged protein. In fact, experience has shown that evident alterations in the protein molecule result far more quickly and extensively under the influence of minute quantities of acid than they do under the influence of equivalent quantities of alkali. Ritthausen showed that many of

the preparations which he obtained from seeds by neutralising alkaline extracts were soluble in sodium chloride solutions, and others have also found that, in many cases, the protein thus dissolved could be obtained in the same crystalline form and with the same chemical and physical properties as after direct extraction with sodium chloride solutions. It is, therefore, almost certain that, if the concentration in alkali does not exceed a proper limit, many, if not all, seed proteins can be obtained by its use with unchanged properties.

The greatest danger in employing this method comes not from the action of the alkali but from that of the acid which is used in neutralising it. In precipitating the proteins from alkaline extracts a complete separation is usually obtained only when an excess of acid is added beyond that required to neutralise the alkaline, or in other words, only when sufficient acid is added to form a salt of the protein insoluble in water. In neutralising alkaline extracts it is exceedingly difficult to add just enough acid to effect precipitation without exceeding this amount. If the excess of acid be even exceedingly small the protein is rapidly converted into products which are no longer soluble in neutral saline solutions. (Compare Chapter VII., p. 39.)

The amount of protein extracted by alkalies is usually greater than that extracted by neutral saline solutions or by water, in fact, in many cases it is very much greater. Sufficient attention has not yet been directed to the cause of this difference, and for this reason our present knowledge of the character of the total protein constituents of the greater number of seeds which have been studied is still incomplete. This difference may be due to any one of several causes. The alkali may dissolve protein which in its native condition is insoluble in salt solutions but soluble in alkali. It may dissolve protein enclosed within unruptured cells and hence inaccessible to the action of neutral solvents. It may dissolve compounds of the globulin with non-protein substances which are insoluble in neutral solvents. The precipitate produced by neutralisation may consist of globulin which undergoes changes during the process of extraction with and isolation from salt solutions whereby a large part of the original globulin is converted into proteoses which on dialysis are lost by diffusion, or even into polypeptide combinations or free amino-acids which are not precipitable by saturating their solutions with ammonium sulphate, and are thus lost in the ordinary processes employed for isolating the dissolved protein.

According to the older view the proteins soluble in alkalies dissolved in consequence of the formation of soluble alkaline salts, hence these proteins were designated caseins. Although protein may some-

times be thus dissolved by alkalies, apparently this is not generally the case as the majority of seed proteins have more pronounced basic properties than acid, and the reason that these dissolve when treated with alkalies is often due to the fact that combined acid is neutralised and the protein set free in a soluble condition. (Compare Chapter IV., p. 28.)

When compounds of protein insoluble in neutral or acid solutions exist in the seed the acid and the protein both pass into solution on treatment with alkali, and when such solutions are neutralised or acidified, and if a relatively insoluble combination of the protein with the organic acid is possible, the two recombine, and a salt of the protein with the organic acid, rather than with the acid employed for neutralising, results. Thus if the protein is combined with nucleic acid, solution may result from double decomposition of protein nucleate with the formation of soluble alkaline salts of nucleic acid from which the nucleate is regenerated on adding acid. It is almost certain that, in very many cases, the so-called nucleins and nucleo-proteins which have been described are products of this character, that is protein nucleates. (See Chapter X., p. 82.) The alkaline solution used for extracting proteins may be either very dilute solutions of caustic alkalies, usually 0·1 to 0·2 per cent. potassium hydroxide or dilute solutions of alkaline carbonates or bicarbonates, 0·50 to 1·0 per cent. The choice of the proper solvents depends on circumstances which must be determined for each case, all unnecessary excess of alkali being avoided since the concentration of the extract in hydroxyl ions should be kept as low as possible. As most seeds yield slightly acid extracts or contain weak bases combined with acids the extract made with dilute alkalies has a less concentration in hydroxyl ions than the solution employed in making it. The amount of acid contained in the seed or capable of development from it should, therefore, be carefully considered. The practice of employing acid and alkaline solutions for dissolving proteins which contain definite concentrations of acid, and having no regard to the amount of protein or its state of combination with acids or bases, is to be avoided as far as possible in attempting to obtain preparations of proteins with unchanged properties.

D. Extraction with Alcohol.

Extraction with somewhat diluted alcohol, that is alcohol of 70 to 80 per cent., has been employed to remove a part of the protein from the seeds of cereals, but proteins which dissolve in this solvent have not been found in any other seeds. Extracts with alcohol can be made at any

temperature up to the boiling-point of the solvent, for, if the alcohol is sufficiently concentrated, the proteins which dissolve in it are entirely unchanged. At higher temperatures the proteins dissolve more quickly than at lower. The dissolved protein can be separated from the filtered extract either by diluting with water or by concentrating, best under diminished pressure and at a temperature below 50°, whereby the alcohol is removed to such an extent that the protein separates from the nearly aqueous residual solution. It is important in carrying out this concentration to avoid high temperatures toward the end of the process, for in the presence of relatively much water the protein is easily coagulated at high temperatures, and when a part has been thus rendered insoluble the subsequent filtration of the extract becomes exceedingly difficult, if not impossible, although but little insoluble matter may be present. From concentrated alcoholic solutions the protein may also be separated by adding absolute alcohol, for none of these proteins is soluble except in alcohol containing some water. The precipitation by absolute alcohol can be made more complete by adding ether, and this method affords an excellent means for separating from the protein the fats and oils which are invariably extracted from the seed together with it.

CHAPTER IV.

BASIC AND ACID PROPERTIES OF PROTEINS.

A. Basic Properties.

IT is now generally recognised that proteins have both basic and acid properties. Most of the evidence in support of this has, however, been obtained from experiments in which relatively large quantities of acids have been used, whereby the protein has probably been to some extent altered and its original deportment towards acids changed. The grounds for this assumption are given on page 37, where the denaturing effect of acid is discussed in detail.

Ritthausen (416) stated that his preparations of crystallised edestin dissolved to a large extent when washed with water, and this has since been observed by all who have made preparations of this protein.

From this apparently remarkable fact, that the edestin, originally crystallising from a dilute aqueous solution, dissolves on simply washing with water, one would naturally conclude that the protein had either suffered a serious change in the processes employed in isolating it or that the crystalline preparation consisted of at least two distinct proteins of different solubility. Neither of these suppositions is in fact true, for the behaviour of the preparation is wholly due to the basic properties of the edestin, as will be soon shown.

A failure to recognise the effect of the basic nature of vegetable proteins on the solubility and other properties of their preparations has led to much of the confusion that still exists in regard to them. Since a clear understanding of these properties is fundamental to an intelligent study of the plant proteins, and its importance appears as yet not to be fully appreciated, experiments made by the writer with edestin (312, 315) are here given in considerable detail.

Pure edestin, from hemp-seed, when free from combined acid or alkali, is entirely insoluble in water, but in the presence of a very little acid, and in the complete absence of salts, it promptly dissolves to a clear solution. From such a solution the edestin is readily precipitated by the addition of a small quantity of a neutral salt, *e.g.*, sodium chloride,

and the fact that the edestin has precipitated in combination with the acid is conclusively shown by the behaviour of this precipitate. When such a precipitate is washed thoroughly with dilute alcohol, until all the sodium chloride is removed, and is then dissolved in water, a definite quantity of potassium hydroxide is required to render the solution neutral to phenolphthalein. When thus neutralised, the edestin is completely precipitated, and can be removed from the solution by filtration. If this solution is then evaporated to dryness it leaves a residue of potassium chloride containing nearly all of the alkali originally employed for neutralisation. It seems clear from this experiment that the hydrochloric acid used to dissolve the edestin is precipitated with it in the form of a protein salt.

If edestin is extracted from hemp-seed by means of a sodium chloride solution the extract has a slightly acid reaction toward litmus. If this extract is dialysed the edestin is precipitated in a crystalline form, and, when washed with water and alcohol and dried, can be obtained as a powder composed of microscopic crystals. If this powder is suspended in water, as can be very easily done, owing to its physical condition, a certain quantity of potassium hydrate must be added to the suspension before a red colour is produced with phenolphthalein. The edestin then remains as an insoluble mass of decomposed crystals. The solution filtered from this, when evaporated to dryness, leaves a residue of potassium salts of various acids, the greater part of which consists of potassium chloride, but together with this is a small quantity of sulphate and of phosphate and also a small quantity of organic salts of potassium.

This is illustrated by the following experiment in which 7·5 grammes of very carefully washed edestin were suspended in pure water and made neutral to phenolphthalein with 90 c.c. of decinormal potassium hydrate solution, diluted with much water and added gradually. After the mixture was shaken in a closed flask for some hours the undissolved edestin was filtered out and thoroughly washed. The filtrate and washings yielded a residue which, dried at 110°, weighed 0·6700 gramme. After ignition 0·5433 gramme of inorganic matter remained, which analysis showed to have the following composition:—

	Per cent.
Potassium carbonate	18·40
Potassium sulphate	17·81
Potassium chloride	51·54
Potassium phosphate	4·32
Sodium chloride	1·67
Undetermined and loss	6·26
	100·00

From these figures it is seen that most of the substance formed by neutralising this preparation of edestin consisted of potassium salts of mineral acids containing 73 per cent. of the potassium used for neutralisation. It is thus evident that on precipitating edestin by dialysis from a faintly acid solution in the presence of sodium chloride and of the organic and inorganic salts extracted from the seed, this protein combines with some of each of these acids according to the concentration in which the several acid substances were present in the solution at the time of precipitation, and that the crystalline precipitate of edestin consists not of the free protein but of a mixture of its salts. If such a precipitate of crude edestin, as was used for the experiment just described, is recrystallised from a solution of pure sodium chloride, it still contains a similar quantity of acid, but the inorganic salts which result on neutralising contain a larger proportion of potassium chloride. The composition of the salts obtained in such an experiment were as follows:—

	Per cent.
Potassium carbonate	5·7
Potassium sulphate	6·5
Potassium chloride	73·9
Sodium chloride	7·4
Undetermined and loss	6·5
	100·00

These figures show that the proportion of potassium chloride is greatly increased by thus recrystallising the edestin; that the organic acids from which the potassium carbonate originated, as well as the sulphuric acid, are greatly diminished and that the potassium phosphate has entirely disappeared; a result which would be expected if the relative concentration of the different anions in the solution from which the edestin was recrystallised is taken into consideration.

Edestin extracted from the seed with ammonium sulphate instead of sodium chloride yields, on neutralising, chiefly potassium sulphate instead of chloride, as is shown by the following analysis of the salts obtained in this way:—

	Per cent.
Potassium carbonate	18·98
Potassium sulphate	77·34
Potassium chloride	2·61
Undetermined and loss	1·07
	100·00

In this case over 75 per cent. of the recovered potassium was in the form of sulphate and less than 3 per cent. in that of chloride.

If the neutralised edestin obtained in the last experiment, which

originally consisted chiefly of sulphate, is dissolved in sodium chloride solution containing 1 c.c. of decinormal hydrochloric acid for each gramme of dissolved edestin and the resulting solution is dialysed, the edestin separates in crystals which on neutralisation yield chiefly potassium chloride as shown by the following figures:—

	Per cent.
Potassium carbonate	2·01
Potassium sulphate	16·31
Potassium chloride	73·65
Undetermined and loss	8·03
	100·00

Here over 82 per cent. of the potassium is chloride and less than 16 per cent. is sulphate, the proportions being almost exactly the reverse of those in the preparation of the identical protein substance previously precipitated from a solution of a sulphate.

Further, it is probable, in view of the recovery of most of the potassium, used for neutralising, in salts described in the experiments reported above, that such titrations are very nearly true measures of the absolute amount of combined acid. If such be the case edestin, with respect to its first basic group, cannot be a very weak base.

The amount of acid which is thus combined with edestin corresponds, in the very large number of different preparations which have been examined, to from 1·0 c.c. to 1·4 c.c. of decinormal alkali per gramme. Variations in the quantity of this combined acid can be readily determined by titration with potassium hydrate, using phenolphthalein as an indicator, for the end reaction is sharp and easily recognised. Owing to the fact that edestin can be obtained in the form of extremely minute crystals which show no tendency to adhere in lumps, it is possible to suspend it in an exceedingly finely divided state in distilled water and to determine the quantity of combined acid, even in the solid state, by titration with potassium hydrate. The result thus obtained is the same as that found by dissolving the edestin in neutral sodium chloride solution, though the end reaction takes place a little more slowly under the first-named conditions. The whole of the combined acid can therefore be determined by neutralising the crystals when suspended in pure water, although these do not dissolve during the process.

That this acidity can be determined with apparent ease is due to the fact that edestin has such feeble acid properties that they do not interfere with the use of phenolphthalein as an indicator, and also to the fact that the acid which is thus determined by titration is almost wholly strong mineral acid. Toward indicators less sensitive than

phenolphthalein such protein salts show a less degree of acidity. Edestin, even before it becomes neutral to phenolphthalein, when dissolved in sodium chloride solution gives an alkaline reaction with litmus which is undoubtedly caused by the edestin itself and not by organic salts of the alkali, for such preparations yield less than 0·05 per cent. of ash which is neutral to litmus or phenolphthalein. Toward lacmoid edestin reacts distinctly alkaline, even when its solution is noticeably acid toward litmus. It is therefore important, in determining the acidity of a protein solution, to use an indicator which marks the reaction of the protein in question when that substance is perfectly free from both bases and acids.

As has been already stated, many preparations of edestin, which are obtained by extracting hemp-seed with sodium chloride solution, when washed with water dissolve to a greater or less extent. Determinations of the proportion of acid combined with the water-soluble edestin show its amount to be greater than that which is combined with the part which does not dissolve, thus indicating the existence of two different salts of edestin, one of which, soluble in water, contains more combined acid than the other which is insoluble in water. This difference in solubility is due to the proportion of combined acid, for, when this is removed from either the soluble or the insoluble part, no difference can be detected between the neutralised products which result.

If 1 gramme of pure neutral edestin is suspended in water and a quantity of decinormal solution of hydrochloric acid which is just sufficient to dissolve it all is added, it will be found that the amount of acid required corresponds closely with the amount of acid contained in 1 gramme of the water-soluble part of edestin preparations, and this amount is just twice as great as the amount of acid combined with 1 gramme of the insoluble part. This proportion, of two to one, strongly suggests the existence of two different chlorides, one of which contains twice as much acid as the other, although each yields crystalline products of apparently the same form. The importance of recognising the existence of such protein salts having different solubility relations is manifest, for the evidence presented by differences in solubility would indicate that most of our edestin preparations contained at least two different protein substances. Too much emphasis, therefore, cannot be laid on the existence of such protein salts; and the possibility of their occurrence should be kept constantly in mind.

That edestin forms no exception in its behaviour towards acids

is evident from an examination of numerous preparations of other proteins. All of the vegetable globulins which have been tested show a distinct acid reaction towards phenolphthalein. Most of them, however, do not permit of determination of the nature of this acidity so readily as does edestin, for, when made neutral to phenolphthalein, many of them are soluble in water. Other proteins besides globulins, such for instance as repeatedly recrystallised ovalbumin and the proteins soluble in alcohol, also show the presence of combined acid.

Erb (110), who studied the acid-binding capacity of edestin, directed his efforts to a determination of the maximum acid-binding power of this protein, and worked with solutions which in all cases contained hydrochloric acid much in excess of that present in the salts just described. He considers in detail the effect of hydrolytic dissociation of such protein salts on the results obtained in studying their formation. His results indicate the existence of an acid-binding capacity about forty times greater than the quantity of hydrochloric acid required to dissolve edestin. Edestin salts containing such relatively enormous quantities of acid are clearly of a very different order from those which have just been considered and depend on a very high polyacidity of the protein. The equivalent weight of edestin corresponding to this amount of acid is about 172, whereas that calculated from the acidity of the water-soluble edestin, previously described, is more than 7,000, while from that of the water-insoluble part it is over 14,000.

It is commonly assumed that definite combinations of proteins with acid cannot be obtained under such conditions as to permit of a consideration of their molecular relations; for the proteins are supposed to be extremely weak bases. The basic properties of edestin, however, are so pronounced that it forms much more stable and definite compounds with small proportions of acids than do most animal proteins, and consequently conclusions as to its equivalent combining weight deserve more consideration than do those which are based on experiments with ovalbumin, or other proteins of animal origin.

B. Acid Properties.

It has long been known that the proteins form salts with bases, and numerous attempts have been made to prepare such salts, especially with the heavy metals, with the hope of obtaining evidence of the existence of definite combinations between the protein and the metal. All such attempts, however, have failed to yield definite compounds. Experiments with edestin appear to show that definite salts are formed,

and indicate that the molecular proportion of base with which the protein reacts is equivalent to the proportion of acid found in the salts of edestin, which have been already described.

Like the amphoteric amino-acids the proteins have acid properties as well as basic, but the latter are commonly much more manifest. Thus the writer (312, 315) has found that edestin salts, when suspended in water and gradually treated with a decinormal solution of potassium hydrate, begin to dissolve after all of the combined acid has been neutralised, and an excess of alkali is present as indicated by a red colour with phenolphthalein. When a sufficient, but definite, excess of alkali is added, all of the edestin passes into solution. The proportion of alkali required to dissolve a given quantity of neutral edestin is nearly the same as that required to neutralise the acidity of the insoluble edestin chloride, in accord with the view that this solution involves a reaction with alkali in molecular proportions. Furthermore, when neutral edestin is present in excess the amount dissolved is proportional to the amount of alkali added, showing the edestin to be dissolved through the formation of an alkaline salt, the protein functioning as an acid. The strength of this protein acid, however, is slight, and its salts are so readily hydrolysed by water that they show a strong alkaline reaction towards phenolphthalein. Weak bases, like ammonium hydroxide, form less stable salts with edestin, hence 13 c.c. of a decinormal solution of ammonium hydrate are required to dissolve a quantity of edestin which is completely dissolved by 1 c.c. of a decinormal solution of potassium hydroxide.

It was formerly supposed that many proteins were strongly acid in their nature and formed relatively stable salts with bases, as milk casein is now generally considered to do. We, therefore, find many seed proteins described in the older literature as caseins, and among these the so-called legumin from peas and beans was long regarded as a protein of strongly acid character. More recent studies of legumin, however, have shown that the solubility caused by the addition of small quantities of alkali is due to the basic and not to the acid nature of this protein.

Legumin can be extracted from various leguminous seeds by neutral sodium chloride solution, and the preparations obtained by dialysis, like those of edestin, are distinctly acid toward phenolphthalein. This acidity is probably not caused by the protein itself but by the combined acid of the legumin salt. Such preparations usually require about 2 c.c. of decinormal potassium hydroxide solution per gramme to make them neutral to phenolphthalein, a quantity slightly greater than that

required by similar preparations of edestin. Owing to the fact that legumin, when thus neutralised, is soluble in water, neither the presence nor the nature of the combined acid has yet been demonstrated, but there is little doubt that these legumin salts are similar to those of edestin. It has been shown that the slightest excess of alkali added to edestin, beyond the quantity needed to neutralise the combined acid, produces a red colour with phenolphthalein, through hydrolytic dissociation of the potassium edestinate, and it is in the highest degree improbable that if legumin were dissolved by forming a soluble potassium salt, the latter would be so stable as to yield a solution neutral to phenolphthalein under the conditions given.

In view of these facts there is little doubt that legumin, in the free state, is soluble in water, but when combined with acids, forms salts that are insoluble therein, and the idea that it is a strong acid, insoluble in water in the free state, but forming water-soluble salts with alkalies, is no longer tenable.

CHAPTER V.

SOLUBILITY OF VEGETABLE PROTEINS.

A. Solubility in Water.

ALL seeds, when ground fine and treated with water, yield extracts which in most cases contain only a small quantity of protein substance. Some of this consists of proteose and albumin, soluble in pure water; some may be globulin, dissolved in the dilute saline solution formed by the soluble mineral constituents of the seed; and some may be protein, insoluble in water alone, but dissolved by the acids extracted from the seeds.

From some of the leguminous and other seeds, when freshly ground, water extracts a considerable quantity of protein which, after a time, begins to separate from solution in consequence of a development of acid in the extract. This separation can also be effected by adding a small quantity of acid. We have just shown, page 28, that there are proteins, *e.g.*, legumin, which in the free state are soluble in water, but when combined with a small amount of acid, as a protein salt, are insoluble in water, but soluble in neutral saline solutions.

It is, consequently, necessary to consider carefully the conditions under which the dissolved protein exists in its solution before definite conclusions can be reached as to its true solubility.

