Chapter 17

Peanut Proteins

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In this chapter, the authors have attempted to cover most contributions on the purification and characterization of peanut proteins. The first part gives a brief introduction to the functions and chemistry of proteins. For further information, the reader is referred to a number of excellent treatises published earlier: Neurath (1963), Haurowitz (1963), and Schultz and Anglemier (1964). Subsequent sections discuss the early work on the classical method of preparing proteins based on their solubility properties, and finally, a report on current research including immunochemical techniques, electron microscopy, and subcellular localization. The authors have not attempted to discuss the massive literature on the numerous enzymes that have been extracted from dormant or germinating peanut seedlings.

General Consideration Of Protein

Proteins may be defined as substances essential to all life-forms such as we know them, from the great whales to submicroscopic viruses. In animals, the structural material of muscles and various organs consists primarily of proteins. Proteins also function in the capacity of biological catalysts, enzymes, which promote the innumerable chemical reactions and energy transfers necessary for maintaining all phases of the complex living machinery, from digestion, absorption, and utilization of foods to the incredible light-detecting reactions in the retina of the eye. In many instances these enzymatic proteins operate in combination with other chemical substances. Many of the hormones of the animal body are proteins. These hormones serve as a system of "chemical mes-

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sengers" that helps to control and coordinate many of the body's life processes. The main structural material of plants is likely to be a polysaccharide such as cellulose, but the complex systems of chemical and energy transformations involved in germination, growth, photosynthesis, and other phases of plant activities are also controlled by specialized enzyme proteins.

Proteins are of vital dietary importance, particularly for monogastric animals including man. That the nutritive value of proteins is dependent upon their amino acid content, which is required for growth, maintenance, and other metabolic functions was realized from the fundamental studies of Osborne and Mendel (1914). The nutritional significance of peanut proteins, however, is described elsewhere in this book.

A review of the chemical structure of proteins reveals that they are complex organic polymers of high molecular weight (from 15,000 to more than 1,000,000), consisting of one or more polymeric chains of much smaller molecules termed amino acids. Generally, these chains are folded and interconnected in some specific pattern. Each chain can be visualized as a string of beads of various colors and shapes, each bead corresponding to a particular amino acid. Although there are thousands of proteins, there are only about 26 amino acid "beads." The properties of a given protein are determined to a great extent by what amino acids are present and their sequential distribution in the chain. Besides amino acids, proteins frequently contain small amounts of other organic components such as carbohydrates, pigments, and lipids.

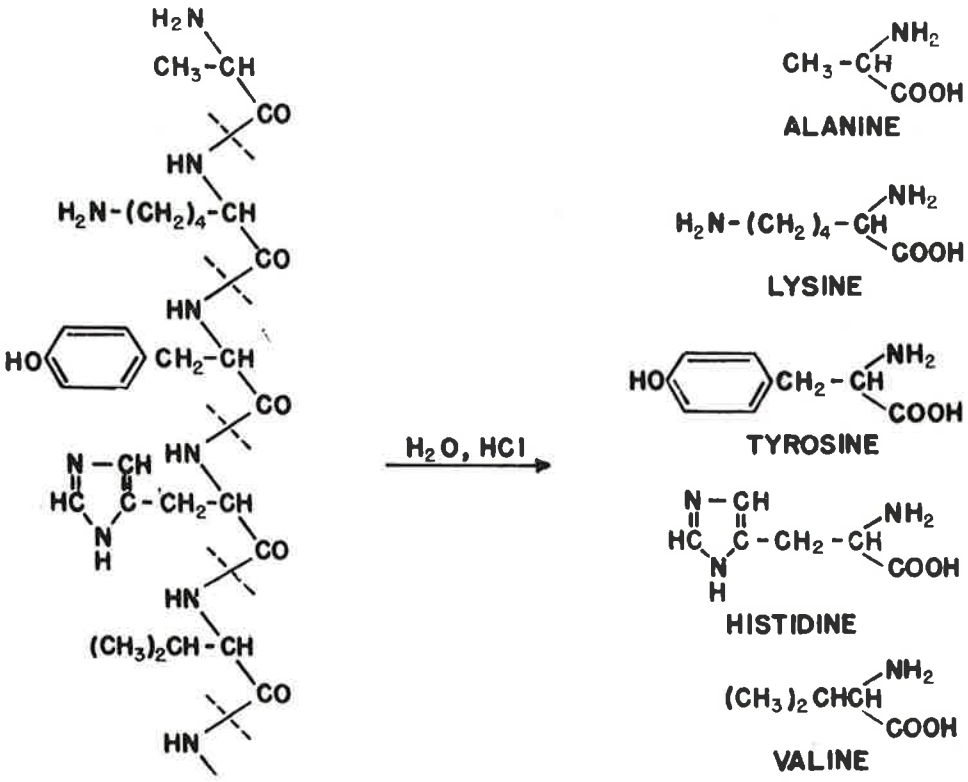
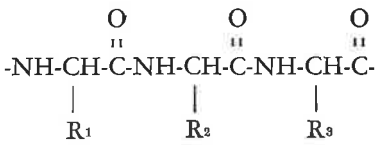


Figure 1. Schematic equation for protein hydrolysis. (From Mann, G. E. 1964. The amino acids of commercial cottonseed meals, p. 65-69. In Proceedings of 1964 Cottonseed Processing Clinic, U. S. Department of Agriculture, Agricultural Research Service, ARS 72-40. October 1965).