Most, perhaps all, seeds, when extracted with water, yield a small proportion of proteose which may either be a constituent of the seed or be derived from the other proteins by the action of enzymes. Besides this proteose a small amount of protein is nearly always present which appears to have the properties of a true albumin. Whether this latter is actually an albumin soluble in pure water is difficult to determine definitely.

Leucosin (366) from wheat flour is the only critically studied vegetable albumin which in the free state, as well as when combined with acids, is soluble in water.

It is generally assumed, and seems to be highly probable, that most seeds contain a small proportion of similar albumins, but few are known in which such occur in any considerable quantity.

B. Solubility in Saline Solutions.

As already stated, a large proportion of the different seed proteins are soluble in neutral saline solutions from which they are precipitated by removing the salt by dialysis or by abundant dilution. Proteins having such solubility have been found in small or large proportion in all the seeds thus far examined, and in a majority of them they constitute the greater part of the reserve protein. The concentration of the neutral salt solution required to dissolve these globulins varies very widely, not only with the nature of the salt but also with that of the protein. Some of the seed globulins are but slightly soluble at the room temperature in sodium chloride solutions when these contain less than 2 per cent. of this salt, while others are readily soluble in solutions containing only a few tenths of 1 per cent. The degree of solubility of many seed globulins depends much on the temperature of the solution and increases rapidly at about 30°.

The solubility of these proteins in solutions of various salts has been studied by Osborne and Harris (357) with edestin. As this protein is entirely insoluble in water, the solvent effects of the addition of various quantities of different salts were determined in the following manner. Portions of 2 grammes each of pure crystallised edestin were suspended in sufficient water to make a final volume of 20 c.c. with the different quantities of molar solutions of the several salts which were afterwards added, the edestin being in each case in excess of the amount dissolved. After agitating for some time, the solutions were filtered, nitrogen was determined in 10 c.c. of each, and the amount of edestin dissolved was calculated from the nitrogen in solution. It was thus found that the amount of dissolved edestin was closely proportional to the concentration of the salt solution. Its solubility in solutions of sodium, potassium or cæsium chlorides was nearly the same. In solutions of magnesium, calcium, strontium or barium chloride its solubility was twice as great as it was in the solutions of the chlorides of the monovalent bases, with the exception of lithium chloride, in solutions of which it did not dissolve as abundantly as in those of the chlorides of the other monovalent bases. The sulphates of potassium, sodium, lithium and magnesium had a solvent power corresponding closely with that of the chlorides of the divalent bases.

Bromides and iodides did not behave like chlorides, for sodium and potassium iodides had a solvent power twice as great as that of the corresponding chlorides, agreeing in this respect with the chlorides of the divalent bases. The bromides were less energetic solvents than the

iodides but more energetic than the chlorides. Barium and calcium bromides were equal to one another in solvent power, but this was less than that of sodium or potassium iodide and greater than that of sodium or potassium bromide, the two latter being somewhat less powerful solvents than the corresponding chlorides. Lithium bromide was a much better solvent than lithium chloride, but less energetic than either sodium or potassium bromide.

Salts of *strong* bases with *weak* acids which are dissociated in solution with an alkaline reaction had a solvent power approximately proportional to their hydrolytic dissociation. Sodium sulphite and sodium thiosulphate were alike in their solvent power, both being much better solvents than the sulphates. Potassium chromate dissolved edestin more readily than did either the sulphite or the thiosulphate, and was but little less powerful in its solvent effect than sodium carbonate.

Salts of *weak* bases with *strong* acids, which are hydrolytically dissociated with an acid reaction, had a less solvent power than those of strong bases with strong acids, corresponding in this respect to their hydrolytic dissociation. Thus manganese chloride, manganese sulphate and ferrous sulphate dissolved edestin to about the same extent as did sodium chloride, having, therefore, about one-half the solvent power of salts of strong bases with strong acids, the two manganese salts being, in fact, better solvents than ferrous sulphate.

Solutions of acetates behave anomalously, for the acetates of potassium, sodium or ammonium had no solvent action at 20° on edestin, while those of manganese, barium, strontium, calcium and magnesium dissolved it freely, the degree of solubility in solutions of each of these acetates being in the order named. In solutions of acetates of lead, copper and silver, which are commonly supposed to be precipitants of proteins, edestin, in the complete absence of other salts, dissolves almost as freely as it does in solutions of pure acetic acid. The solvent power of each of these metallic acetates is equal, and evidence was obtained which showed that the metallic ion of the acetate was united in organic combination with the protein. The solvent effect of lead, copper and silver acetates is manifestly due to a different reaction with the protein than that which takes place when the protein is brought into solution by means of any of the other salts previously mentioned. Solutions of edestin, produced with each of these other salts, are precipitated by dilution, but those made with solutions of lead, copper or silver acetate are not, behaving in this respect like those made with free acid.

The degree of solubility of edestin is influenced by the amount of the acid which is united with the preparation to form an edestin salt.

Neutral edestin and "edestin monochloride" have the same solubility, but are more soluble than "edestin dichloride".

The results obtained with sodium sulphate, which in sufficient concentration precipitates edestin, are of interest, for, while solutions of this salt up to a certain degree of concentration dissolve edestin in the same proportion as do solutions of a corresponding molecular concentration of potassium, lithium or magnesium sulphates, the solvent power of more concentrated solutions diminishes with increasing concentration, until by a molar solution of this salt practically no edestin is dissolved. The curve showing the solubility of edestin in solutions of different concentrations of potassium sulphate is the same as that of corresponding solutions of sodium sulphate up to the point of saturation of the solution with potassium sulphate.

The solubility of potassium sulphate is so small, however, that molar solutions cannot be made, but if sodium sulphate is added to the saturated solution of potassium sulphate in such quantity that the solution contains as many molecules of the two sulphates as does the corresponding solution of sodium sulphate alone, the solubility of edestin in the solution of these mixed sulphates is the same as that in a solution containing only sodium sulphate.

C. Solubility in Acids and Alkalies.

As the solubility of the isolated proteins in acids and alkalies has already been discussed in dealing with their basic and acid properties, and will be further considered in other aspects in connection with the question of denaturing and also in describing the properties of the group of glutelins, it need not be dealt with here further than to mention the fact that zein, the alcohol-soluble protein of maize, can be dissolved in boiling glacial acetic acid without undergoing apparent change (308), for on pouring the acetic acid solution into water and treating the resulting precipitate *at once* with 70 per cent. alcohol, it dissolves in the same manner as does zein similarly precipitated from an alcoholic solution [*cf.* also Kjeldahl (190)].

D. Solubility in Alcohol.

The seeds of cereals, with the exception of rice, contain much protein soluble in alcohol of from 70 to 90 per cent. In this respect these proteins show a marked contrast in their solubility to all the other proteins of animal or vegetable origin. They dissolve in alcohol of sufficient strength in all proportions, so that their solutions, under proper conditions, can be concentrated to thick syrups, from which the protein

separates on further evaporation in the form of a transparent film. The addition of alcohol to the concentrated alcoholic solution precipitates these proteins when the concentration of the alcohol reaches a certain degree, which depends on the nature of the dissolved protein, for none of them is at all soluble in alcohol free from water. On the other hand, these proteins are precipitated on adding water to their alcoholic solution when the concentration in alcohol is reduced to a certain degree, which depends on the nature of the protein in solution. The limits of concentration in either direction have not yet been determined with accuracy, but, in general, solutions containing less than 50 per cent. of alcohol or over 90 per cent. of alcohol dissolve very little of these proteins with the exception of zein of maize, which is readily soluble in alcohol of 92 to 93 per cent.

Other alcohols than ethyl alcohol dissolve gliadin and zein and possibly also the other alcohol-soluble proteins, although experiments with these have not yet been made. Zein is readily soluble in methyl alcohol and in commercial propyl alcohol. Kjeldahl (190) has shown that gliadin is soluble in phenol and Mathewson (269) that it is soluble in dilute methyl and propyl alcohol, in paracresol, and in benzyl alcohol, and that from its solution in phenol it is precipitated by adding ether, acetone, pyridine, benzene or chloroform. Mathewson also found that methyl, ethyl, propyl and amyl alcohols all produce precipitates in the phenol solution, the amount required being less the greater the molecular weight of the alcohol, but precipitates are not produced by adding several volumes of aniline, phenylhydrazine or nitrobenzene, although gliadin is not soluble in these latter substances. When dissolved in phenol the solution can be heated to 140° without producing any notable effect on the specific rotation of the dissolved gliadin.

CHAPTER VI.

PRECIPITATION OF VEGETABLE PROTEINS.

A. Precipitation by Neutral Salts.

MANY of the seed proteins, like those of animal origin, are precipitated by adding neutral salts to their solutions up to sufficient concentration. A few of them are precipitated by saturating their solutions with sodium chloride or with magnesium sulphate. All of them are precipitated by saturating their aqueous solutions with ammonium sulphate or with sodium sulphate at 33°. Fractional precipitation with ammonium sulphate is discussed in detail in Chapter VIII.

B. Precipitation by Dilution or by Dialysis.

Precipitation by dilution or by dialysis is frequently employed in separating many of the seed proteins. From what has been said before it is evident that the degree of precipitation obtained by this means depends largely on the reaction of the solution. Most of the precipitates are protein salts and usually separate only from solutions which contain a small quantity of acid. It is for this reason that many diluted solutions of proteins yield a precipitate only after carbonic acid has been passed through them, for the small proportion of hydrogen ions thus liberated in the solution is sufficient to lead to the formation of their insoluble salts. That such salts are formed by the action of carbonic acid has been shown by Osborne (313) by the following experiment with neutral edestin, which is more soluble in a very dilute saline solution than are its salts. Such a solution when diluted with water until it shows a slight turbidity and then saturated with carbonic acid yields a crystalline precipitate of edestin chloride. This precipitate when washed free from chlorine requires a considerable quantity of decinormal potassium hydrate solution to make it neutral to phenolphthalein. The solution in which the neutralisation is effected contains over 90 per cent. of the added potassium in the form of chloride, which shows that the precipitate produced by carbonic acid is edestin chloride, formed in consequence of the slightly acid reaction imparted to the solution by the carbonic acid. Neutral solutions of legumin and many

other seed globulins when dialysed yield no precipitates, but slightly acid solutions yield such abundantly.

C. Precipitation by Acids.

Precipitation by acids may be due either to the acid uniting with a base, already combined with the protein to form a soluble compound, or by the formation of a salt of the protein insoluble in water. The formation of a precipitate on neutralisation, therefore, depends on the nature of the protein. Edestin dissolved in dilute potassium or sodium hydrate is precipitated by adding enough acid to combine with all of the alkali that is present, for the free edestin is insoluble in water. A solution of legumin is not thus precipitated, for the free legumin is soluble, but a very little more acid added to the solution precipitates an insoluble salt of legumin. A further addition of acid beyond the amount required to form this salt dissolves the legumin, the quantity necessary depending upon the proportion of mineral salts contained in the solution, for the soluble salts of most seed proteins are insoluble in the presence of small amounts of inorganic salts. Thus edestin dissolved in the least possible quantity of hydrochloric acid necessary for its solution is precipitated by traces of most mineral salts, but a slight excess of acid requires the addition of more salt for precipitation. The precipitation of most seed proteins by acids, therefore, depends largely on the presence of mineral salts in their solutions. An excess of acid appears to act in much the same way, although a larger molecular concentration of acid than of salt is necessary to effect precipitation.

CHAPTER VII.

DENATURING OF VEGETABLE PROTEINS.

A. Denaturing by Acids.

PROTEINS are subject to changes whereby their molecules are slightly altered and their solubility is changed. Our knowledge of the nature of these changes and the various products which result from the denaturing influence of the agents which bring them about, is very limited. It is commonly stated that the action of acids on proteins produces "acid albumin," which is described as insoluble in water but readily soluble in the slightest excess of either acid or alkali. Products of such solubility appear to be formed by the action of acid on nearly all kinds of proteins, and the "acid albumin" which results corresponds in its constitution with the protein from which it originated, for the changes involved in its formation are slight and do not lead to any profound decomposition of the protein molecule. Little attention appears to have been paid to the possibility of the formation of intermediate products between the native protein and this "acid albumin," and much confusion exists in the literature in connection with the action of acids on proteins. The only attempt known to the writer that has yet been made to study this question is one made by him some years ago (314, 311), in which the effect of small quantities of acid on edestin was studied with some care. As the results of these experiments appear to have a general application and to shed considerable light on the denaturing effect of acids on proteins they will here be described in detail.

Crystallised edestin, when dissolved by the least possible quantity of hydrochloric acid, yields a solution which is precipitated by the addition of a little sodium chloride. This precipitate when treated with a strong solution of sodium chloride never wholly redissolves. If the part that does dissolve is recrystallised and the experiment repeated, a part of the preparation originally soluble in a neutral salt solution again remains undissolved, and this occurs each time the experiment is tried. This insoluble derivative, which cannot be made soluble

again in a neutral salt solution by any known means, is not "acid albumin," for it is not soluble in a slight excess of potassium hydrate solution. Similar products result from nearly all the seed proteins, and their formation may be considered to be a general property of these substances. As no suitable name had ever been suggested for such insoluble products, the writer has proposed that they be called in general *proteans*, that that obtained from edestin be called *edestan*, and that similar derivatives from other proteins be given corresponding names.

Preparations of nearly all proteins, made by the customary methods, contain more or less substance of such altered solubility, and there is little doubt, in view of what we have learned in regard to the formation of these products from edestin, that they are the result of the action of a slight quantity of acid present in the solutions from which they were originally obtained. The following experiments, made with edestin, give the evidence on which this belief is founded.

Several 1 gramme portions of neutral edestin, which contained 2·16 per cent. of edestan, were suspended in 10 c.c. of pure water and kept at different temperatures for six hours with frequent shaking. An equal volume of 20 per cent. sodium chloride solution was added and the solution at once made neutral to phenolphthalein. The solution was then filtered and nitrogen determined in the thoroughly washed residue collected on the paper, from which the quantity of edestan was calculated.

Percentage of Edestan Formed by Contact with Water.

Treated at once with 10 per cent. NaCl at 20°.	Treated with sodium chloride solution after six hours.			
	Water + CO_2 at 20°.	Pure Water at 20°.	Pure Water at 30°.	Pure Water at 50°.
2·16	6·75	4·32	7·11	29·00

These figures show that even at 20° the quantity of edestan which is formed by water alone is twice as great as in the original preparation and that the quantity is decidedly greater if the water contains carbonic acid. At 50° about four times as much edestan is formed as at 30° and nearly eight times as much as at 20°; showing the velocity of the reaction to be doubled by each increase of 10° in the temperature. In view of the larger amount of edestan produced by the water containing carbonic acid we should expect to find that the proportion of

this insoluble product would be increased by the addition of small quantities of strong acid to the solution. This has been found to be the case, and the following figures give the result of experiments which have shown this :—

PERCENTAGE OF EDESTAN FORMED BY ACIDS AT 20°.

9 c.c. $\frac{N}{100}$ HCl		14 c.c. $\frac{N}{100}$ HCl		18 c.c. $\frac{N}{100}$ HNO_3	19 c.c. $\frac{N}{100}$ HNO_3	20 c.c. $\frac{N}{100}$ HNO_3
3 hours	20 hours	3 hours	20 hours	24 hours	24 hours	24 hours
9·01	12·15	29·80	33·55	68·38	75·20	79·02

These figures, compared with those of the preceding table, show that edestin yields much more edestan in contact with acids than in contact with water. In the experiment in which 9 c.c. of centinormal hydrochloric acid were used the proportion of edestan that formed was very much less than in the experiment containing 14 c.c. of the same acid. In the first experiment the amount of acid used was less than that with which the edestin can combine to form a water-soluble compound, and the solution therefore contained only so much free acid as was produced by hydrolytic dissociation of the edestin salts. In the experiment with 14 c.c. of acid a small amount of acid in excess of that required to form a soluble compound was present, and the effect of this larger quantity of free acid produced by the greater hydrolysis of the weaker acid compounds of edestin is made very manifest in the greatly increased quantity of edestan which was produced.

That the amount of edestan which is formed depends on the degree of ionisation of the acid which produces it is shown by the following experiments, in which portions of neutral edestin, each weighing 1 gramme, were suspended in 6 c.c. of water and 14 c.c. of centinormal hydrochloric, phosphoric and acetic acids were respectively added. The quantity of edestan that had formed after frequently agitating during two hours is given in the following table :—

PERCENTAGE OF EDESTAN FORMED BY EQUIVALENT QUANTITIES OF DIFFERENT ACIDS UNDER THE SAME CONDITIONS.

14 c.c. $\frac{HCl}{100}$	14 c.c. $\frac{H_3PO_4}{100}$	14 c.c. $\frac{H_4C_2O_2}{100}$
19·29	16·02	5·65

The solution of phosphoric acid contained 0·98 gramme of H_3PO_4 per litre and was made on the assumption that this acid would behave toward edestin as a monobasic acid. The quantity of edestan formed by acetic acid as compared with that formed by hydrochloric acid corresponds with the lesser ionisation of the former acid.

The edestan which is contained in preparations of edestin, made by

the usual methods, has the same properties as the edestan which results from the action of acids on the unchanged edestin, for if preparations of edestin, made by the commonly employed methods, are treated with water and the solution of the soluble part is precipitated by the addition of a little sodium chloride, the precipitate produced, when treated with a larger proportion of sodium chloride, leaves an insoluble residue which in all respects is the same as that obtained by the action of acids on the unchanged edestin.

The ultimate composition of edestan differs little, if at all, from that of edestin, as is shown by the following analyses of three samples of edestan made under somewhat different conditions and also by an analysis of a sample of unchanged edestin. Preparation I. was made by suspending 10 grammes of crystallised edestin chloride in water and gradually adding 30 c.c. of a decinormal solution of hydrochloric acid. After the clear solution which resulted had stood at 20° for about two hours it was made neutral to phenolphthalein with 38 c.c. of decinormal potassium hydrate solution, and the precipitate produced was washed with 10 per cent. sodium chloride solution until all of the unchanged edestin was removed, and then with water until free from chlorine. After dehydrating with absolute alcohol, the preparation was dried at 110°. Preparation II. was made in the same way, except the acid solution was kept at a temperature below 10° for about twenty hours, when 50 c.c. of decinormal potassium hydrate solution was added. Although this excess of alkali was more than sufficient to dissolve the entire quantity of the precipitate produced by neutralisation, had this been unchanged edestin, nevertheless very little protein matter was dissolved by it. Preparation III. represents the insoluble edestan which was obtained directly from a preparation of edestin and is the insoluble substance which is commonly found in such preparations.

	Composition of Edestan.			Composition of Edestin.
	I.	II.	III.	
Carbon	51·48	51·91	51·69	51·50
Hydrogen	6·91	6·96	6·98	7·04
Nitrogen	18·51	18·49	18·49	18·69
Sulphur	1·00	0·99	0·92	0·88
Oxygen	22·10	21·65	21·92	21·89
	100·00	100·00	100·00	100·00

It is to be noticed that the amount of nitrogen found in the three samples of edestan is very constant and slightly less than that found

in edestin, and it may be that in the formation of edestan a slight loss of nitrogen occurs. The difference, however, between these analyses is too slight to warrant a definite conclusion on this point.

The edestan, thus prepared for analysis, is a voluminous white powder which swells somewhat in water and forms a colourless, transparent jelly with very dilute hydrochloric acid. In the dry state it is slightly, if at all, soluble in strong ammonia, but the gelatinous mass formed by treating the substance with very dilute hydrochloric acid is slightly soluble therein and yields a solution which gives a precipitate with ammonium chloride. Consequently when hydrochloric acid is added to its ammoniacal solution a precipitate forms, even when much of the ammonia is still unneutralised. The ammoniacal solution is not precipitated by sodium chloride.

Edestan exists in preparations of edestin chloride in combination with acid. The amount of acid required to form a compound sparingly soluble in water appears to be definite, as the following experiments show. A quantity of edestan obtained from a preparation of edestin chloride was suspended in water and dialysed until free from chloride. The dialyser then contained an opalescent fluid and a voluminous precipitate. The acidity of the substance dissolved in this solution was found in two experiments to correspond to 21·5 and 23·4 c.c. of a centinormal solution per gramme. Other experiments gave similar results, from which it appears that the acidity of the edestan chloride was almost exactly three times that of the edestin chloride insoluble in water or one and one-half times that of the edestin chloride soluble therein. From these results we may conclude that the basic property of this altered product is greater than that of the original protein, and as this substance originates so readily and in such large proportion in the presence of a small amount of free acid, it is evident that experiments which have been made to determine the acid-combining power of proteins, in which an excess of acid has been employed, do not necessarily show the acid-combining power of unchanged proteins.

The reactions of edestan are similar to those which are considered to be characteristic of the histone group, but there is manifestly no connection between this substance and the true histones. The globin obtained from hæmoglobin by the action of dilute acids is generally designated a histone on the ground of the similarity of the reactions of the two substances. It is possible, if not probable, that globin more nearly resembles edestan than the true histones and that it is a similar product of alteration of the protein constituent of the hæmoglobin

which has been produced by the action of acids, in the same way as edestan is produced from edestin.

Products similar to edestan are apparently formed from some of the animal proteins with great ease, as, for example, from the myosins of muscle tissue which rapidly become insoluble in neutral salt solution during the development of acid which occurs in these tissues soon after death. Whether other products than edestan are formed by the action of acids on proteins before true "acid albumin" results has not yet been determined; but it is not improbable that such may occur.

B. Denaturing by Alkalies.

It has long been known that proteins undergo a change when dissolved in solutions containing a moderate quantity of caustic alkali. A product of this change which is known as "alkali albumin" or "alkali albuminate" resembles in solubility the acid albumin which results from proteins through the action of dilute acids. No satisfactory study has ever been made of these two substances and little, therefore, is known in respect to their relations to one another. Whether one or more intermediate products are formed by the action of alkali before the so-called alkali albumin results has not been determined.

No study of this action of alkali on vegetable proteins has been made. Such scattered observations as are on record indicate that the vegetable proteins are less easily affected by alkalies than are the animal proteins. Ritthausen's experience in extracting seed proteins with dilute caustic alkali solutions showed that the precipitates produced by neutralisation still retained, to a large extent, their solubility in neutral saline solutions, from which it was clear that much of the protein thus extracted had not been converted into alkali albumin.

Chittenden and Osborne (72) found that zein was particularly resistant to the action of alkali, for even after digesting with 2 per cent. potassium hydrate solution at 40° for twenty-four hours, the zein still retained its original solubility in alcohol and gave no evidence of the formation of any "alkali albumin". In this experiment the possibility, however, is not excluded of the formation from zein of an alkali albumin soluble in alcohol, for it may be that the zein had suffered a change quite analogous to that which results in the formation of alkali albumin without producing a substance whose solubility was like that of the "alkali albumin" obtained from other proteins.

Experience indicates that seed proteins are less easily altered by small quantities of alkali than they are by acids, a fact which is contrary

to the generally accepted view in regard to the action of alkalies and acids on proteins in general. This subject is greatly in need of further critical study, and with the means at present available more definite information than we now have may be obtained in regard to the changes which the protein undergoes.

C. Denaturing by Alcohol.

Alcohol produces a marked denaturing effect on many of the animal proteins, and the ease with which such changes are effected is different with the different proteins. Thus ovalbumin is quickly converted into a product insoluble in water, but serumalbumin resists this change for a longer time. Many of the proteins of seeds appear to be but little affected by a long treatment with alcohol, and the evidence that any change whatever is caused by alcohol is of such an uncertain character that definite statements in regard to the action of alcohol cannot at present be made. Zein shows an apparently unique behaviour toward alcohol, for when dissolved in strong alcohol the original solution gradually becomes gelatinous and finally is converted into a firm jelly which is a combination of the zein with alcohol similar to that formed by gelatin with water when its hot solutions are cooled. The formation of this combination depends on the concentration of the solution in zein and apparently also on other conditions, the nature of which is not yet known. Whether an actual denaturing of the protein here occurs is uncertain, but this appears to be the case, for it has not yet been found possible, by any of the numerous means that have been employed, to restore the zein to its original solubility in alcohol after this change has once taken place. Such a change has not yet been observed with any of the other alcohol-soluble proteins.

D. Denaturing by Metallic Salts.

It is generally recognised that the addition of salts of the heavy metals to solutions of a protein result in the denaturing of the protein. It is probable, from experiments which have been made with edestin, that this denaturing is largely if not wholly due to the fact that such metallic salts are hydrolytically dissociated with a strong acid reaction and that, in the presence of the acid thus set free, the protein is rapidly denatured. Solutions of ferric chloride behave toward edestin in almost exactly the same manner as pure hydrochloric acid, the edestin being denatured with the formation of a product soluble in dilute acid but not precipitated by an excess of ferric chloride. It is probable that the acid set free by hydrolytic dissociation of the metallic salts is

the chief cause of the difficulty encountered in attempting to prepare definite salts of the protein with metals.

E. Denaturing by Heat.

It appears to be commonly believed that all proteins having the properties of globulin are completely coagulated by heating their slightly acid solutions and that this property is also shared by nearly all of the other native proteins. In this respect the seed proteins differ in a marked degree from the animal proteins, for most of them are very incompletely coagulated by heating their solutions, even to boiling, and many of them are not coagulated at all under these conditions. In this connection should be considered the part which acid plays in the production of a heat coagulum, for it is well known that alkaline solutions of proteins cannot be coagulated by heat and that neutral solutions are usually coagulated with some difficulty. It is customary in attempting to separate a protein from its solution by heat to add a very small quantity of acetic acid. From what we have said in relation to the denaturing effect of acid on protein it is evident that acid cannot be added to the solutions of many of the seed proteins without of itself causing a change in the solubility of the protein, and it becomes difficult in such cases to distinguish between the denaturing effect of the acid and that of the heat. It has, therefore, been the practice in determining the effect of heat on vegetable proteins to heat them in solution without the addition of any more acid than that which has combined with them during the process of isolation. Osborne and Campbell (601) have shown that crystallised ovalbumin, which has an acid reaction toward phenolphthalein similar to that of the preparations of the seed proteins, is completely coagulated when its solutions are sufficiently heated. If, however, alkali is first added in sufficient quantity to exactly neutralise the acid combined with the ovalbumin, using phenolphthalein as an indicator, its solution when heated yields no coagulum, although the albumin has suffered a chemical change which is made evident by cooling its solution and then adding a quantity of acid corresponding to that originally present in the crystalline substance.

The behaviour of edestin solutions toward heat is similar to that of a large number of other seed globulins, and in this connection the experiments of Chittenden and Mendel (71) are of interest, for they show that the acid combined with the crystallised edestin is insufficient to effect its complete coagulation, as the part of the edestin which is not coagulated remains unchanged even after long heating in a boiling solution. Chittenden and Mendel employed a sodium chloride solution

of edestin which had been obtained by extracting hemp-seed with a solution of this salt at 60°, a process which yields preparations consisting chiefly of the more acid salt of this protein, which is to some extent hydrolysed when in solution. When this solution was heated to boiling, a part of the edestin was coagulated. On removing the coagulum and dialysing the solution the edestin was precipitated unchanged, as shown by its complete crystallisation. When a very little acetic acid was cautiously added to the filtrate from the coagulum and this again heated to boiling, a second coagulum resulted at the same temperature, namely, 95°, as that at which the first coagulum began to form. The coagulation in this case, as in the first, was still incomplete, and the filtrate from the coagulum required a further addition of acid in order to give a coagulum on again heating to boiling. From this we might conclude that, in the complete absence of acid, edestin would not be affected by simply heating its solution, were it not for the fact that the writer has found that neutral edestin which contains no combined acid whatever behaves in the same way as the edestin chloride.

Some seed proteins, as, for instance, leucosin obtained from wheat, are more easily coagulated than the seed globulins, but whether these can be completely separated from their solution by heating to a few degrees above the temperature at which a flocculent coagulum is formed, is still an open question. The aqueous extract of wheat flour becomes turbid on heating to 48° to 50° and a flocculent coagulum is formed at 52°. Whether the leucosin is thus completely coagulated is difficult to decide, for after heating the solution for some time at 65° a second but smaller coagulum begins to separate at 73°, which gradually increases in amount as the temperature is raised to 82°. Above this temperature no more coagulum is formed even on boiling. Whether this second small coagulum, formed at the higher temperature, is a residue of leucosin which remains uncoagulated at the lower temperature or a distinctly different protein having a higher coagulation point, remains to be determined.

Most seed extracts behave in a similar manner on heating, and in view of the incomplete coagulation of edestin and other seed proteins it is a question whether or not the coagula obtained at the several temperatures are really formed from different protein substances. The temperature at which a coagulum separates in such solutions depends much upon the rate of heating, and, unless the temperature is raised very slowly, the first coagulum is obtained at a much higher degree. The presence of sodium chloride in the aqueous extract of wheat flour has little effect on the temperature at which the coagulum separates.

In respect to its behaviour toward heat leucosin resembles the animal proteins more closely than do the globulins, which constitute the greater part of the reserve protein of most seeds. We have already stated the reasons for believing leucosin to be chiefly contained in the embryo of the seed, and it is probable that the physiologically active seed proteins, in this respect as well as in others, more closely resemble the physiologically active animal proteins than do the true reserve proteins of the seed.

CHAPTER VIII.

PHYSICAL CONSTANTS OF VEGETABLE PROTEINS.

A. Specific Rotation of Vegetable Proteins.

THE specific rotation of only a few of the vegetable proteins has been determined. These are given in the following table:—

SPECIFIC ROTATION OF VEGETABLE PROTEINS.

		Solvent	$(\alpha)_D$
Edestin, hemp-seed,	Chittenden and Mendel (71),	10 % NaCl	− 43·48°
,, ,,	Alexander (15),	,,	− 41·5°
,, ,,	Osborne and Harris (352),	,,	− 41·7°
Excelsin, Brazil-nut,	Alexander (15),	,,	− 40·5°
,, ,,	Osborne and Harris (352),	,,	− 42·94°
Globulin, flax-seed,	Alexander (15),	,,	− 38·5°
,, ,,	Osborne and Harris (352),	,,	− 43·53°
Globulin, squash-seed,	,, ,,	,,	− 38·32°
Amandin, almond,	,, ,,	,,	− 56·44°
Corylin, hazel-nut,	,, ,,	,,	− 43·09°
Juglansin, English walnut,	,, ,,	,,	− 45·21°
Juglansin, American black walnut,	,, ,,	,,	− 44·43°
Juglansin, butter-nut,	,, ,,	,,	− 45·40°
Phaseolin, kidney-bean,	,, ,,	,,	− 41·46°
Legumin, horse-bean,	,, ,,	,,	− 44·09°
Ricin, castor-bean,	Osborne, Mendel and Harris (365),	water	− 28·85°
Gliadin, wheat,	Osborne and Harris (352),	80 % alcohol	− 92·28°
,, ,,	Kjeldahl (190),	55 % alcohol	− 92·0°
,, ,,	,,	glacial acetic acid	− 81·0°
,, ,,	,,	5 % – ·1 % acetic acid	− 111·0°
,, ,,	,,	phenol	− 130·0°
,, ,,	Mathewson (269),	70 % methyl alcohol	− 95·65°
,, ,,	,,	70 % ethyl alcohol	− 91·95°
,, ,,	,,	60 % ethyl alcohol	− 96·66°
,, ,,	,,	50 % ethyl alcohol	− 98·45°
,, ,,	,,	60 % propyl alcohol	− 101·10°
,, ,,	,,	70 % phenol	− 123·15°
,, ,,	,,	phenol, anhydrous	− 131·77°
,, ,,	,,	paracresol	− 121·00°
,, ,,	,,	benzyl alcohol	− 53·10°
,, ,,	,,	glacial acetic acid	− 78·60°
Gliadin α, wheat,	Lindet and Ammann (226),	70 % alcohol	− 81·6°
,, β, ,,	,, ,,	,,	− 95·0°
,, rye,	Kjeldahl (190),	55 % alcohol	− 121°
,, ,,	,,	"dilute acid"	− 144°
,, ,,	,,	glacial acetic acid	− 105°
,, ,,	,,	phenol	− 157°
,, ,,	Lindet and Ammann (226),	70 % alcohol	− 87·8°
,, barley,	,, ,,	,,	

SPECIFIC ROTATION OF VEGETABLE PROTEINS (*continued*).

		Solvent	$(\alpha)_D$
Hordein, barley,	Lindet and Ammann (226),	70 % alcohol	− 137·5°
,, rye,	,, ,,	,,	
Zein α, maize,	,, ,,	,,	− 29·6°
,, β, ,,	,, ,,	,,	− 40·0°
,, ,,	Osborne and Harris (352),	90 % alcohol	− 28·0°
,, ,,	Kjeldahl (190),	75 % ,,	− 35·0°
,, ,,	,,	glacial acetic acid	− 28·0°

Alexander's determination of the rotation of edestin was made on edestin chloride and therefore contained some combined acid, but the result was the same as that obtained by Osborne and Harris for a preparation free from combined acid. Alexander concluded that the specific rotation decreased with decreasing concentration of the edestin solution, and his result which is given in the above table is that calculated for a 1 per cent. solution of this protein. Osborne and Harris, however, found that differences in concentration had no effect, while differences in temperature appeared to have but slight effect on the specific rotation of edestin. Alexander found a slightly lower rotation for edestin dissolved in sodium sulphate solution, and an intermediate value when dissolved in one of ammonium sulphate. The addition of alkali to the sodium chloride solution raised the rotation to about − 64° and the addition of acid raised it to about − 85°. Alexander also found that the specific rotation of excelsin was increased by the action of alkali to − 58° and that of the globulin of the flax-seed to − 54·5°.

Lindet and Ammann concluded from the results of their determinations of the rotation of gliadin and zein that preparations obtained in the ordinary way were a mixture of two proteins. Their results, however, are not in accord with the experience of other investigators. It is possible that the differences which they noticed in rotation were caused by the presence of small quantities of acid. From rye and barley they obtained an alcohol-soluble protein which could be separated into two parts of different rotation, one of which with a rotation of − 87·8° they called gliadin, the other with a rotation of − 137·5° they called hordein. This latter name, therefore, does not designate the same substance as that to which the writer had previously applied this name.

B. Heat of Combustion of Vegetable Proteins.

Few determinations of the heat of combustion of vegetable proteins are to be found in the older literature. The earliest were those made by Danilewsky (86), Stohmann (527), Berthelot and André (31), and Stohmann and Langbein (528).

Benedict and Osborne (29) have recently determined the heat of combustion of a number of vegetable proteins which were prepared with special reference not only to their separation from all non-protein bodies but also from other associated proteins. These substances were burned in the Berthelot-Atwater bomb.

The calorimeter room was kept at the most constant temperature possible, and all the minor precautions, suggested by experience with over 10,000 of these combustions, were employed.

The bomb used in these determinations was so adjusted, as regards its hydrothermal equivalent, as to give the heat of combustion of pure, anhydrous cane sugar as 3,959 calories per gramme and of pure, fused benzoic acid as 6,322 calories per gramme.

From 0·5 to 0·8 gramme of substance was used for each combustion, which was weighed after the thoroughly dried material had assumed constant weight by prolonged exposure to the air.

The results of these determinations, calculated for 1 gramme of perfectly dry substance, are given in the following table:—

	C.	H.	N.	S.	O.	Calories per gramme.
Amandin, almond	51·30	6·90	18·90	0·43	22·47	5543
Corylin, hazel-nut	50·72	6·86	19·03	0·83	22·56	5590
Excelsin, Brazil-nut	52·23	6·95	18·26	1·09	21·47	5737
Edestin, hemp-seed	51·36	7·01	18·65	0·88	22·10	5635
Globulin, cotton-seed	51·71	6·86	18·30	0·62	22·51	5596
Vignin, cow-pea	52·64	6·95	17·25	0·42	22·74	5718
Glycinin, soy-bean	52·01	6·89	17·47	0·71	22·92	5668
Legumin, lentil, horse-bean, vetch	51·72	6·95	18·04	0·39	22·90	5620
Phaseolin, kidney-bean	52·57	6·97	15·84	0·33	24·29	5726
Conglutin, blue lupine	51·13	6·86	18·11	0·32	23·10	5475
,, α, yellow lupine	51·75	6·96	17·57	0·62	23·10	5542
,, β, ,, ,,	49·91	6·81	18·40	1·67	23·21	5359
Vicilin, lentil	52·29	7·03	17·11	0·17	23·40	5683
Legumelin, lentil	53·31	6·71	16·08	0·97	22·93	5676
Gliadin, wheat, rye	52·72	6·86	17·66	1·03	21·73	5738
Glutenin, wheat	52·34	6·83	17·49	1·08	22·26	5704
Globulin, wheat	51·03	6·85	18·30	0·69	23·13	5358
Hordein, barley	54·29	6·80	17·20	0·85	20·86	5916
Bynin, barley malt	55·03	6·67	16·26	0·84	21·20	5807

In general the higher heats of combustion are found for those proteins which have a higher carbon content and similarly for those with a lower oxygen content. Many irregularities, however, appear in the preceding table, which are doubtless due to the different proportions of the various amino-acids which constitute the molecules of the different proteins.

C. Ultimate Composition of Vegetable Proteins.

Analyses of seed proteins have shown wider variations in composition than have those of animal proteins. Thus the proportion of carbon in most of them falls between 50 and 55 per cent., nitrogen between 15 and 19 per cent., and sulphur between 0·1 per cent. and 2 per cent. Phosphorus has not yet been proved to be a constituent of any of the seed proteins thus far studied. It is true that many preparations of these substances contain small amounts of phosphorus, but it has been found that this phosphorus disappears from most of them when they are purified by repeated precipitation. We have seen that crude preparations of edestin, when neutralised, yield a small quantity of phosphate in the mixture of salts which results on neutralising and that by reprecipitation this phosphorus disappears. There is little doubt that the phosphorus found in most crude preparations of seed proteins is present in them in the form of combined acid and that it forms no part of their molecule. This question will be further considered in connection with the presence of nuclein and nucleoproteins in seeds.

D. Fractional Precipitation with Ammonium Sulphate.

Saturation with ammonium sulphate is a means frequently employed in separating proteins from solution, and partial saturation at definite concentrations of this salt has been extensively used in separating them from one another. It appears to be generally believed that the different proteins are precipitated by ammonium sulphate at definite concentrations and within narrow limits, and that this salt can be used for characterising the different protein individuals as well as for effecting a sharp separation of mixtures of them. Osborne and Harris (356) found that several of the seed proteins do not show such definite relations to solutions of ammonium sulphate as the statements generally current in protein literature would lead one to expect. Several of the globulins which had been separated from seed extracts by fractional precipitation with ammonium sulphate and purified, as far as possible, by repeated precipitation between narrow limits of concentration of this salt, were found to have limits of precipitation lower and wider apart after being reprecipitated by dialysis. The dialysis precipitates thus obtained gave every evidence that they consisted of unchanged protein, and it would appear that fractional precipitation with ammonium sulphate is not such a definite characteristic of these proteins as is generally assumed. This subject, however, requires further investi-

gation. As an illustration of what has just been said, the behaviour of the crystalline globulin, excelsin, from the Brazil-nut may be given. The proteins extracted by dilute ammonium sulphate solution from this seed were subjected to repeated careful fractionation with ammonium sulphate and two fractions obtained—I. between 40 and 50 per cent. saturation, II. between 50 and 60 per cent. These fractions were then dissolved in one-tenth saturated ammonium sulphate solution and re-precipitated by dialysis. I. yielded a precipitate consisting wholly of hexagonal crystals which when washed with water and alcohol and dried over sulphuric acid weighed 24·5 grammes; II. by a similar treatment, yielded 5 grammes which consisted of a mixture of imperfectly formed crystals and spheroids. The precipitation limits of these preparations were then found to be for I. between 19 and 46 per cent. of saturation, for II. between 21 and 60 per cent. of saturation. Thus preparation II., which before precipitation by dialysis had been entirely freed from all protein precipitable below 50 per cent. saturation, showed a lower limit of precipitation identical with that of preparation I., all of which was originally precipitated below 50 per cent. saturation, and an upper limit of precipitation of 60 per cent. saturation which agreed with its original upper limit. After precipitation by dialysis each of these preparations had, therefore, retained its upper limit of precipitation practically unchanged, but the lower limits were greatly reduced and were the same for each fraction. Although doubt exists as to the extent to which the precipitation limits of proteins can be employed for characterising them as chemical individuals, nevertheless ammonium sulphate is of great use in effecting the separation of proteins from their solutions, as well as from one another.