When proteins are exposed to the action of acids or bases, the chemical bonds between the amino acids are ruptured with the addition of water, and eventually the whole protein chain is broken down into individual amino acids. Figure 1 shows the chemical equation describing the hydrolytic breakdown of a hypothetical section of a protein. The repeating units of the protein chain are the "peptide bonds": where



R_1, R_2, and R_3 are the "amino acid residues" that confer upon the intact protein such properties as basicity or acidity, migration in electric fields, ability to crosslink, solubility, etc. The dotted lines in Figure 1 indicate where the peptide bonds are broken by hydrolysis to yield the five different amino acids. In general, hydrolysis of an actual protein yields a great variety of amino acids. Much effort has been directed towards the quantitative determination of the amino acid compositions of various proteins, but it is only within the last decade that such determinations have been performed with any degree of reliability and accuracy. Certain "proteolytic enzymes," e.g., pepsin, trypsin, are capable of effecting hydrolytic breakdown of other "substrate proteins" under much milder conditions of temperature and acidity than used in acid or base hydrolysis. Since some proteolytic enzymes show some degree of specificity regarding the peptide bonds they attack, they have been utilized to provide information concerning the sequence of amino acids in the peptide chains of purified proteins.

Almost all of the nitrogen in dormant seeds is present in the form of storage protein nitrogen. The factor of 6.25 is normally used to convert percent nitrogen to percent protein, based on the assumption that proteins contain 16% nitrogen. The use of this factor is, at best, only a rough estimate. There is no valid evidence for this assumption, but it is a widely used convention, especially in the commercial formulation of animal feeds (Bondi, 1958). For a more accurate nitrogen-to-protein conversion factor for oilseeds and cereal grains, one should consult reports of Johns (1931) and Tkachuk (1969). Since peanut protein consists almost entirely of two globulins, both of which contain approximately 18.3% nitrogen, the conversion factor used should be 5.46 (Johns, 1931).

Seeds vary widely in their protein content (Table 1) from a low concentration, as in cereals, to a high concentration, such as found in legumes. The peanut, a legume, has a relatively high content of crude protein, being exceeded only by the soybean. The subject of utilization of vegetable proteins and their value in foods and feeds has been reviewed by Altschul (1958, 1964, 1965).

Early Work

Solubility, Isolation, and Composition. Possibly the earliest report on the isolation of peanut protein is the work of Ritthausen (1880). Proteins were obtained from oilseed meals by solubilization with dilute alkalis followed by precipitation with acid. Dilute salt solutions were also used to solubilize the proteins. The proteins prepared by use of dilute potassium hydroxide from peanut, sunflower, and sesame had approximately the same elemental compositions (C, H, N, S, O) as proteins prepared from the cor-

Table 1. Protein content of some seeds[a]

		Crude protein content, %[b]
Legumes		
Peanut (groundnut)	*Arachis hypogaea*	25-28
Soybean	*Glycine max*	32-42
Chick pea	*Cicer arietinum*	20-28
Lentil	*Lens esculenta*	23-27
Oilseeds		
Cottonseed	*Gossypium hirsutum*	17-21
Sesame seed	*Sesamum indicum*	25
Sunflower seed	*Helianthus annuus*	27
Walnut	*Juglans regia*	15-21
Cereals		
Wheat	*Triticum aestivum*	12-14
Corn	*Uea mays*	7-9
Rice	*Oryza sativa*	7.5-9

[a] From Altschul, A. M. 1964. Seed proteins, p. 295-313. *In* H. W. Schultz and A F. Anglemier (ed.) Symposium on foods: proteins and their reactions. Avi Publisning Co., Westport, Conn.
[b] Nitrogen multiplied by 6.25.

responding oilseeds by extraction with dilute sodium chloride solutions. Lichnikov (1913) extracted peanuts with water, 70% ethanol, 10% sodium chloride solution, and 0.25% potassium hydroxide solution. The extracted materials were classified as albumin, glutin, and globulin. The glutin and globulin were hydrolyzed and the amino acids histidine, arginine, and lysine separated.