Osborne and Harris (351) determined the precipitation limits of preparations of a number of different proteins which had been precipitated by dialysis from sodium chloride solutions and dried over sulphuric acid, after being washed with water and alcohol in the usual way. A weighed quantity of each of the proteins was dissolved in one-tenth saturated solution of ammonium sulphate, the solution filtered clear and 2 c.c. mixed with enough two-tenths saturated sulphate solution to make a final volume of 10 c.c. with the saturated sulphate solution to be afterwards added. Successively greater quantities of the saturated sulphate solution were used and the points noted at which the solution first became permanently turbid and that at which the protein was all precipitated, as shown by saturating the filtered solution with ammonium sulphate. The results are stated in the following table in cubic centimetres of a saturated solution of am-

monium sulphate required for the precipitations indicated, account being taken of the one-tenth saturated solution used to dissolve the protein, as well as that used to bring the final volume to 10 c.c.

	Lower limit.	Most precipitated.		Upper limit.
	c.c.	c.c.	c.c.	c.c.
Juglansin, English walnut	2·8	2·8	4·6	6·6
,, black walnut	2·8	2·8	4·6	6·6
Edestin	3·0	3·0	3·9	4·2
,, monochloride	3·0	3·0	3·9	3·9
Globulin, flax-seed	3·1	3·3	4·6	4·7
,, castor-bean	3·1	3·3	4·3	4·5
,, squash-seed	3·3	3·5	4·1	4·4
Amandin	3·5	3·5	5·0	5·3
Corylin	3·7	3·7	5·3	6·6
Excelsin	3·8	4·0	5·0	5·5
Conglutin (α)	4·2	4·3	6·0	7·3
,, (β)	7·0	7·0	9·0	?
Globulin, cotton-seed	4·6	5·0	6·0	6·4
Legumin	5·4	5·5	6·5	7·5
Phaseolin	6·4	6·5	8·2	8·8

CHAPTER IX.

PRODUCTS OF HYDROLYSIS OF VEGETABLE PROTEINS.

A. Hydrolysis by Acids.

SEED proteins are decomposed by heating with acids or by the action of enzymes, and yield products of the same general character as those obtained from proteins from animal sources. The experiments of Chittenden (68) and his associates have shown that proteoses and peptones of the same general character as those obtained from animal proteins are formed from vegetable proteins by the action of pepsin hydrochloric acid.

Underhill (548) has shown that both the natural and artificial proteoses of vegetable origin have the same physiological effect when injected into the circulation of animals as have those of animal origin, namely, a lowering of the blood pressure, rendering the blood uncoagulable, accelerating the flow of lymph, deep narcosis, and other toxic symptoms.

Fisher and Abderhalden (111) obtained by tryptic digestion of edestin a resistant product which contained all of the glycocoll, proline and phenylalanine together with other amino-acids, and (112) from the products of acid hydrolysis of gliadin a dipeptide of leucine and glutaminic acid. Osborne and Clapp (339) similarly isolated a dipeptide of proline and phenylalanine from this latter protein after prolonged hydrolysis with sulphuric acid.

Whether the vegetable proteins are in general more difficult to completely hydrolyse than the animal has not yet been definitely determined, but it has been found that a much longer hydrolysis is necessary to completely decompose several of them than has been employed by most of those who have studied the animal proteins. Thus Osborne, Leavenworth and Brautlecht (364) found that distinctly more of the basic amino-acids was obtained after boiling several of the seed proteins with 25 per cent. sulphuric acid for twenty-four hours than after boiling for twelve hours, a time usually considered to be quite sufficient to effect complete decomposition of all proteins. That other combinations hydrolysable with difficulty may occur is shown by the pre-

sence of the above-mentioned dipeptide of proline and phenylalanine, which required several hours' heating in a closed tube with concentrated hydrochloric acid before it was completely hydrolysed. Gliadin when boiled with 25 per cent. sulphuric acid for twelve hours yields a considerable quantity of an insoluble product which resembles the substance usually regarded as humus. The writer, however, found that this substance could be further decomposed by boiling with strong hydrochloric acid and that it then yielded several amino-acids, among which glutaminic acid and cystine were conspicuous. Whether further study of the time of hydrolysis required to completely decompose the animal proteins will show them to be equally resistant to hydrolysis is a question which cannot at present be answered.

The amino-acids which have been obtained from vegetable proteins are the same as those yielded by animal proteins, with the exception of diamino-tri-oxy-dodecanic acid, which has thus far been found only in casein (see Plimmer, *The Chemical Constitution of the Proteins*). Ammonia is also a constant product of their hydrolytic decomposition. It is thus plain that no fundamental chemical difference exists in the character of the amino-acids from which the molecules of the vegetable proteins are constructed and those which form the molecules of the animal proteins. The ideas which at one time appear to have been held by some, that the vegetable proteins are fundamentally different in their constitution from the animal proteins, has no foundation in fact, for such differences as have been found consist chiefly in the proportion in which some of the amino-acids are yielded by the vegetable proteins as compared with the animal. In general the plant proteins yield more glutaminic acid than the animal and many of them also yield more ammonia. Proline has been obtained in relatively large amount from a number of the plant proteins, and arginine is yielded in larger proportion by many of them than by most animal proteins. The proteins soluble in alcohol, on the other hand, yield these basic amino-acids in remarkably small proportion, and, as Kossel and Kutscher showed (205), are the only proteins, with the exception of the protamines, which have yet been found to yield no lysine. It has long been recognised that many vegetable proteins contain more nitrogen than the animal proteins, and it has been recently shown (364) that this difference is chiefly caused by the relatively larger proportion of arginine which most of the seed proteins yield. A few, however, of the seed proteins which are rich in nitrogen yield but little arginine. In these the high nitrogen content is due to a larger proportion of amide nitrogen,

B. Hydrolysis by Alkalies.

Alkalies have been frequently used to effect hydrolytic decomposition of proteins, notably by Schützenberger and Emil Fischer, but in comparison with acids they have been little used, for some of the amino-acids, notably arginine and cystine, are destroyed by heating with them. Osborne, Leavenworth and Brautlecht (364) found that continued boiling with a strong solution of sodium hydroxide yields a quantity of ammonia corresponding to the sum of the amide nitrogen and one-half of the nitrogen of the arginine. With these exceptions the primary products of alkaline hydrolysis are, so far as is now known, the same as those produced by acids. In regard to the proportion of ammonia eliminated by alkaline hydrolysis, and the forms of union of nitrogen in the molecule of some vegetable proteins, the reader is referred to page 62.

C. Colour Reactions.

From what has just been said of the products of protein decomposition it is evident that the colour reactions of the vegetable proteins are practically the same as those of the animal proteins. None of these reactions, therefore, deserves special consideration except those showing the presence of carbohydrates. In the case of vegetable proteins, associated so intimately with a variety of carbohydrates, Molisch's reaction is of especial interest, for this indicates the presence of even minute quantities of carbohydrates. Preparations of proteins which do not give this reaction are entirely free from any carbohydrate or from any substance which can yield a carbohydrate or furfurol, as, for instance, glucosides or nucleic acid. The fact that a large number of the vegetable proteins give absolutely no trace of colour with Molisch's test, shows that a carbohydrate complex is not a constituent of the molecules of a large number of vegetable proteins. Whether protein preparations which give Molisch's reaction contain a carbohydrate group as a constituent of their molecules, or as a constituent of some group organically united with the protein molecule, or as simply a contamination, cannot yet be definitely decided. Attempts to isolate a carbohydrate from such proteins have, up to the present time, failed, and we have no good ground to believe that any of the seed proteins actually contain a carbohydrate group as a constituent of their molecules. On the other hand, we have no conclusive evidence that some of them do not contain such a group, for it is extremely difficult to isolate carbohydrate from a mixture of protein decomposition products, and

the fact that this has not yet been accomplished is by no means conclusive evidence of the absence of such substances. Molisch's reaction is not given by most of those proteins whose physical properties favour their purification. Preparations of the crystalline globulins from the squash, hemp and flax-seeds and Brazil-nut, the juglansin from the walnut, corylin from the hazel-nut, amandin from the almond, and legumin from the pea or vetch, have been obtained which give no trace whatever of this reaction. On the other hand preparations of other proteins, especially those from the leguminous seeds, frequently give very strong reactions, but it has been noted that when a number of different preparations of one or the other of these proteins are tested under uniform conditions the intensity of the reaction varies greatly. In such cases it is highly probable that this reaction is caused by a small amount of some contaminating substance which is difficult to separate from the protein.

D. Nitrogen in Vegetable Proteins.

Partition of Nitrogen in Seed Proteins.

The different forms in which the several seed proteins yield their nitrogen when completely decomposed by boiling with strong hydrochloric acid has been extensively studied by Osborne and Harris (349).

Hausmann's method (150), slightly modified, was used for these determinations, the results of which are given in the table (p. 57), together with a few obtained with animal proteins which are introduced for the sake of comparison.

The most striking feature shown by this table is the wide range in the amount of basic nitrogen obtained from the different proteins, namely, from one-third to one-thirtieth of the total nitrogen of the protein, while the proportion of ammonia differs from one-fourth to one-sixteenth of the total nitrogen. The non-basic nitrogen, on the other hand, is more constant even than the total nitrogen and forms from about one-half to three-fourths of the latter.

Comparison of the Nitrogen Precipitated by Phosphotungstic Acid with that in the Basic Amino-acids.

The great differences thus indicated in the constitution of these different proteins make it important to know with what accuracy such figures as these in the table (p. 57) can be obtained. Unfortunately there is no method by which they can be directly tested, but the constancy of the results can be established and indirect evidence obtained which makes it highly probable that they closely agree with

PARTITION OF NITROGEN IN DIFFERENT PROTEINS.

Protein.	Source.	In per cent. of protein.				Total N.	In per cent. of nitrogen.		
		N as ammonia.	Basic N.	Non-basic N.	N in MgO pp.		N as ammonia.	Basic N.	Non-basic N.
Globulin,	cocoanut	1·36	6·06	10·92	0·14	18·48	7·3	32·8	59·0
,,	squash-seed	1·28	5·97	11·04	0·22	18·51	7·4	32·3	59·6
Edestin,	hemp-seed	1·88	5·91	10·78	0·12	18·69	10·1	31·6	57·7
Excelsin,	Brazil-nut	1·48	5·76	10·89	0·17	18·30	8·0	31·5	57·8
Corylin,	hazel-nut	2·20	5·75	10·89	0·16	19·00	11·6	30·3	59·5
Globulin,	cotton-seed	1·92	5·71	11·01	—	18·64	10·3	30·6	59·1
,,	castor-bean	1·96	5·64	11·03	0·12	18·75	10·5	30·1	58·7
Juglansin,	walnut	1·78	5·41	11·50	0·15	18·84	9·4	28·7	61·2
Conglutin,	lupine α	2·12	5·20	10·40	0·18	17·90	11·8	29·1	58·1
	lupine β	2·65	5·13	10·29	0·14	18·21	14·0	28·2	56·6
Legumin,	pea [1]	1·68	5·11	11·10	0·15	18·04	9·3	28·3	61·5
,,	lentil	1·69	5·16	11·10	0·11	18·06	9·3	28·5	61·3
,,	horse-bean	1·62	4·92	11·41	0·11	18·06	9·0	27·2	63·2
,,	vetch	1·75	5·17	10·92	0·18	18·02	9·7	28·7	60·7
Globulin,	flax-seed	2·00	4·77	11·49	0·22	18·48	10·8	25·8	62·0
Vicilin,	pea	1·70	4·92	10·22	0·21	17·05	10·0	28·8	60·0
,,	lentil	1·75	4·59	10·77	0·13	17·24	10·1	26·6	62·5
,,	horse-bean	1·93	4·53	10·35	0·23	17·04	11·3	26·6	60·7
Vitellin,	egg-yolk [1]	1·29	4·36	10·04	0·59	16·28	7·9	26·7	61·6
Vignin,	cow-pea	1·91	4·28	10·81	0·25	17·25	11·1	24·8	62·6
Globulin,	sunflower	2·57	4·27	11·50	0·24	18·58	13·8	23·0	61·9
Conalbumin,	egg-white [1]	1·00	3·76	10·93	0·42	16·11	6·2	23·3	67·8
Amandin,	almond	3·05	4·15	11·63	0·17	19·00	16·0	21·8	61·2
Phaseolin,	kidney-bean	1·69	3·62	10·56	0·33	16·20	10·4	22·3	65·2
,,	adzuki-bean	1·74	4·18	10·01	0·27	16·20	10·7	25·8	61·7
Glycinin,	soy-bean	2·11	3·95	11·27	0·12	17·45	12·1	22·6	64·6
Legumelin,	lentil	1·08	3·59	10·97	0·42	16·06	6·7	22·3	68·3
,,	horse-bean	0·96	3·42	11·10	0·44	15·92	6·0	21·4	69·5
,,	adzuki-bean	1·03	3·84	10·93	0·30	16·10	6·4	23·9	67·9
,,	soy-bean [1]	1·18	3·08	11·44	0·39	16·09	7·3	19·1	71·1
Leucosin,	wheat	1·16	3·50	11·84	0·43	16·93	6·8	20·6	69·6
Casein,	cow's milk	1·61	3·49	10·31	0·21	15·62	10·3	22·4	66·0
Ovalbumin,	egg-white [1]	1·34	3·20	10·68	0·29	15·51	8·6	20·6	69·0
Glutenin,	wheat	3·30	2·05	11·95	0·19	17·49	18·8	11·7	68·3
Gliadin,	,,	4·33	1·09	12·17	0·07	17·66	24·5	6·2	68·9
,,	rye	4·08	0·91	12·56	0·11	17·66	23·1	5·2	70·0
Hordein,	barley	4·01	0·77	12·20	0·23	17·21	23·3	4·5	70·9
Zein,	maize	2·97	0·49	12·51	0·16	16·13	18·4	3·0	77·5

[1] Revised figures, given in view of later unpublished determinations.

the quantity of these substances actually yielded by the protein. In regard to the constancy of the determination of the ammonia nitrogen the following results obtained recently in the writer's laboratory serve to illustrate how closely repeated determinations by different analysts using different preparations may be expected to agree. [*Cf.* Osborne, Leavenworth and Brautlecht (364)].

NITROGEN AS AMMONIA IN PER CENT. OF THE PROTEIN.

Gliadin	4·40,[2] 4·44,[2] 4·35, 4·30, 4·33, 4·33, 4·30.[1]
Hordein	4·10,[2] 4·01.[1]
Zein	2·99,[2] 2·97.[1]
Edestin	1·83,[2] 1·86, 1·88,[1] 1·93,[1] 1·86, 1·81, 1·80, 1·87.
Globulin, squash-seed	1·35,[2] 1·28.[1]
Glycinin	2·14,[2] 2·11.[1]
Vignin	1·89, 1·86, 1·91,[1] 1·82.
Globulin, cotton-seed	1·94, 1·96, 1·92.[1]
Legumin, pea	1·68, 1·68.[1]
Vicilin, pea	1·64, 1·78, 1·60.[1]
Vitellin, hen's egg yolk	1·24, 1·29, 1·28,[1] 1·24.[1]
Conalbumin, hen's egg	1·13, 1·21.[1]

[1] Figures given in preceding table.
[2] Distilled *in vacuo* at 40°.

Is the Nitrogen Yielded as Ammonia Amide Nitrogen?

As the amino-acids which result from protein hydrolysis yield no ammonia by long boiling with strong hydrochloric acid, this ammonia must arise from some other form of binding of the nitrogen in the protein molecule. That this nitrogen may be in amide union seems probable from the following experiments in which five portions of gliadin, each weighing 1 gramme, were dissolved in 50 c.c. of 20 per cent. hydrochloric acid, and the solutions boiled for the times indicated in the following table:—

Boiled for 30 min., nitrogen as NH_3 = 4·30 per cent.
Boiled for 1 hour, nitrogen as NH_3 = 4·35 per cent.
Boiled for 2 hours, nitrogen as NH_3 = 4·33 per cent.
Boiled for 3 hours, nitrogen as NH_3 = 4·33 per cent.
Boiled for 4 hours, nitrogen as NH_3 = 4·33 per cent.
Boiled for 6 hours, nitrogen as NH_3 = 4·40 per cent.

When treated with strong hydrochloric acid at 20° for two hours, only 0·22 per cent. of nitrogen as ammonia was obtained, while after seventeen hours at the same temperature 1·67 per cent. was found. Under similar conditions asparagine yielded 1·4 per cent. of nitrogen as ammonia after seventeen hours at 20° and one-half of its nitrogen after boiling for thirty minutes.

In regard to the accuracy of the figures given for the nitrogen precipitated by phosphotungstic acid we are able to form some judgment, since the only strongly basic amino-acids yielded by proteins are arginine, histidine and lysine, and these are, therefore, precipitated from a dilute solution by phosphotungstic acid. The nitrogen thus precipitated should, consequently, be equal to the nitrogen contained in these three basic amino-acids, if no other basic substances are present among their decomposition products. That such an agreement actually exists is shown from the following table:—

	Histidine.	Arginine.	Lysine.	Basic N Calculated.	Basic N Precipitated.	Difference.	
	Per cent. of the protein.						Basic N. in p.c. of that precipitated.
Globulin, squash-seed	2·42	14·44	1·99	5·69	5·99	−0·30	95·00
Excelsin, Para-nut	2·50	14·29	1·64	5·57	5·76	−0·19	96·70
Edestin, hemp-seed	2·19	14·17	1·65	5·48	5·91	−0·43	92·70
Globulin, cotton-seed	3·46	13·51	2·06	5·69	5·71	−0·02	99·65
Globulin, castor-bean	2·74	13·19	1·54	5·29	5·64	−0·35	93·80
Amandin, almond	1·87	12·16	0·72	4·56	4·15	+0·41	109·90
Legumin, pea	1·69	11·73	4·98	5·20	5·11	+0·09	101·50
Legumin, vetch	2·94	11·06	3·70	5·07	5·17	−0·10	98·07
Conglutin-α, yellow lupine	2·51	10·93	2·74	4·77	5·16	−0·39	92·50
Vicilin, pea	2·17	8·91	5·40	4·50	4·92	−0·42	91·46
Glycinin, soy-bean	2·10	7·69	3·39	3·59	3·95	−0·36	90·90
Vitellin, hen's egg yolk	1·90	7·46	4·81	3·82	4·36	−0·54	87·62
Vignin, cow-pea	3·08	7·20	4·31	3·98	4·28	−0·30	92·90
Glutelin, maize	3·00	7·06	2·93	3·63	3·52	+0·11	103·10
Ovalbumin, hen's egg	1·71	4·91	3·76	2·76	3·20	−0·44	86·25
Leucosin, wheat	2·83	5·94	2·75	3·25	3·50	−0·25	92·87
Conalbumin, hen's egg	2·17	5·07	6·43	3·45	4·16	−0·71	83·00
Legumelin, pea	2·27	5·45	3·03	2·95	3·45	−0·50	85·50
Legumelin, soy-bean	2·04	5·35	4·91	3·21	3·08	+0·13	104·22
Phaseolin, kidney-bean	2·62	4·87	4·58	3·15	3·62	−0·47	87·00
Glutenin, wheat	1·76	4·72	1·92	2·37	2·05	+0·32	115·60
Casein, cow	2·46	3·39	5·95	2·99	3·49	−0·50	85·70
Gliadin, wheat	0·58	3·16	0·00	1·18	1·09	+0·09	108·30
Gliadin, rye	0·39	2·22	0·00	0·83	0·90	−0·07	90·20
Hordein, barley	1·28	2·16	0·00	1·05	0·77	+0·28	136·40
Zein, maize	0·82	1·35	0·00	0·65	0·49	+0·16	132·60

Seventeen of the twenty-six proteins given in this table contain an amount of bases in which the nitrogen does not fall below 90 nor above 110 per cent. of that precipitated by phosphotungstic acid. Three of the others show differences which are relatively great though absolutely small; but as they contain very little base these differences are unquestionably caused by unavoidable errors of analysis; for the phosphotungstates of the bases are somewhat soluble, and, therefore, when the amount of base is small the nitrogen precipitated by this acid is less than that actually contained in them. On the other hand, the bulky precipitate of the phosphotungstates, which is obtained when the amount of base is large, carries with it some of the mono-amino-acids, thereby compensating the error caused by solubility. The six remaining proteins yield a quantity of bases containing an amount of nitrogen less than that precipitated by phosphotungstic acid by a little more than 10 per cent. of the latter. Two of these, namely, phaseolin and legumelin from the pea, yield more bases when hydrolysed for twenty-four hours than when hydrolysed for twelve hours, hence this difference may possibly be due to an incomplete hydrolysis even after this longer

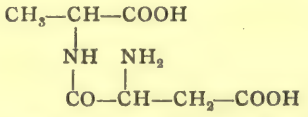
time of boiling. The deficiency found for casein may be caused by diamino-tri-oxy-dodecanic acid which Fischer has shown to be precipitable by phosphotungstic acid.

It is evident from the figures given in the table that in most cases the agreement between the nitrogen in arginine, histidine and lysine and that precipitated by phosphotungstic acid is so close that Hausmann's method can be employed for controlling the results of determinations of the bases by the method of Kossel, Kutscher and Patten; for where a wide difference between the nitrogen obtained by these two methods is found the accuracy of the direct determination of the bases can be established by careful repetition.

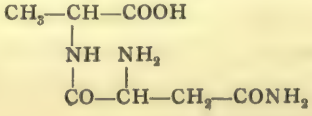
The accuracy of the determinations of the individual bases, that is, the completeness with which they can be separated from one another, as well as from other substances, is shown by the evident purity of the products obtained in the course of analysis. The arginine copper nitrate double salt separates completely from its solution on slow evaporation, leaving no trace of any other substance in the final liquid. The histidine solutions readily yield pure histidine dichloride, and the character of the crystallisation of the lysine picrate, in which form this substance is weighed, is such as to leave no doubt of its purity, which can, moreover, be further established by analysis of the product in the form in which it is actually weighed. The agreement between duplicate determinations of the several bases made on one and the same protein is further evidence of their accuracy.

Ratio of Ammonia to Glutaminic and Aspartic Acids.

The work of Emil Fischer has made it almost if not quite certain that the amino-acids are for the most part united in the protein molecule in polypeptide union; that is by the union of the NH_2 group of one amino-acid with the carboxyl group of another; thus



which represents a peptide of alanine and aspartic acid. The dibasic acids would therefore afford carboxyl groups with which nitrogen might unite in amide union, as shown by the following formula, which represents the amide of the peptide just mentioned:—



A relation may consequently exist between the quantity of amide nitrogen which the different proteins yield and their content in glutaminic and aspartic acids. Fischer has already suggested this, and Osborne and Gilbert (348) have shown that a large proportion of glutaminic acid is in many cases accompanied by a similar large proportion of amide nitrogen. In the following table the amount of ammonia which would correspond to each molecule of glutaminic and aspartic acid found in the different proteins is calculated, and the percentage of ammonia found by distillation is given for comparison. The results show in most cases a close agreement, but some proteins, for example those of the cereals and those from the pea, show marked differences.

Ratio of Ammonia to Glutaminic and Aspartic Acids.

	Percentage of NH_3 calculated for 1 mol. of NH_3 to 1 mol. of glutaminic and aspartic acids.	Percentage of NH_3 found by distillation.	Difference between amount calculated and that found.
Globulin, squash-seed	1·84	1·64	+0·20
Edestin	2·19	2·28	−0·09
Globulin, cotton-seed	2·40	2·33	+0·07
Globulin, sunflower-seed	2·93	3·13	−0·20
Excelsin	1·99	1·80	+0·19
Amandin	3·36	3·70	−0·34
Conglutin-α	2·63	2·55	+0·08
Legumin, vetch	2·27	2·16	+0·11
Legumin, pea	2·64	2·05	+0·59
Vicilin, pea	3·15	2·03	+1·12
Legumelin, pea	2·02	1·23	+0·79
Vignin	2·46	2·34	+0·14
Phaseolin	2·35	2·06	+0·29
Glycinin	2·75	2·56	+0·19
Leucosin	1·23	1·41	−0·18
Glutenin	2·83	4·01	−1·18
Maize glutelin	1·54	2·12	−0·58
Gliadin	4·39	5·11	−0·72
Hordein	5·02	4·87	+0·15
Zein	3·72	3·61	+0·10
Casein	1·38	1·61	−0·23
Ovalbumin	1·34	1·63	−0·29

As we have good reason to believe that the proportion of glutaminic and aspartic acids obtained from gliadin and glutenin agrees closely with the quantities actually present in these proteins, it is probable that they differ in some way in structure from all the others which have been examined, and that they may possibly contain some other dibasic acid not yet isolated from their decomposition products.

In the case of the proteins of the pea the difference, which is in

the opposite direction, might indicate that a part of the nitrogen yielded as ammonia was in the combination R—CO—NH—CO—R, as recently suggested by Bergell and Feigl (588), for in this case two molecules of the dibasic acid would be united to only one NH. This, however, is probably not the case, for distillation of these proteins with sodium hydroxide solution has given no evidence of the presence of this grouping, which Bergell and Feigl have shown to be stable in acid solutions but to yield ammonia on boiling with alkalies. None of the other proteins, when distilled with alkali, gave any indication of this diamide binding, and we have, as yet, no reason to suppose that it occurs in the protein molecule.

This marked agreement between the ammonia as determined and that calculated for the proteins of seeds, other than those of the wheat and the pea, indicates that this ammonia exists in these proteins as amide nitrogen in combination with one of the carboxyl groups of the dibasic acids.

Nitrogen Converted into Ammonia by Alkaline Hydrolysis.

Cystine and arginine are the only known decomposition products of the proteins which are not stable in alkaline solutions. Arginine, theoretically, should yield one-half of its nitrogen as ammonia on boiling with fixed caustic alkalies. If the proteins yield no other products sensitive to alkalies, the amount of nitrogen which they should give as ammonia, when distilled with a strong solution of sodium hydroxide, ought to be equal to the sum of their amide nitrogen and one-half the nitrogen of the arginine which they contain.

Experiments by Osborne, Leavenworth and Brautlecht (364) show how some vegetable proteins behave when subjected to alkaline hydrolysis. One gramme, air dry, of each of the proteins given in the table below was distilled with 300 c.c. of decinormal sodium hydroxide solution, and when 200 c.c. had distilled over, the distillate was titrated. The residual solution was then made up to 300 c.c. with decinormal sodium hydroxide solution and the distillation repeated. The solution was then again made up to 300 c.c. with water and again distilled, the process being repeated until no more ammonia came over. The results obtained are given in the following table:—

Distillation.	Gliadin, wheat, 0·9198 gm. Mgr. N.	Legumin, pea, 0·8979 gm. Mgr. N.		Vicilin, pea, 0·9264 gm. Mgr. N.		Excelsin, Para-nut, 0·9024 gm. Mgr. N.
1	27·2	15·4	17·6	17·8	15·4	11·4
2	12·0	4·4	5·8	3·6	5·2	4·5
3	1·6	2·2	2·6	2·2	3·0	3·5
4	2·6	1·8	1·6	1·6	1·2	2·4
5	0·4	1·8	1·6	1·2	1·6	1·4
6	—	1·0	1·0	1·0	0·8	1·8
7	—	1·3	1·2	1·0	1·0	1·6
8	—	1·0	0·8	0·8	1·0	1·6
9	—	0·6	0·8	0·4	0·6	1·6
10	—	0·8	0·4	0·4	0·4	1·6
11	—	1·4	1·0	1·2	0·8	1·6
12	—	0·8	0·6	0·2	0·4	0·8
13	—	0·6	0·8	—	—	0·4
14	—	0·2	0·4	—	—	0·9
15	—	—	—	—	—	0·0
Total	43·8	33·3	36·2	31·4	31·4	35·1
Per cent. of dry and ash-free protein	4·76	3·71	4·04	3·39	3·39	3·72
Amide N	4·30	1·69	—	1·70	—	1·48
½ Arginine N	0·51	1·88	—	1·42	—	2·25
Sum	4·81	3·57	—	3·12	—	3·73

Gliadin, which contains much amide nitrogen and but little arginine nitrogen, and excelsin, which contains little amide nitrogen and very much arginine nitrogen, both gave results in very close agreement with the calculation, while legumin and vicilin yielded slightly more nitrogen by alkaline hydrolysis than that calculated.

Most of the nitrogen came over in the first two distillations, corresponding to the ease with which amide nitrogen is converted into ammonia by caustic alkalies. The nitrogen subsequently coming off as ammonia was evolved slowly in much the same way as from arginine, although a little more quickly. The close agreement between the results thus obtained and those calculated shows that these proteins contain little or no nitrogen except the amide and one-half the arginine nitrogen which can be thus converted into ammonia.

The Undetermined Nitrogen of Protein Hydrolysis.

The nitrogen of the protein, other than the amide and the basic nitrogen, largely exists, so far as is now known, in the form of *a*-amino-acids. It is not probable that all of the decomposition products of the proteins are yet known, for attempts to determine the amount of each of the known substances has in no case given a result which did not

fall far short of the total of the protein. A considerable part of this deficit is doubtless made up of the known substances that are determined, for losses necessarily occur in the processes of isolating and separating them [*cf.* Osborne and Heyl (360)]. This loss, however, probably does not account for all of the unknown residue. If it is assumed that the amino-acids that are found in a well-conducted analysis of the decomposition products of a protein are united in the molecule with the elimination of water, and that the dibasic acids are united to an amide group which replaces one hydroxyl, and the sum of the percentages of these radicals is subtracted from 100, the percentage of the unknown residue can be obtained. If the nitrogen unaccounted for in the same analysis is calculated as per cent. of this unknown residue, it is found that in the case of gliadin this unknown part contains 13·3, in excelsin 14·0, and in legumin 14·3 per cent. of nitrogen. Among the known decomposition products of the proteins this percentage of nitrogen is equalled only by glycocoll, alanine, tryptophane and serine, even if the calculation is made for the radicals of the monoamino-acids in polypeptide union, that is, after subtracting one molecule of water from their molecular weights. In making this calculation no account is taken of the fact that the amount of the amino-acids isolated in a condition fit for weighing is distinctly less than the quantities actually yielded by hydrolysis. As this loss falls mostly on substances which contain less than 13 per cent. of nitrogen, the actual proportion of nitrogen in the unknown residue of the protein must be even higher than that indicated by the above calculation. As it is improbable that this unknown residue is wholly made up of undetermined quantities of the four amino-acids above mentioned, it is fair to presume that the proteins contain a considerable amount of some still unknown substance or substances relatively rich in nitrogen [*cf.* Emil Fischer (591)].

E. Sulphur in Vegetable Proteins.

The small but constant quantity of sulphur which proteins contain has especial interest in connection with the numerous attempts that have been made to ascertain their molecular weights and to establish empirical formulas for them. The fact that sulphur exists in two forms in the protein molecule was long ago indicated, and it has since been assumed that the protein molecule contains at least two atoms of sulphur. It has been recently demonstrated that many proteins yield cystine on hydrolysis, and the probability has become great that much, if not all, of the sulphur of some of the proteins is cystine sul-

phur. The assumption of the presence of two atoms of sulphur in the protein molecule may be considered to be established if the cystine which results on hydrolysis is an actual constituent of the molecule. Proteins which contain 0·4 per cent. of sulphur must therefore have a molecular weight of at least 15,000 if the preparations in which this quantity of sulphur is found do not consist of mixtures of sulphur-containing and sulphur-free proteins.

With the exception of one or two unsatisfactory observations, no proteins have been described which contain no sulphur whatever. Only one carefully studied vegetable protein has yet been obtained which contains so small a proportion of sulphur as to make the existence of sulphur-free protein in any way probable. This protein is vicilin, obtained from several leguminous seeds, some preparations of which have been found to contain as little as 0·1 per cent. of sulphur. The sulphur content of various preparations of vicilin, which were obtained by Osborne and Campbell (330, 331, 332, 334) by fractional precipitation, fell between 0·2 and 0·1 per cent. The accuracy of the determinations in these different fractions was established by repeated closely agreeing determinations, and there can be no doubt that differences in the sulphur content of these fractions actually existed. In view of this variable content in sulphur and of its very small total amount, it is possible that these preparations of vicilin were mixtures of different proportions of sulphur-free and sulphur-containing protein. In no other case has any evidence of the possible existence of sulphur-free protein yet been obtained. In all other carefully studied and properly analysed proteins the sulphur content has been found to be very constant and in many of them it has been established with a high degree of accuracy.

Numerous attempts have been made to establish a definite ratio between the sulphur which can be thus split off as sulphide by boiling with alkalies, and the total sulphur of the protein. In every case the amount of sulphide sulphur thus obtained was less than the total sulphur, and it was for a long time assumed that this fact showed the presence of two different forms of sulphur in the protein molecule. Experiments with cystine show that not more than two-thirds of the sulphur which this substance contains can be converted into sulphide by boiling with alkali, and that, unless special care is taken, the proportion obtained is but little more than one-half. The fact that only a part of the protein sulphur can be converted into sulphide gives, in most cases, no evidence of the existence of two forms of sulphur in the protein molecule, but the fact that the proteins yield cystine on hydro-

lysis is good evidence that at least two atoms of sulphur must be present in their molecules.

A study made by Osborne (316) of the sulphur content of a number of thoroughly purified preparations of seed proteins has shown that it is possible to determine the total sulphur of the protein with a high degree of accuracy. The sulphur content of preparations of edestin obtained from chloride solutions agreed closely with one another and in all cases was less than that of preparations of edestin obtained from sulphate solutions. The preparations from chloride solutions consist chiefly of edestin chloride, while those from sulphate solutions are chiefly edestin sulphate. The difference in the sulphur content of these two salts of edestin was almost exactly equal to the sulphur of the combined sulphuric acid as actually determined in the products of neutralisation of the preparations in question.

It has already been shown that preparations obtained from sodium chloride solutions contain a very small amount of combined sulphuric acid in addition to the combined hydrochloric acid, and in agreement with this fact preparations of pure neutral edestin from which all the combined acid had been carefully removed yielded a little less sulphur than the preparations which still retained their combined acid. The slight differences found between twenty-one preparations of edestin (see p. 67), which amounted to only a few hundredths of a per cent., were undoubtedly caused by differences in the relative proportions of combined sulphuric and hydrochloric acids which these preparations contained. Other similar determinations of the total sulphur in a number of different proteins led to equally constant results, so there is no doubt that these proteins contained definite quantities of sulphur. Determinations, by Schulz' method, of the sulphur of different seed proteins, converted into sulphide by boiling with caustic soda, yielded uniform results which are given in the following table, together with similar results obtained with some animal proteins which are introduced for comparison.

RATIO OF SULPHIDE SULPHUR TO TOTAL SULPHUR.

	Total Sulphur.	Sulphide Sulphur.	Per cent. of total Sulphur as Sulphide.	
Seralbumin	1·930 (605)	1·280 (605)	66	
Oxyhæmoglobin, dog	0·568 (596)	0·335	59	
Serglobulin, horse	1·110 (592)	0·630 (605)	57	
Gliadin	1·027	0·619	60	
Conglutin, blue lupine I.	0·393	0·262	66	$\frac{2}{3}$
Conglutin, blue lupine II.	0·359	0·239	66	$\frac{2}{3}$
Conglutin, yellow lupine I.	0·530	0·344	65	
Conglutin, yellow lupine II.	0·954	0·558	58	
Conglutin, yellow lupine III.	1·378	0·889	64	
Oxyhæmoglobin, horse	0·380 (606)	0·190 (605)	50	
Vignin	0·426	0·214	50	
Amandin	0·429	0·217	50	
Globin	0·420 (605)	0·200 (605)	48	$\frac{1}{2}$
Glycinin	0·710	0·320	46	$\frac{1}{2}$
Vicilin	0·200	0·092	46	
Legumin	0·385	0·165	41	
Edestin	0·880	0·346	40	
Zein	0·600	0·212	35	
Ovovitellin	1·028	0·348	34	
Fibrin	1·100 (592)	0·380 (597)	34	$\frac{1}{3}$
Excelsin	1·086	0·350	32	$\frac{1}{3}$
Ovalbumin	1·616	0·491	30	$\frac{1}{3}$
Phaseolin	0·312	0·072	23	$\frac{1}{4}$
Casein	0·800 (590, 593)	0·101	13	$\frac{1}{8}$

As an example of the uniformity with which total sulphur and sulphide sulphur may be determined the following figures are given :—

PERCENTAGE OF TOTAL SULPHUR IN DIFFERENT PREPARATIONS OF EDESTIN FROM HEMP-SEED.

Edestin chloride.

I.	II.	III.	IV.	V.	VI.	VII.
0·931	0·934	0·980	0·895	0·983	0·960	0·944
0·942	0·924	0·938	—	0·963	—	0·941
0·910	0·937	—	—	—	—	—
VIII.	IX.	X.	XI.	XII.	XIII.	XIV.
0·990	0·990	0·997	0·943	0·874	0·864	0·900
0·972	—	0·987	—	0·900	0·866	—
0·963	—	—	—	—	—	—
XV.	XVI.	XVII.	XVIII.	XIX.	XX.	XXI.
0·902	0·893	0·934	0·941	0·964	0·939	0·932

Neutral edestin.

0·880
0·887
—

Edestin sulphate.

XXII.	XXIII.
1·125	1·084
1·110	—
1·103	—

PERCENTAGE OF SULPHIDE SULPHUR IN EDESTIN.

Five grammes heated with 30 p.c. NaOH and $Pb(C_2H_3O_2)_2$ at 165°.

XXIV.	XXV.
0·339	0·363
0·344	0·347
—	0·340
—	0·337

PERCENTAGE OF TOTAL SULPHUR IN DIFFERENT PREPARATIONS OF EXCELSIN.

I.	II.	III.	IV.	V.
1·06	1·12	1·07	1·083	1·109

PERCENTAGE OF SULPHIDE SULPHUR IN EXCELSIN.

One gramme boiled with 30 p.c. NaOH, Zn and $Pb(C_2H_3O_2)_2$ 7½ hours.		Heated with 30 p.c. NaOH. One gramme at 135° 2 hours.		One gramme at 165° 4½ hours.
IV.	V.	IV.	V.	V.
0·339	0·347	0·294	0·293	0·350
0·321	0·344	—	—	—
0·289	0·297	—	—	—
0·277	0·290	—	—	—
0·257	0·284	—	—	—
—	0·281	—	—	—

PERCENTAGE OF TOTAL SULPHUR IN DIFFERENT PREPARATIONS OF GLIADIN.

I.	II.	III.	IV.
1·002	1·022	1·030	1·058
—	1·005	—	1·027

PERCENTAGE OF SULPHIDE SULPHUR IN GLIADIN.

One gramme boiled with 30 p.c. NaOH, Zn and $Pb(C_2H_3O_2)_2$ 7½ hours.	One gramme with 30 p.c. NaOH and $Pb(C_2H_3O_2)_2$ at 135° 4 hours.	Five grammes with 30 p.c. NaOH and $Pb(C_2H_3O_2)_2$ at 165° 5 hours.
0·627	0·635	0·624
—	—	0·611
—	—	0·600

PERCENTAGE OF TOTAL SULPHUR IN DIFFERENT PREPARATIONS OF OVALBUMIN.

I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.
1·610	1·612	1·572	1·644	1·613	1·619	1·613	1·634	1·590	1·651

These figures agree well with those given by several other investigators, namely:—

Hammarsten (594)	1·64
Bondzynski and Zoja (589)	1·66
Krüger (597)	1·66
Hopkins (595)	1·57

PERCENTAGE OF SULPHIDE SULPHUR IN OVALBUMIN.

One gramme boiled with 30 p.c. NaOH, Zn and $Pb(C_2H_3O_2)_2$ 7½ hours.	1 gramme with 30 p.c. NaOH and $Pb(C_2H_3O_2)_2$ at 135° 2 hours.	5 grammes with 30 p.c. NaOH and $Pb(C_2H_3O_2)_2$ at 165° 4½ hours.
0·523	0·523	0·491
0·511	0·518	—
0·504	0·505	—
0·471	0·459	—
0·455	0·441	—
0·425	—	—

The result obtained with 5 grammes heated at 165° agrees very closely with the average of the others and is also in accord with the results obtained by Krüger (597), Malerba (599) and Schulz (605), who found respectively 0·44, 0·49 and 0·49 per cent. of sulphide sulphur in ovalbumin.

If the sulphide sulphur obtained by Schulz' method originates from a cystine complex in the protein molecule, and if this complex yields

two-thirds of its sulphur, as does free cystine, many of the proteins in the preceding table appear to contain a relatively large proportion of sulphur in some other complex than cystine. The following table shows the amount of cystine sulphur calculated on the above assumption and the amount of sulphur unaccounted for :—

	Total Sulphur, per cent.	Cystine Sulphur calculated, per cent.	Difference unaccounted for, per cent.
Oxyhæmoglobin, horse	0·385	0·285	0·100
Vignin	0·426	0·321	0·105
Amandin	0·429	0·326	0·103
Globin	0·420	0·300	0·120
Glycinin	0·710	0·480	0·230
Vicilin	0·200	0·138	0·062
Legumin	0·385	0·248	0·137
Edestin	0·880	0·519	0·361
Zein	0·600	0·318	0·282
Vitellin, hen's egg-yolk	1·028	0·522	0·506
Fibrin	1·100	0·570	0·530
Excelsin	1·086	0·525	0·561
Ovalbumin	1·616	0·737	0·879
Phaseolin	0·312	0·108	0·204
Casein	0·800	0·150	0·650

For many of these proteins the quantity of sulphur thus unaccounted for is relatively large, and this makes it probable that many proteins contain sulphur in some other combination than cystine.

If the generally uniform physical properties of all these proteins is considered as well as their similar relations toward extremely small quantities of base and acid, it seems more probable that they have similar high molecular weights and contain different numbers of sulphur atoms than that, as has frequently been assumed, they each contain two sulphur atoms and have correspondingly different molecular weights. Six of these twenty-one proteins contain approximately 0·4 per cent. of sulphur and must therefore have molecular weights of at least 15,000. If the empirical formulas and molecular weights which correspond most nearly to 15,000 are calculated for these proteins on the assumption of a definite number of sulphur atoms, the following results are obtained :—

COMPOSITION AND POSSIBLE EMPIRICAL FORMULAS OF SOME PROTEIN BODIES.

Composition.							Vegetable Proteins.	Formula.							Molecular Weight.
C.	H.	N.	S.	Fe.	P.	O.		C.	H.	N.	S.	Fe.	P.	O.	
51·30	6·90	18·90	0·429	—	—	22·471	Amandin	638	1030	202	2	—	—	209	14922
52·64	6·95	17·25	0·426	—	—	22·734	Vignin	660	1040	185	2	—	—	214	15038
51·72	6·95	18·04	0·385	—	—	22·905	Legumin	718	1158	214	2	—	—	238	16642
55·23	7·26	16·13	0·600	—	—	20·78	Zein	736	1161	184	3	—	—	208	15993
52·01	6·89	17·47	0·710	—	—	22·88	Glycinin	780	1240	226	4	—	—	257	18004
54·29	6·80	17·21	0·847	—	—	20·53	Hordein	675	1014	181	4	—	—	194	14880
51·36	7·01	18·65	0·88	—	—	22·10	Edestin	622	1020	193	4	—	—	201	14530
55·03	6·67	16·26	0·84	—	—	21·20	Bynin	706	1026	180	4	—	—	204	15410
52·72	6·86	17·66	1·027	—	—	21·733	Gliadin	685	1068	196	5	—	—	211	15568
52·18	6·92	18·30	1·086	—	—	21·514	Excelsin	642	1018	192	5	—	—	198	14738
53·02	6·84	16·80	1·280	—	—	22·06	Leucosin	663	1026	180	6	—	—	207	15006
							Animal Proteins.								
54·98	7·20	16·89	0·42	—	—	20·51	Globin	700	1098	184	2	—	—	196	15274
52·68	6·83	16·91	1·10	—	—	22·48	Fibrin	645	1004	178	5	—	—	207	14708
52·71	7·01	15·85	1·11	—	—	23·32	Serglobulin, horse	628	1002	160	5	—	—	209	14310
52·93	6·90	16·66	1·25	—	—	22·26	Fibrinogen	679	1062	183	6	—	—	207	15276
52·82	7·11	16·67	1·27	—	—	22·03	Myosin	660	1074	181	6	—	—	208	15048
52·75	7·10	15·51	1·616	—	—	23·024	Ovalbumin	696	1125	175	8	—	—	220	15703
52·19	7·18	15·77	1·73	—	—	23·13	Lactalbumin	644	1064	166	8	—	—	214	14792
52·99	7·01	15·93	1·930	—	—	22·14	Seralbumin, horse	662	1051	171	9	—	—	207	14989
52·25	6·65	15·88	2·25	—	—	22·95	Seralbumin. human	684	1045	178	11	—	—	225	15697
54·64	7·09	17·38	0·39	0·335	—	20·165	Oxyhæmoglobin, horse	758	1181	207	2	1	—	210	16655
54·57	7·11	16·38	0·568	0·336	—	21·036	Oxyhæmoglobin, dog	758	1185	195	3	1	—	219	16667
53·13	7·06	15·78	0·800	—	0·86	22·37	Casein	708	1130	180	4	—	4	224	15982
51·56	7·12	16·23	1·028	—	0·82	23·242	Ovovitellin	671	1112	182	5	—	4	227	15628

The figures in the above table show a degree of uniformity that is highly suggestive, but they must not be understood to actually represent the true molecular weights or empirical formulas of any of these proteins. The number of sulphur atoms in some of these formulas does not correspond with that which would be required by the figures given in the preceding table for the probable amount of cystine sulphur which they contain. Thus the cystine sulphur in gliadin, some of which is known to be present in this protein, requires an even number of atoms if all the sulphur is cystine sulphur. The formula in the above table gives five atoms of sulphur in this protein. For other proteins, however, the calculated cystine sulphur is in full harmony with the requirements of the formula. It is possible, and in fact not improbable, that the actual molecular weights are at least twice as great as those given in the table, as the proportion of sulphur found in vicilin is so small that, if this element is a constituent of all the molecules of the preparations of this protein, the molecular weight must be much in excess of 30,000. The extremely small proportion of sulphide sulphur obtained from phaseolin and casein also require a molecular weight in excess of 30,000. It is therefore evident that definite conclusions cannot be obtained from calculations based on the sulphur content of even the most carefully prepared and carefully analysed proteins, and such calculations are to be taken as simply showing that the molecular weights are probably very high if the preparations of these proteins represent a single substance.

CHAPTER X.

CLASSIFICATION OF VEGETABLE PROTEINS.

A CHEMICAL classification should be based on definite properties of individual substances, but such a treatment of the proteins is at present manifestly impossible. It is, however, desirable to have some scheme by means of which the proteins can be brought together in an orderly fashion. All attempts, thus far made, to classify them have been based chiefly on their solubility under different conditions. This method of classification has proved in many ways unsatisfactory and inadequate, but seems, for the present, to be the best available. Attempts have recently been made to establish greater uniformity throughout the world in the classification of the proteins and to attach more definite and generally recognised meanings to the various terms and designations which have been used in describing and classifying them. To this end committees were recently appointed by societies in England (608) and America (607) for the purpose of agreeing on a scheme of classification for the proteins. These committees have reported plans which in the main are in agreement with one another, no serious point of difference existing between them. As the scheme of classification of the American committee is more detailed and was prepared to include the vegetable proteins, it is used in this monograph. This scheme provides for the following groups :—

I. The Simple Proteins.
   - (*a*) Albumins.
   - (*b*) Globulins.
   - (*c*) Glutelins.
   - (*d*) Alcohol-soluble proteins (Prolamins).
   - (*e*) Albuminoids.
   - (*f*) Histones.
   - (*g*) Protamines.

II. Conjugated Proteins.
   - (*a*) Nucleoproteins.
   - (*b*) Glycoproteins.

(*c*) Phosphoproteins.
(*d*) Hæmoglobins.
(*e*) Lecithoproteins.

III. Derived Proteins.

1. Primary Protein Derivatives.
   (*a*) Proteans.
   (*b*) Metaproteins.
   (*c*) Coagulated proteins.
2. Secondary Protein Derivatives.
   (*a*) Proteoses.
   (*b*) Peptones.
   (*c*) Peptides.

The vegetable proteins belong to groups which were first established in connection with studies of animal proteins, but the definitions of these groups, as usually found in text-books dealing with proteins, must be modified to some extent if they are to include those vegetable proteins which have properties in the main agreeing with those of the animal proteins heretofore assigned to such groups. The properties of the various vegetable proteins which are here included in the different groups must, therefore, be considered somewhat in detail.

I. Simple Proteins.

(*a*) *Albumins.*

After Beccari's discovery of the existence of a protein substance in wheat flour, the presence of coagulable protein was soon recognised in the juices from many parts of different plants. The similarity of this substance to the albumin of hen's egg caused it to be long known as albumin. After advances had been made in the study of the proteins the term albumin was restricted to those proteins which are soluble in water and coagulable by heat. It has, however, of late years become a nearly universal practice among physiological chemists to classify the albumins and globulins on the basis of their behaviour towards a half-saturated solution of ammonium sulphate, and to consider that albumins remain dissolved when this salt is added in this proportion to their solutions. Such a method cannot be employed in differentiating those vegetable proteins which are here considered to be albumins, for some of them at least are thus precipitated by ammonium sulphate. The animal albumins are not precipitated by saturating their neutral solutions with sodium chloride or with magnesium sulphate. The vegetable albumins on the other hand are, in many cases,

precipitated by saturation with these salts, and can be included in the group of albumins only on the basis of their solubility in water at neutral or slightly acid reaction and their coagulability by heat. It is not always easy to decide whether, or not, a vegetable protein actually belongs with the albumins, as it is difficult, in many cases, to determine whether the protein substance in question is soluble in water alone or whether the presence of minute quantities of salts, bases or acids has caused its solubility. Thus, some leguminous seeds contain legumelin, which appears to be soluble in pure water but is partially precipitated by long-continued dialysis. Such precipitates, however, are not soluble in saline solutions and it is probable that they result from a denaturing of the protein. Whether in these cases denaturing occurs before precipitation, or the precipitation results from a complete removal of the salts and denaturing occurs after precipitation, is difficult to establish, and doubt exists, therefore, in regard to the real solubility of such proteins. Owing to the small quantity in which albumins have thus far been obtained from seeds, and the difficulties presented in completely separating them from associated globulins, conclusive evidence in regard to the albumin nature of some of them has not yet been obtained, and they must, for the present, be regarded as albumins since their properties seem to agree best with those characteristic of this group. Leucosin of wheat has been most carefully studied in respect to its solubility in water, and it has been found to be completely soluble in solutions which contain but a mere trace of mineral matter. Most seeds and, probably, most plant juices yield proteins which are as well entitled to be placed in the group of albumins as any of those of animal origin. The best characterised vegetable albumins are :—

Leucosin found in the seeds of	Wheat, *Triticum vulgare* (366, 336).
	Rye, *Secale cereale* (304).
	Barley, *Hordeum vulgare* (305).
Legumelin (334) found in the seeds of	Pea, *Pisum sativum* (330).
	Horse-bean, *Vicia faba* (332).
	Vetch, *Vicia sativa* (333).
	Soy-bean, *Glycine hispida* (335).
	Lentil, *Ervum lens* (331).
	Adzuki-bean, *Phaseolus radiatus* (329).
	Cow-pea, *Vigna sinensis* (327).
Phaselin found in the seeds of	Kidney-bean, *Phaseolus vulgaris* (303).
Ricin „ „ „	Castor-bean, *Ricinus communis* (365).

Small quantities of albumins are also found in most other seeds but have not been studied or given distinctive names.

(*b*) *Globulins.*

That seeds contain protein matter soluble in neutral saline solutions was first shown by Denis (88), and later confirmed by Hoppe-Seyler

(167), who found that such substances were extracted from vegetable tissues by solutions of sodium chloride. In 1877 Weyl (569, 570) examined a large number of seeds and found that all of them contained protein soluble in solutions of sodium chloride. He divided the globulin thus extracted into two groups, the vitellins, soluble in saturated solutions of sodium chloride, and the myosins, insoluble therein. Vines (561, 563) soon after investigated the action of salt solutions on the aleurone grains in different seeds and proposed a classification of these based on his observations. The criticism made by Weyl of the results which Ritthausen had obtained by extracting seeds with dilute alkalies led Ritthausen to apply extraction with sodium chloride solutions to a number of the seeds which he had previously studied, and he showed that most of the seed proteins were globulins. The experience of all who have since worked with the proteins of seeds has fully confirmed this conclusion.

Globulins, as here defined, are proteins insoluble in water but soluble in saline solutions. In this group must be included, for convenience, a large number of vegetable proteins which do not strictly conform to this definition. We have already discussed at some length the effect of combined acid on the solubility of many seed proteins, and have shown that a number of these have the properties of globulins only when combined with a small amount of acid, as a protein salt. When freed from this acid by complete neutralisation, these proteins are entirely soluble in water and in this condition lack the most essential properties of the globulins. As, by the current methods of preparation, these proteins are almost invariably obtained in the form of their salts, it is much more convenient to include them with the globulins, for it must be remembered that our present method of classification has been devised simply for the convenience of those who write and teach about these substances.

It has been shown that the solubility of edestin chloride depends not only on the proportion of combined acid, but also on the proportion of mineral salts contained in the solution. Those preparations which have an acidity to phenolphthalein greater than about 0·7 c.c. per gramme are soluble in water in proportion to their degree of acidity, while those containing an amount of acid equal to 1·4 c.c. of decinormal alkali are soluble therein. The aqueous solutions of the acid salts of edestin are precipitated by adding to them a small quantity of sodium chloride, or other inorganic salt, and the precipitate, thus produced, dissolves again when the quantity of added salt is sufficiently increased. Such acid compounds of edestin are therefore precipitated by dialysis

when the quantity of salt is so far reduced that the acid compound is no longer soluble therein and the quantity of salt remaining in the solution within the dialyser is sufficient to prevent its re-solution in water. Other so-called seed globulins behave in much the same way, so that, in considering the solubility of globulins in saline solutions, it is important to take accurate account of the quantity of acid present.

Saturation with sodium chloride was formerly made the basis for a division of the globulins into two groups, myosins and vitellins. This distinction has been applied extensively to the vegetable globulins, and is included, even to this day, in many of the text-books which deal with vegetable proteins. Experience has shown that such a distinction cannot well be made, inasmuch as many of the proteins which have been thus designated as myosins are, in fact, albumins, and some of those which have been designated vitellins are not soluble in saturated sodium chloride solutions. Thus, the so-called myosin in the seeds of the cereals consists entirely of the albumin leucosin, and the globulin of the castor-bean, which is partially precipitated by saturating its solution with sodium chloride, has been found to consist not of two proteins, one of which is soluble in saturated sodium chloride solution and the other insoluble therein, but of one protein which is less soluble in a saturated saline solution than in a more dilute one (326).

Palladin (368) stated that myosin, *i.e.*, the protein precipitated by saturation with sodium chloride, is a calcium salt of vitellin, and his assertion has been repeated in most of the accounts of vegetable proteins that have since appeared. The indirect evidence of this, which Palladin offers, affords no basis for such a conclusion. His further statement that a 10 per cent. sodium chloride solution of vitellin is not precipitated by mercuric chloride, while one made with a dilute salt solution gives with this mercury salt a precipitate which is soluble in a 10 per cent. sodium chloride solution, is easily explained by the dilution of the vitellin solution with the water in which the mercuric chloride is dissolved. The same quantity of water without the mercury salt will give the same precipitate, and if some sodium chloride is added to the mercuric chloride solution no precipitate results.

While the animal globulins are precipitated by saturating their solutions with magnesium sulphate, many of the vegetable globulins cannot be thus precipitated, but saturation with sodium sulphate, at 33°, precipitates all of them as yet thus tested. The vegetable globulins are precipitated by partial saturation with ammonium sulphate at very different degrees of saturation. Although many of them are precipitated by adding an equal volume of ammonium sulphate to their

solutions, many are not precipitated until their solutions are nearly saturated with this salt. The distinction, therefore, which is at present almost universally made between globulins and albumins, based on the complete precipitation of the former at half saturation of their solutions with ammonium sulphate, cannot be applied to the vegetable globulins.

The animal globulins are all coagulated by heating their solutions to various temperatures. Most of the seed globulins are only imperfectly coagulated by heating their solutions even to boiling, and some of them can be thus heated for a very long time without showing any apparent change.

A number of the vegetable globulins can be readily obtained in crystals; some of them crystallise with difficulty, and nearly all of those which do not crystallise are obtained in the form of minute spheroids. Crystallisation can be effected in various ways. Globulins which crystallise readily are usually precipitated by dialysis in well-formed crystals. Such are edestin from the hemp-seed, excelsin from the Brazil-nut, and the globulins of the squash-seed, the flax-seed, the oat-kernel and the castor-bean. The globulin of the castor-bean is commonly obtained by dialysis in the form of spheroids, but frequently mixtures of spheroids and crystals result, although sometimes completely crystalline separations occur. Phaseolin, from *Phaseolus vulgaris*, frequently precipitates on dialysis in the form of minute spheroids, mixed with a few octahedral crystals which sometimes are of unusual size and very perfect form, but completely crystalline preparations of this globulin have never yet been obtained. Crystals of such globulins as crystallise easily can generally be obtained by diluting their sodium chloride solutions with water heated to 50° or 60° until a slight turbidity forms. After warming the diluted solution until this turbidity disappears and then allowing it to cool slowly, the protein separates in well-developed crystals.

Schmiedeberg (457), who obtained crystals of the globulin from the Brazil-nut, by treating its solution with magnesia and evaporating it slowly, considered the crystals of this substance to be the magnesium salt of the protein. This view of the nature of the crystals of this globulin has been generally accepted, and is frequently found, in the current literature dealing with this protein, although Osborne (302), many years ago, showed that much more perfectly crystalline preparations of this globulin than are described by Schmiedeberg are obtained by simply dialysing its faintly acid saline solutions in running water. These crystals are unquestionably salts of the globulin with the acid

of the extract, and are not compounds with magnesia or any other base.

The following is a list of the principal globulins:—

Legumin (334), found in the seeds of	Pea, *Pisum sativum* (330). Horse-bean, *Vicia faba* (332). Vetch, *Vicia sativa* (333). Lentil, *Ervum lens* (331).
Vignin found in the seeds of	Cow-pea, *Vigna sinensis* (328).
Glycinin „ „ „	Soy-bean, *Glycine hispida* (335).
Phaseolin, Crystalline, found in the seeds of	Kidney-bean, *Phaseolus vulgaris* (303). Adzuki-bean, „ *radiatus* (329). Lima-bean? „ *lunatus*.
Conglutin found in the seeds of	Lupines, *Lupinus* (325, 356).
Vicilin found in the seeds of	Pea, *Pisum sativum* (330). Horse-bean, *Vicia faba* (332). Lentil, *Ervum lens* (331).
Corylin found in the seeds of	Hazel-nut, *Corylus avellena* (324).
Amandin found in the seeds of	Almond, *Prunus amygdalus* (324). Peach, „ *persica* (324). Plum? „ *domestica* (97). Apricot? „ *armeniaca* (97).
Juglansin found in the seeds of	European walnut, *Juglans regia* (324). American black walnut, *Juglans nigra* (353). „ butter-nut, *Juglans cinerea* (353).
Excelsin, Crystalline, found in the seeds of	Brazil-nut, *Bertholletia excelsa* (302, 570).
Edestin, „ „ „ „	Hemp-seed, *Cannabis sativa* (416, 302).
Avenalin, „ „ „ „	Oat, *Avena sativa* (299, 324).
Castanin, found in the seeds of	European chestnut, *Castanea vulgaris* (24).
Maysin, „ „ „	Maize, *Zea mays* (308).
Tuberin, „ „ tubers of	Potato, *Solanum tuberosum* (586, 322).

Globulins have also been isolated in considerable quantity from the following seeds and have been the subject of more or less study, but distinctive names have not yet been given to them.

Globulin, Crystalline, from	Flax-seed, *Linum usitatissimum* (301).
„ „ „	Squash-seed, *Cucurbita maxima* (143, 302).
„ „ „	Castor-bean, *Ricinus communis* (416, 302).
„ „ „	Cocoa-nut, *Cocos nucifera* (415, 189).
„ „ „	Sesame-seed, *Sesamum indicum* (416).
„ „	Cotton-seed, *Gossypium herbaceum* (367).
„ „	Sunflower-seed, *Helianthus annuus* (415, 327).
„ „	Candle-nut, *Aleurites triloba* (418).
„ „	Radish-seed, *Raphanus sativus* (418).
„ „	Pea-nut, *Arachis hypogea* (415).
„ „	Rape-seed, *Brassica campestris* (570).
„ „	Mustard-seed, *Brassica alba* (570).

Globulins which have not yet been named have also been isolated in small quantity from the seeds of wheat, *Triticum vulgare* (366, 88); rye, *Secale cereale* (304); barley, *Hordeum vulgare* (305); rice, *Oryza sativa* (439); and maize, *Zea mays* (72, 308, 570), and in larger amount from those of the oat, *Avena sativa* (299, 300, 570). In the wheat-kernel most, if not all, of the globulin is contained in the embryo (297, 336), and, from analogy, it is probable that most of it occurs in this part of the seeds of the other cereals except in those of oats, in which the quantity is relatively so great that it is probable that, in this seed, it forms a part of the reserve protein.

(*c*) *Glutelins.*

This group includes those proteins which are not dissolved by neutral aqueous solutions, by saline solutions, or by alcohol. The glutenin of wheat is the only well-characterised representative of this group which has yet been obtained. The seeds of other cereals apparently contain protein of similar character, but, owing to the difficulties encountered in extracting this protein, no preparations have been made which appeared to be at all definite. That such proteins exist in the seeds of other cereals is assumed from experience obtained in studying wheat. Wheat, rye and barley yield similar quantities of albumins and globulins which appear to be identical and also nearly the same quantity of protein soluble in alcohol. Protein matter can be extracted from rye and barley flour by treating the residue, from which the other proteins have been removed, with dilute alkaline solutions, but the preparations obtained are manifestly impure, and, owing to the difficulty of filtering the alkaline extracts, only very small quantities of any of them have ever been obtained. Since much nitrogen remains in the extracted residue, it is fair to presume that the greater part of it belongs to protein matter, as is the case with wheat. From a bye-product of maize starch manufacture, which is known as "gluten," a considerable quantity of protein can be extracted with alkaline solutions after the alcohol-soluble zein has been removed. In its products of hydrolysis this protein differs, both quantitatively and qualitatively, from zein, and preparations which have thus far been made are probably impure preparations of the glutenin of this seed, but these have not yet been sufficiently studied to justify definite statements. A similar protein has been described by Rosenheim and Kajiura (439) from rice under the name of oryzenin, which they state represents the greater part of the protein of this seed.

The residues of most seeds after extraction with neutral solvents usually contain a small quantity of nitrogen which may or may not belong to protein matter. It is probable that most of this nitrogen is protein nitrogen, as alkalies usually extract from such residues a small quantity of impure protein which may be either a protein of the properties of glutelin, or a portion of the proteins which failed to be extracted by neutral solvents, either because this was contained in unruptured cells which were afterwards destroyed by the alkaline solution or was retained in the meal residue in combination with other substances, such as nucleic acid or tannin, which rendered it insoluble in neutral solutions. Although it is possible that proteins

of the character of glutelins are widely distributed among the different seeds there is no conclusive evidence that this is in fact so.

The only well-defined glutelins are thus :—

Glutenin found in the seeds of Wheat, *Triticum vulgare* (366).
and
Oryzenin found in the seeds of Rice, *Oryza sativa* (439).

(*d*) *Prolamins.*

Alcohol-soluble proteins were among the first recognised in seeds, having been described as early as 1805 by Einhof as occurring in the seeds of rye (105) and barley (106). Taddei (537) found, in 1819, that a part of the gluten of wheat was soluble in alcohol, and Gorham (135) reported in 1821 a similar protein, which he called zein, in the seeds of maize. Kreusler (211) in 1869 found in the oat-kernel an alcohol-soluble protein, and the writer later found that the seeds of sorghum, *Andropogon sorghum* (not published), also contain a considerable quantity of such protein. Rosenheim and Kajiura (439) have found that the seeds of rice are free from any protein soluble in alcohol. Alcohol-soluble proteins have thus been found in the seeds of all the cereals that have been examined with the exception of rice, but have never been found in the seeds of any other family of plants.

The group of alcohol-soluble proteins deserves a definite name, for it is one of the best characterised groups yet found in either plants or animals. It has recently been proposed to call these proteins "gliadins," but as this name has been used to designate a definite protein obtained from wheat, a more distinctive name should be adopted. The writer has proposed (319) to call this group "prolamins," for all its members, which have thus far been hydrolysed, yield a relatively large quantity of both proline and amide nitrogen. The prolamins are characterised by their solubility in alcohol of from 70 to 90 per cent. They are nearly or wholly insoluble in water, but their salts with acids or alkalies dissolve freely therein. They yield much glutaminic acid, proline and ammonia, and, as Kossel and Kutscher (205) have shown, small amounts of arginine and histidine and no lysine.

The prolamin of wheat, *Triticum vulgare*, was named gliadin by Taddei (507). Ritthausen (409) concluded that the alcohol-soluble protein of wheat consisted of three distinct proteins, gliadin, mucedin and gluten-fibrin, but subsequent investigations have not supported this view (*cf.* 317). The prolamin of rye, *Secale vulgare*, is also known as gliadin, for no positive difference has yet been established between it and the gliadin of wheat (304, 347). The prolamin of maize was

named zein by Gorham (135) and maize fibrin by Ritthausen. Zein has been the subject of special study by Chittenden and Osborne (72), by Osborne (308), and by Osborne and Clapp (346). Hordein, which is the prolamin of barley, resembles gliadin in solubility [Osborne (305)] but differs distinctly in the proportion of the amino-acids which it yields on hydrolysis [Osborne and Clapp (342)].

The principal prolamins are therefore:—

Gliadin found in the seeds of	Wheat, *Triticum vulgare* (366).
	Rye, *Secale cereale* (304).
Hordein found in the seeds of	Barley, *Hordeum vulgare* (305).
Zein „ „ „	Maize, Zea mays (72, 308).

(*e*) *Albuminoids.*

No representatives have yet been found in plants of the remaining groups of simple proteins, namely, the albuminoids, histones and protamins. That many of the reserve proteins of seeds show relations to the albuminoids has been already pointed out, but the differences in their behaviour toward solvents is so great that none of them can properly be considered to belong to this group.

(*f*) *Histones.*

The large amount of basic amino-acids which many of the seed proteins yield is similar to that which is considered to be characteristic of the histones, and the reactions of edestan (*cf.* p. 41) are similar to the reactions of the histones. Whether any real relation exists between histones and seed proteins high in base, or whether the similarity alluded to is merely accidental, must be the subject of further study. It is possible that the proteins commonly included among histones do not, in fact, differ so widely from other simple proteins as has been generally supposed.

(*g*) *Protamines.*

No substances in any way similar to the protamines have ever been found in plants, and there is no reason to expect to find them among the reserve proteins of seeds. It is possible that such substances occur in pollen grains, but neither the early investigations by Fourcroy (123), John (180) and Braconnot (55), nor the later ones by v. Planta (379*a*) of hazel pollen, and by Kammann (183) of rye pollen, have given evidence of their presence. These investigators, however, made no attempts to discover protamine in pollen and its presence is by no means excluded by their work.

II. Conjugated Proteins.

(*a*) *Nucleoproteins.*

The nucleoproteins deserve especial attention because they are among the most important constituents in the cells of animals and plants. They were first described as existing in vegetable cells by Hoppe-Seyler (169), who obtained a preparation from yeast which was very similar to those then recently obtained by Miescher from animal sources. Kossel (201, 202) next investigated this substance and found that the phosphorus content of different preparations varied widely, but that most of them contained about 3·5 per cent. of phosphorus, which fact he regarded as evidence of a more stable combination with this proportion of phosphorus. He also found hypoxanthin among its decomposition products, but as he later obtained adenin from yeast nuclein he considered this to be the mother substance from which the hypoxanthin had originated. Liebermann (220) in 1890 obtained reactions with yeast nuclein which, as he thought, showed the presence of metaphosphoric acid in this substance.

Altmann (587), in 1889, discovered nucleins to be compounds containing both nucleic acid and protein. It is difficult to determine just what views are now held concerning the character of the union of the nucleic acid with the protein, but, from what appears in the literature of this subject, most writers evidently consider nuclein to be something other than a protein nucleate. The available evidence in regard to the nature of this union is very scanty and practically all that is definite relates to the formation of protein nucleates. It is not impossible that other forms of union may exist, but the conditions under which nucleoproteins and nucleins are at present obtained make it extremely difficult, if not impossible, to prove the existence of an organic combination between the nucleic acid and protein. The methods thus far employed in isolating these substances depend on processes which would give a salt of protein and nucleic acid if these two substances were present in the solution. No hydrolytic splitting of the nuclein thus obtained appears to be necessary to set its component parts free, unless hydrolysis is effected with extraordinary ease and with great rapidity. Furthermore, the experiments of Milroy (600) and Löbisch (598) show that artificial mixtures of phosphorus-free protein and free nucleic acid yield products which have the properties usually considered to be characteristic of the nucleins, although from the conditions of their production these artificial compounds could have been nothing other than protein nucleates.

Such preparations of nucleoproteins as have been thus far obtained from vegetable sources can, in the writer's opinion, be considered only as protein nucleates; and they in no sense represent actual constituents of the vegetable cells, although it is not at all impossible that similar products *may* exist there.

The only extensive study of the character of the "nucleoproteins" obtained from plants which has been made since the true character of the nucleic acids has been established and the basic properties of the proteins recognised, was that of Osborne and Campbell (336), who obtained a large number of products from the wheat embryo, which consisted of protein combined with very different proportions of nucleic acid. The aqueous extract of the wheat embryo contains a large proportion of nucleic acid which has been isolated in quantity and its composition and properties determined by Osborne and Harris (602).

The freshly prepared aqueous extract of the embryo-meal is neutral to litmus, alkaline to lacmoid and decidedly acid to phenolphthalein. If heated at once in a water bath to 98° no coagulation occurs unless a very little acid is previously added. On standing at the room temperature for a few hours, protected with thymol, the extract gradually becomes acid, and, if then heated, a large coagulum forms between 50° and 55°.

In consequence of this continuous development of acid in the extracts, the conditions which determine the proportion in which bases and acids can combine are constantly changing, and, as the proteins are polyacid bases and nucleic acids are polybasic acids, the salts which are formed under the various existing conditions may be very various. We must therefore expect to find the combinations of protein and nucleic acid which are separated from the extracts of the embryo-meal under different conditions to contain very different proportions of these two substances; in fact just such products as Osborne and Campbell (336) obtained, for details of the preparation of which the original paper must be consulted.

The ultimate composition of these products is shown in the four following tables. The first table shows the ultimate composition of different preparations obtained from the aqueous extract of the wheat embryo, many of which might be called nucleoproteins. The second table shows the composition of the protein part of these preparations after deducting ash and nucleic acid, the amount of the latter being calculated from the phosphorus content of the preparation. It also gives the average composition of the protein part of all the preparations, and, for com-

parison, the composition of leucosin, the phosphorus-free albumin obtained from the entire seed.

Composition of Preparations Extracted by Water from the Wheat Embryo.

	1.	2.	3.	4.	5.	6.	7.	8.	9.
Carbon	51·13	50·52	50·17	52·39	51·77	52·13	52·73	43·59	52·28
Hydrogen	6·85	6·81	7·01	6·83	6·81	7·04	7·11	5·77	6·97
Nitrogen	16·28	16·47	16·66	16·20	16·11	16·48	16·00	15·16	16·38
Sulphur	1·18	1·17	1·00	1·32	1·30	1·49	1·53	0·90	1·39
Phosphorus	0·72	0·97	0·91	trace	0·17	0·06	none	3·38	0·07
Ash	2·73	2·90	3·03	0·35	1·39	0·43	0·39	13·04	0·44
P_2O_5 in Ash	1·88	2·09	1·91	trace	0·47	trace	none	6·73	trace

	10.	11.	12.	13.	14.	15.	16.	17.	18.
Carbon	51·21	46·67	51·87	—	51·95	51·65	—	52·02	49·59
Hydrogen	6·85	6·19	6·89	—	6·86	6·66	—	7·00	6·68
Nitrogen	16·18	15·89	16·65	16·31	16·08	16·02	16·09	16·45	16·34
Sulphur	1·10	0·93	1·19	1·35	1·60	1·13	1·12	1·24	0·91
Phosphorus	0·46	2·53	trace	trace	trace	trace	trace	none	1·85
Ash	2·19	8·17	0·38	0·45	0·32	1·09	2·83	0·56	2·50
P_2O_5 in Ash	1·11	5·71	trace	trace	trace	trace	trace	none	1·79

Composition of Leucosin Contained in the above Preparations from Water Extracts of the Wheat Embryo.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Carbon	52·93	52·75	52·41	52·57	52·57	52·47	52·93	53·23	52·64	52·63
Hydrogen	7·12	7·16	7·38	6·85	6·91	7·08	7·13	7·09	7·02	7·06
Nitrogen	16·45	16·68	16·94	16·26	16·27	16·55	16·06	16·30	16·46	16·40
Sulphur	1·29	1·32	1·13	1·32	1·34	1·50	1·53	1·60	1·41	1·17
Oxygen	22·21	22·09	22·14	23·00	22·91	22·40	22·35	21·78	22·47	22·74
	100·00	100·00	100·00	100·00	100·00	100·00	100·00	100·00	100·00	100·00

	11.	12.	13.	14.	15.	16.	17.	18.	Average of preparations.	Leucosin.
Carbon	52·44	52·06	—	52·11	52·16	—	52·30	53·45	52·54	53·02
Hydrogen	7·10	6·92	—	6·88	6·73	—	7·04	7·30	7·04	6·84
Nitrogen	16·26	16·71	16·38	16·13	16·20	16·56	16·54	16·57	16·43	16·80
Sulphur	1·34	1·19	1·35	1·60	1·14	1·15	1·24	1·16	1·26	1·28
Oxygen	22·86	23·12	—	23·28	23·77	—	22·88	21·52	22·73	22·06
	100·00	100·00		100·00	100·00		100·00	100·00	100·00	100·00

Of these preparations 1, 2, 3, 4, 13, 14, 15, 16 and 17 were obtained, under various conditions, by coagulation by heat, 5 and 10 by coagulation with alcohol, 8 and 11 by saturation with sodium chloride, 6, 7,

9 and 12 by dialysing sodium chloride solutions into water and 18 by direct dialysis of the aqueous extract.

Considering the conditions under which these preparations were obtained and the impossibility of accurately determining their true ash content, the agreement between the different preparations in respect to the ultimate composition of their protein constituents is very striking. This protein constituent also agrees closely in composition with the phosphorus-free albumin, leucosin, obtained by extracting the entire seed with water, and also agrees with leucosin in many of its properties, especially its deportment on heating. It is thus highly probable that they are one and the same substance.

When the embryo-meal was extracted with sodium chloride solution heated to 70°, whereby most of the water-soluble constituents are coagulated, extracts were obtained which yielded globulin. Many preparations of this globulin, made under various conditions, contained different proportions of phosphorus. The composition of the protein part of these preparations was wholly different from that of the preparations obtained from the aqueous extract, and agreed closely with that of the globulin obtained from the entire seed, as shown in the second of the two following tables:—

Composition of Preparations Extracted by Sodium Chloride Solutions from the Wheat Embryo.

	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.
Carbon . . .	—	—	48·77	50·03	50·23	48·17	49·39	48·75	49·79	48·67
Hydrogen . .	—	—	6·44	7·04	6·89	6·54	6·78	6·52	6·76	6·56
Nitrogen . . .	18·14	18·21	18·12	18·39	18·23	18·06	17·95	18·16	18·01	17·97
Sulphur . . .	0·49	0·56	0·51	0·60	0·53	0·55	0·48	0·63	0·61	0·61
Phosphorus . .	1·15	1·03	1·35	0·76	0·56	1·41	1·17	1·41	1·11	1·55
Ash	2·29	1·86	2·25	1·30	1·22	3·85	2·60	2·66	1·11	2·94
P_2O_5 in Ash . .	1·66	1·34	1·68	0·84	0·80	2·00	1·82	2·00	0·68	2·30

Composition of the Globulin Contained in the Preparations Extracted from the Wheat Embryo by Sodium Chloride Solution.

	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	Average.	Globulin Wheat.
Carbon . .	—	—	51·37	51·58	51·40	51·56	51·86	51·40	51·98	51·70	51·60	51·03
Hydrogen .	—	—	6·83	7·31	7·08	7·07	7·19	6·94	7·12	7·05	7·07	6·85
Nitrogen .	18·59	18·59	18·62	18·70	18·45	18·85	18·41	18·71	18·37	18·53	18·58	18·39
Sulphur .	0·57	0·63	0·60	0·66	0·57	0·67	0·55	0·75	0·70	0·75	0·65	0·65
Oxygen .	—	—	22·58	21·75	22·50	21·85	21·99	22·20	21·83	21·97	22·10	23·08
			100·00	100·00	100·00	100·00	100·00	100·00	100·00	100·00	100·00	100·00

We thus have well-defined cases where the nucleic acid separates in combination with two distinctly different proteins. In each case the protein exerts a controlling influence on the general properties of the compound formed, those compounds containing the albumin, leucosin, having in the main the properties of albumin, while those containing the globulin have the properties of this group of simple proteins. In this respect the compounds of protein with nucleic acid behave much like the salts of the simple proteins with strong mineral acids, *e.g.*, hydrochloric and sulphuric, as already described on page 22.

Since the proteins are polyacid bases and the nucleic acids are polybasic acids, the number of different salts which can be formed is exceedingly large, and we may well expect to obtain from solutions, in which these two substances occur, preparations containing every possible proportion of phosphorus, in other words, just such products as have been actually obtained.

This must, however, not be understood to mean that definite organic combinations between proteins and nucleic acids do not exist in nature, but simply that, so far as the writer's experience has gone, no evidence has yet been obtained which indicates that any of the preparations of so-called nucleoproteins from plants are anything else than protein nucleates.

From what has just been said it is clear that definite statements based on a study of isolated products cannot yet be made concerning the occurrence of nucleoproteins in plants. Concerning the evidence of the existence of such compounds which is furnished by microscopic observations of stained tissues, nothing will here be said, for this lies outside of the scope of this monograph as well as of the personal experience of the writer.

From such knowledge as we now possess it is evident that, at the most, only small quantities of nucleoprotein occur in the entire seed, and that this will be found chiefly in the tissues of the embryo in which the nuclei of the cells are far more abundant than in the tissues of the endosperm. Nucleoproteins have been described as constituents of fungi and bacteria, but no critical study of any of these has yet been made (*cf.* 287, 169, 201, 202, 203, 198, 529, 549, 220, 137, 254, 215, 129, 582, 443, 17, 172, 544).

(*b*) *Glycoproteins.*

Little that is definite can be said concerning the occurrence of glycoproteins in plants. It is certain that many of the known seed

proteins do not belong to this group, for they give no Molisch reaction and therefore contain no carbohydrate. Krakow (210) obtained an osazone from "pea albumin," but in the absence of conclusive evidence that the preparation of this "albumin" was free from admixed carbohydrate, little importance attaches to this observation. Ishii (171) has described a substance obtained from the tubers of yams which had physical properties and an ultimate composition similar to the mucins of animal origin. As he makes no statements concerning the presence of carbohydrate in his preparations its relations to the true mucins are yet to be demonstrated. Wróbleski (582) has stated that mucin is one of the constituents of yeast, but gives no experimental evidence of this. Several observations are on record indicating that many bacteria produce a mucin-like substance in the culture medium in which they grow, but such substances are hardly to be considered as vegetable proteins.

(*c*) *Phosphoproteins.*

It seems to be believed by many writers on vegetable proteins that a large number of the proteins of seeds contain phosphorus and should be consequently assigned to the group of phosphoproteins [*cf.* Wiman (573)]. The fact that the reserve food protein of the egg yolk consists largely of such a phosphorised globulin-like protein, and that the protein of milk consists chiefly of the phosphorised casein belonging to this group, has apparently led many to assume, by analogy, that a large part of the reserve food protein of seeds consists of similar substances (*cf.* Czapek, 82*a*, p. 59).

Hoppe-Seyler (167) suggests the presence of protein substances in the Brazil-nut and pea which may be similar to the vitellin of the yolk of hens' eggs, but he bases this suggestion solely on the fact that he had extracted a lecithin-like substance from crude preparations obtained from these seeds. He says nothing of the presence of phosphorus in the protein which remained after extracting with warm alcohol, and consequently gives no evidence which shows these to be vitellin-like proteins.

In discussing the relations of edestin to acids it was shown that the preparations of crude edestin which were obtained by a single precipitation from the extract of the seeds contained a small amount of phosphorus, but that this disappeared completely after a second precipitation by dialysis or by dilution. The *crude* preparations of most seed proteins, like those of edestin, contain traces of phosphorus, but, when

sufficiently purified, by repeated precipitation, they are obtained wholly free from this substance. A careful examination of well-purified preparations of nearly all the known seed proteins has shown that these can easily be obtained by reprecipitation entirely free from phosphorus, and there is no ground whatever for believing phosphorus to be a constituent of their molecules. The vitellin of the egg yolk under these conditions retains its phosphorus completely and in this respect differs in a marked degree from edestin. No evidence has as yet been obtained that phosphoproteins occur in plants, and in view of what is now known it seems probable that these occur in very small quantity if they occur at all.

(*d*) *Hæmoglobins.*

Whether proteins which resemble hæmoglobins are to be found in plants is still an open question, although the coloured crystals of phycoerythrin and phycocyan which Molisch (277, 278) obtained from some of the sea algæ appear to be similar in many respects to hæmoglobin.

(*e*) *Lecithoproteins.*

Lecithoproteins have not been isolated from plants, and satisfactory evidence of their existence has not yet been brought forward. Schulze and Likiernik (496) and Schulze and Winterstein (506) assume the presence of lecithalbumin in seeds from the fact that a part of the lecithin always remains undissolved when the powdered seeds are extracted with ether.

Hoppe-Seyler (167), as just stated on page 87, obtained a lecithin-like substance from crude preparations of the proteins of the Brazil-nut and pea, but his brief statement is not sufficient to warrant the conclusion that this lecithin was anything other than a contamination of the preparations which he examined.

III. Derived Proteins.

1. Primary protein derivatives.

The members of the various groups of derived proteins are all represented by corresponding products obtained from vegetable proteins. As these have been discussed in Chapter VII. in connection with the denaturing of these proteins they require no further mention here.

2. SECONDARY PROTEIN DERIVATIVES.

(*a*) *Proteoses.*

The first observation which indicated the presence of proteoses in seeds was made in 1879 by Vines (561, 562), who obtained a proteose-like product from lupine-seeds which he called "hemialbumose". He also noted the presence of a similar substance in the seeds of vetch, hemp and flax. Schulze and Barbieri (485) soon afterwards examined a number of different parts of many kinds of plants, and concluded from the results which they obtained that plant juices and extracts often contain "peptone" (proteose?) in small quantity. Even during germination they found but a small quantity of "peptone," and stated that a storing up of this substance does not occur. They confirmed an earlier observation of Kern (186), made on lucerne and vetch hay, that plants contain ferments which peptonise protein during extraction. Proteoses have been frequently found in the extracts of seeds after the other proteins had been removed. Whether these proteoses were original constituents of the seeds or resulted from the action of proteolytic enzymes is still an open question, for it is extremely difficult to conduct the extraction of the seed and separation of the other proteins in such a way as to certainly exclude the formation of proteoses. That changes occur in seed extracts is shown by many facts already recorded. Osborne (301) found that the extract of the flax-seed yielded a nearly constant quantity of diffusible non-protein nitrogen during several days' dialysis. The fact that the nitrogen diffused during the first dialysis period was only about one-half of that diffused during the subsequent periods, which were equally long, is evidence that the diffusible nitrogen did not exist as such in the seed but was formed from some other substance, presumably protein. Experiments recently made by the writer have also shown that more non-protein nitrogen is found in the diffusate from extracts of the seeds of *Phaseolus* made at 20° than from extracts made with solvents heated to 70°.

That the proteins in many extracts undergo changes in the process of purification is shown by the constant loss of material which is so frequently met with. A striking instance of this was encountered by Osborne and Harris (356), who subjected 600 grammes of crude conglutin from the yellow lupine to fractionation with ammonium sulphate and finally separated the different fractions by dialysis. Although the mechanical losses during these operations were small, only 314 grammes

of conglutin were recovered. According to Mack (250, 251) the seeds of the yellow lupine contain an enzyme which at neutral or acid reaction attacks the protein, and it is probable that the large loss of conglutin was due to the action of this enzyme.

As the formation of proteose would almost certainly accompany the formation of diffusible products which do not give protein reactions, the proteoses found in the extracts cannot be considered to be original constituents of the seeds until more convincing evidence is obtained than any as yet given.

(*b*) *Peptones.*

The facts which lead to the uncertainties respecting the pre-existence of proteoses in seeds apply also to the peptones. The most elaborate investigation of this subject has been made by Mack (251), who obtained peptone from lupine-seeds under conditions which he thought entirely excluded its formation by enzyme action. He worked with very large quantities of seeds and employed Siegfried's method of isolating and purifying peptones by means of ferric-ammonium alum. The several products obtained had a nearly constant composition corresponding to the formula $C_{32}H_{56}N_8O_6$, while the barium salt contained an amount of barium in close accord with the calculated. When hydrolysed with hydrochloric acid this peptone yielded lysine, arginine and glutaminic acid.

By artificially digesting vegetable proteins with pepsin or trypsin, proteoses and peptones have been obtained by Chittenden (68), Chittenden and Hartwell (69, 70), Chittenden and Mendel (71), and Chittenden and Smith (73).

(*c*) *Peptides.*

Only two well-characterised peptides have thus far been obtained from seed proteins. The first of these was a dipeptide of proline and phenylalanine, which Osborne and Clapp (339) isolated from the decomposition products of gliadin which had been decomposed by boiling with 25 per cent. sulphuric acid for many hours. This peptide was obtained in beautiful mother-of-pearl crystals of definite form, and yielded a copper salt, the crystals of which were so large and well formed that the measurement of their angles served to definitely characterise the substance. By hydrolysis in a sealed tube with strong hydrochloric acid it yielded proline and phenylalanine in molecular

proportions. The identity of this peptide with a synthetic polypeptide composed of these amino-acids has not been established, as no peptide of proline and phenylalanine has yet been prepared.

Fischer and Abderhalden (112) soon after obtained l-leucyl-d-glutaminic acid from gliadin by partial hydrolysis at the room temperature with 70 per cent. sulphuric acid for sixteen hours and then for three days in an incubator. This peptide agreed in properties with the synthetic product.

CHAPTER XI.

SOME PHYSIOLOGICAL RELATIONS OF VEGETABLE PROTEINS TO THE ANIMAL ORGANISM AND THE BIOLOGICAL RELATIONS OF SEED PROTEINS TO ONE ANOTHER.

A. Toxalbumins.

THE existence of toxic substances in the seeds of *Ricinus* and *Abrus* was long ago recognised, but the first attempt to isolate the toxin was made in 1884 by Warden and Waddell (566), who obtained from the seeds of *Abrus precatorius* a substance which they considered to be a protein, and to which they gave the name abrin.

In 1887 Dixson (92) obtained, by neutralising a hydrochloric acid extract of the seeds of *Ricinus communis* with sodium carbonate, a precipitate which contained the toxic substance of this seed, and he made extensive experiments with a view to purifying the product thus prepared. His final preparation, which was very toxic, contained much protein.

In 1887 Sidney Martin (263) found that the protein substance forming the abrin of Warden and Waddell consisted of two proteins, one of which was a globulin coagulating at 75° to 80° and precipitated by saturating its solutions with sodium chloride or magnesium sulphate, and the other an albumose.

Stillmark (525) in 1888 ascribed the toxic action of *Ricinus* seeds to a protein which he obtained by precipitating a sodium chloride extract of the seed with magnesium sulphate or sodium sulphate, dissolving the precipitate in water and dialysing away the salts. Stillmark named this substance ricin and considered it to be either a globulin or a ferment. He also found that, in addition to its toxic properties, it caused agglutination of a suspension of red blood corpuscles.

Martin and Wolfenden (266) in 1889 described the physiological activity of the globulin of *Abrus*, and at the same time Martin (265, 264) studied the toxic action of the albumose which he separated from the globulin by coagulating the latter by long digestion with alcohol.

Ehrlich (102) made the important discovery, in 1891, that animals

could be immunised against ricin and abrin and that an animal immunised against abrin is still sensitive to ricin, thus showing the two substances to be different.

Ehrlich's discovery was soon followed by numerous investigations of the physiological effects of these toxalbumins which were then recognised to have close relations to the bacterial poisons [*cf.* Schaer (449), Tichmiroff (545), Werhovsky (568), Stepanoff (523), Ehrlich (103), Heuseval (159), Kobert (199), Lau (216), Roemer (435), Hausmann (151), Jacoby (174), Kraus (209), Rehns (390, 391), Braun and Behren (58), Brieger (59), Arrhenius (16), Fraenkel (124), Jacoby (175), Jodlbaur (178), Pascucci (372), Woronzow (580), Sachs (444), Cornevin (79)].

Siegel (516) discovered and described in 1893 a toxic substance from the seeds of *Jatropha curcas* which he named curcin.

Elfstrand (109) discovered in 1897 another toxic substance in the seeds of *Crotin eluteria* which appeared to be of protein nature and to which he gave the name crotin. This substance agglutinated the blood corpuscles of certain animals and was toxic. The preparation contained both albumin and globulin and Elfstrand supposed that both were toxic substances. The toxicity was destroyed by heating their solution to 70° and also by pepsin digestion.

In 1898 Cushny (82) undertook an extensive study of various methods for isolating ricin and obtained preparations of much greater toxicity than those before described. All his attempts to separate a toxic substance from the protein preparations failed, and he states that when the preparation contained protein it was toxic, but when the protein was removed it was not. He ascribed the failure of his predecessors to detect protein reactions in solutions which were highly toxic to the fact that the ricin is fatal in such extremely small amounts that such solutions were too dilute to give a protein reaction. He concludes that ricin is either a protein or is so intimately associated or combined with the protein that it could not be separated by any of the means then available. As evidence of its protein nature he found that it was precipitated by saturating its solutions with ammonium sulphate, was coagulated by heating its solutions and thereby rendered non-toxic, and that the toxicity of the solutions decreased as the protein was removed from them. He further found that saturation with magnesium sulphate removed all of the ricin from its solution, and that the albumose which was not thus precipitated had no toxic or agglutinating properties.

In 1901 Power (381) discovered and described a somewhat similar

toxin in the bark of *Robinia pseudacacia*. This substance he considered to be a nucleoprotein and he named it robin.

Dunbar (99, 100) in 1903 ascribed the effect which extracts of rye pollen had on hay-fever patients to a toxic protein contained in the pollen. In 1904 Kammann described the proteins obtained from rye pollen and stated that one was a toxalbumin which when administered in extremely small doses produced the symptoms of hay fever in patients afflicted with this disease. Prausnitz (382) described in 1905 several other toxic proteins related to the hay-fever toxins.

Osborne, Mendel and Harris (365) in 1905 made an extensive study of the proteins of *Ricinus* with special reference to the isolation of ricin. They found that the globulin, which constitutes the greater part of the protein of this seed, and which can be precipitated by dialysing the sodium chloride extracts, is entirely free from toxic properties, and that from the solution from which this globulin had been separated by fractional precipitation with ammonium sulphate the ricin was separated between narrow limits of concentration in this salt.

Most investigators of this subject have been impressed with the idea that the toxic properties belonged in fact to the protein substance. That the protein substance should have such properties is, however, extremely difficult to believe, and doubt has been frequently raised as to the real nature of these toxins. The principal experimental evidence which has been employed to solve this question has involved the effect of proteolytic enzymes on the toxic preparations, it being assumed that, if the toxin were a protein, digestion with proteolytic enzymes would destroy its physiological activity. As long ago as 1889, Stillmark (526) showed that in one experiment the toxic power of his ricin was not destroyed by digesting with trypsin for eighteen hours. In 1895 Repin (395) stated that abrin was not rendered inactive by pancreatic digestion. Müller (280) in 1899 subjected ricin to the action of very active trypsin, and found that after twenty-four hours it was quantitatively as active as at first. He concluded from his experiment that ricin was not a protein, though he stated that the possibility was not excluded that toxic proteoses might have been formed. Jacoby (173) showed in 1901 that ricin was precipitated between one-tenth and six-tenths saturation with ammonium sulphate, and he digested a precipitate obtained under such conditions for five weeks with active pancreatic juice, and reported that, although the protein reactions had vanished, the precipitate which he obtained from the digested solution, by six-tenths saturation with ammonium sulphate, contained as much toxin as the original preparation of ricin. The experiments of

Dzierzgowski and Sieber-Schoumoff (101) in 1901 indicated a great resistance of abrin to the digestive enzymes. Hausmann (151) in 1902 found that the toxic and agglutinating properties of abrin were not impaired by trypsin digestion. Sieber and Schumoff-Simonowski (515) showed that neither erepsin nor intestinal juice destroyed the toxicity of abrin, nor did digestion with trypsin followed by that with intestinal juice. The slight weakening of the toxicity which they observed they attributed to the long exposure to the relatively high temperature employed in the digestion. Rochat (432) stated that the agglutinating power of ricin was destroyed by digesting with gastric juice prepared according to Pekelharing's method and that trypsin did not have so strong an action on the ricin. Osborne, Mendel and Harris (365) found that the agglutinating power of their preparations of ricin was destroyed or diminished by prolonged digestion with trypsin and that the toxicity was also either destroyed or greatly diminished.

The evidence thus recorded apparently strongly favours the view that these toxalbumins are not destroyed by proteolytic enzymes, but an examination of the details of the experiments which have been tried leaves one much in doubt as to the weight which should be attached to them. The earlier experiments with ricin were all made with crude preparations which have since been shown to contain but little toxine, and were made at a time when the enormous toxicity of this substance was not appreciated. It is therefore almost certain that the relatively short periods of digestion employed in most of these experiments would leave much ricin unattacked. The same is true of experiments with abrin.

It is evident from all the experiments which have been made that ricin is not easily altered by proteolytic enzymes. Assuming, however, that it is not thus altered it would even then be questionable whether or not this could be considered as good evidence of its non-protein nature, for proteolytic enzymes act very differently on different forms of protein. Thus erepsin easily hydrolyses the primary proteoses and casein, but has no action on other native proteins. Trypsin digests many of the proteoses with the formation of free amino-acids, but a part of the polypeptide-like products which result from its action on the native protein resists its further action. It also readily digests many of the synthetic polypeptides, but is without action upon others. That there should exist in nature a form of protein which cannot be hydrolysed by proteolytic enzymes would not be surprising, and the fact that these toxalbumins produce such remarkable and powerful physiological effects upon animals would indicate that they differ in

some pronounced manner from other known proteins. All the other facts which we know in regard to these toxalbumins speak strongly in favour of their protein nature, and in view of the experiments of Osborne, Mendel and Harris (365) it is difficult to believe that they should not be classed with the other protein substances.

These experiments were directed to a separation of all the different proteins of the seed of *Ricinus*, and resulted in showing that the toxic properties are associated only with those proteins which are soluble in water and coagulable by heat. By dialysing the sodium chloride extract of the seed the greater part of the protein separates as a globulin which is free from toxic properties. The aqueous solution from which this globulin separates, when fractionally precipitated with ammonium sulphate, yields the toxic substance sharply concentrated in fractions obtained within comparatively narrow limits of concentration in this salt. The toxicity of these fractions is closely proportional to the amount of coagulable albumin which they contain, and, by further fractionation with ammonium sulphate, preparations result which consist largely of albumin. The most toxic of these, as yet obtained, and at the same time the richest in albumin, consisted of a mixture of over 70 per cent. of albumin with nearly 30 per cent. of proteose. The toxicity of this preparation was far in excess of any preparation of ricin before described, for ·0005 of a milligramme per kilo of body weight was fatal to rabbits when subcutaneously injected. An examination of the properties, ultimate composition, partition of nitrogen and colour reactions showed this preparation to have in all respects the properties characteristic of pure protein, and no evidence whatever was obtained which would indicate that the preparation contained more than the most insignificant quantity of any other substance. The conclusion appears, therefore, to be justified that the toxic property belongs to the protein, and in view of the close relation found for the degree of toxicity to the proportion of coagulable albumin contained in various fractions of the *Ricinus* protein, it seems almost certain that this latter protein has the toxic power and that true toxalbumins occur in seeds. Similar studies of the other toxalbumins have not been made, and therefore definite conclusions in regard to the actual existence of a toxalbumin in the seeds from which these are obtained are at present not possible, but the facts available strongly indicate that these seeds also contain a toxalbumin similar to ricin.

B. Anaphalaxis.

The toxicity of ricin indicates that there are some protein substances which, when introduced into the animal, cause profound physiological changes resulting ultimately in death. It has recently been discovered that under certain conditions many, if not all, protein substances produce fatal effects when injected into animals, even in small quantities. It has been shown that solutions containing protein, such as blood serum, white of egg, or milk, when injected into an animal, even in large quantities, produce no apparent effect, but if, after ten or twelve days or more, a second injection of a protein solution from the same source is injected, even in very small quantity, the animal quickly dies. The quantity of protein solution required for the first, or sensitising, dose in order to produce a fatal effect when followed at a proper interval by the second dose, is excessively small, smaller even than that required by ricin to produce a fatal effect with a single dose. The quantity required for the fatal second dose is somewhat larger, but still astonishingly small. The toxic effect of these protein solutions appears to be specific, since the serum of a horse sensitises the animal to a second dose of horse serum only, for if the second dose is serum of a rabbit, or a cow, or any other animal, no apparent effect is produced. Whether or not this reaction is specific for individual protein substances has not yet been definitely determined, but the scanty evidence now on record indicates that this is probably the case. Little attention has yet been directed to this reaction with vegetable proteins. The only experiments thus far recorded being those of Rosenau and Anderson (438), who attempted to use edestin for this purpose but failed, owing to the fact that they used this protein dissolved in strong sodium chloride solution, and those of Wells (567), who tried experiments with gliadin and zein, and found that zein showed this reaction to a high degree, while the preparation of gliadin which he used failed to give similar results.

C. Hæmagglutination by Seed Proteins.

Landsteiner and Raubitschek (213) and Mendel (271) have recently shown that the extracts of several seeds when added to a suspension of blood corpuscles rapidly caused them to agglutinate, and also that the agglutinating power of the extracts of various seeds differed greatly and was wholly lacking in some. Whether this effect is to be ascribed to the protein constituents of these extracts remains to be demonstrated,

but, in view of the physiological relations of the toxalbumins and the apparent sensitising power of proteins in the anaphalaxis reaction, it is not improbable that the proteins in these extracts will be ultimately found to be the agglutinating agent.

D. The Precipitine Reaction.

The production of precipitines by means of vegetable proteins has been described by Kowarski (207), Jacoby (174), Schütze (461), Bertarelli (30), Osborne, Mendel and Harris (365), Gasis (131) and Relander (394). No special study appears to have been made as to the degree of specificity of the reaction obtained with different vegetable proteins, the preparations used by those who mention this feature of the reaction having consisted of crude products or of vegetable extracts. Obermayer and Pick (296) have made the interesting observation that by iodising proteins the precipitine reaction is lost for the specific protein employed and that the iodised protein produces a precipitine for all other iodised proteins whatever their source, those of animal origin producing reactions with iodised proteins of even vegetable origin.

E. Biological Relations of Seed Proteins.

If the chemical and physical properties of the different proteins obtained from seeds are compared with one another, it will be noticed that they show many relations which are in harmony with the recognised botanical relations of the seeds from which they were obtained. The most marked instance of this agreement is shown by the protein constituents of the seeds of cereals. These contain a relatively large proportion of prolamins, which, as we have already stated, are characterised by yielding no lysine and much proline, as well as much glutaminic acid and ammonia and also by yielding relatively little arginine and histidine. The physical properties and general behaviour of all these proteins are much alike and present marked differences from the proteins obtained from other groups of seeds. The proteins from leguminous seeds resemble one another in many respects, but differ noticeably from those of the cereals. The proteins of the pea, horse-bean, lentil and vetch yield preparations of legumin, which have thus far been found to be so nearly alike that no certain distinction can be made between them. The proteins of these seeds, while in the main resembling those from *Phaseolus*, are not the same, for distinct but slight differences in properties, composition and products of hydrolysis have been estab-

lished between them. The proteins of the cow-pea and soy-bean, while closely resembling, in many respects, the proteins of the legumes just mentioned, are not exactly the same, while the proteins from different species of *Lupinus* present common characteristics but differ from the proteins of the other leguminous seeds. The proteins of *Lupinus*, however, resemble proteins of other leguminous seeds more closely than those found in non-leguminous seeds. The proteins of different species of *Juglans* appear to be identical so far as they have been examined, but differ in one respect or another from the proteins of other plants. We thus find similar proteins only in seeds which are botanically closely related, and it would seem that these differences in the reserve food substances of the seeds must have an important bearing on the development of the embryo which derives its first food from them. This food substance, as well as the tissues of the embryo itself, are the final products of the metabolism of the plant which produced them. When the embryo first develops it is supplied with a definite food which for each individual of the same species is the same, but for those of different species is different. Each member of a species thus begins its individual life under similar chemical conditions, but under chemical conditions which are different from those of every other species. It seems probable, therefore, that when the plant has reached a stage of development at which its organs of assimilation are able to furnish it with nutriment from its external surroundings, its chemical processes have already been established along definite lines which it must follow throughout the rest of its life.

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