Johns and Jones (1916) separated total protein from the peanut into two major fractions which they named arachin and conarachin. These names are still used today. A 500 gram portion of defatted peanut meal was suspended in 2.5 liters of 10% sodium chloride, the mixture was filtered, and the arachin precipitated by addition of ammonium sulfate to 0.2 of saturation. After being filtered, arachin was dissolved in 10% sodium chloride, dialyzed, and dried. The filtrate from arachin was saturated with ammonium sulfate to precipitate the conarachin. The greatest difference between the two globulins was found in their sulfur contents, 0.40% and 1.09%, respectively. Johns and Jones (1917, 1918) also examined the basic nitrogen in these fractions and hydrolyzed them for amino acid determinations. Jones and Horn (1930) studied the properties of arachin and conarachin, and their proportionate occurrence in the peanut, concluding that they amounted to 25 and 8%, respectively, of the oil-free peanut meal.

Macheboeuf and Tayeau (1942, 1942a) studied the solubilization of peanut proteins and their fractionation, concluding that maximum extraction from the oil-free meal was obtained by using 6 volumes of 6% sodium chloride at pH 7.5 and 20°C. These authors (1942b, 1944) determined the effects of various electrolytes on the solubility of arachin and conarachin, and estimated the isoelectric points of these fractions as 5.2 and 3.9, respectively.

O'Hara and Saunders (1937), Basu and Sen-Gupta (1944), Burnett and Fontaine (1944), and Fontaine and Burnett (1944) have examined the use of various salt solutions for the solubilization of peanut proteins. The influence of peanut meal processing

factors on the solubility of the meal proteins has been assessed by Fontaine, Samuels, and Irving (1944). Efforts to improve the color of isolated peanut protein have been described by Fontaine *et al.* (1945) and the effects of certain acids and dialysis on the peptization of peanut protein nitrogen have been studied by Fontaine *et al.* (1946). Figure 2 indicates the influence of dialysis on the peptization of nitrogen from peanut

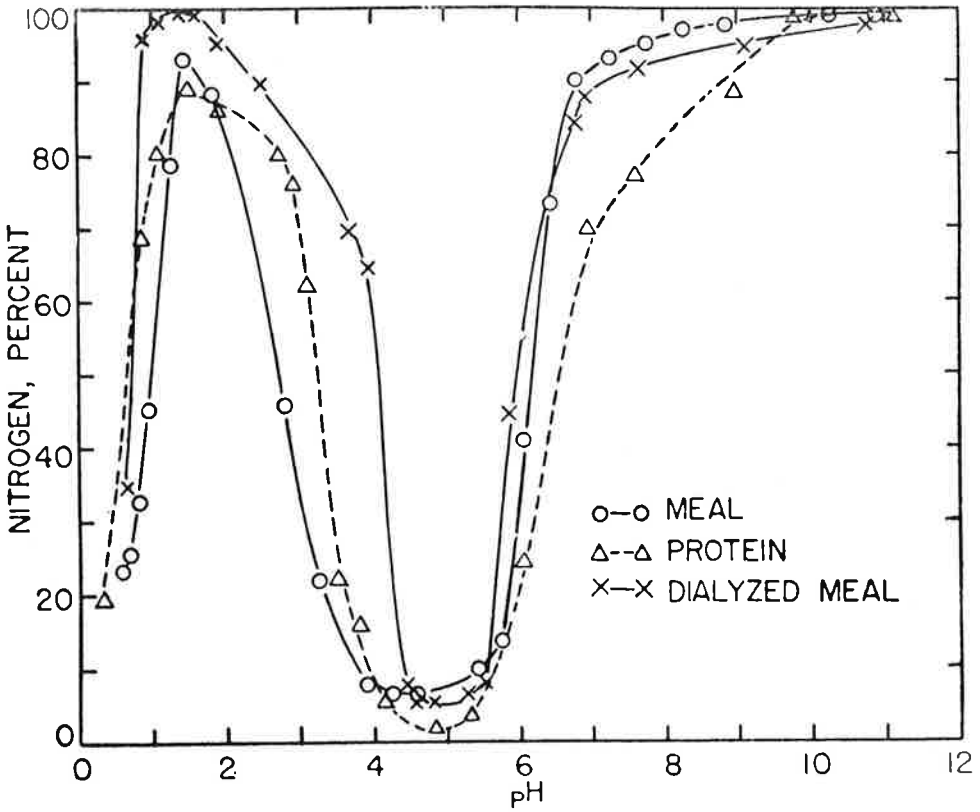


Figure 2. Hydrochloric acid-sodium hydroxide pH-peptization curves for peanut preparations. (From Fontaine, *et al.* 1946. Peptization of peanut and cottonseed proteins. Effect of dialysis and certain acids. Ind. Eng. Chem. 38:658-662.

meal as determined by these authors. Naturally occurring phytic acid in peanuts has been found responsible for the suppression of the solubility of peanut meal proteins at pH values below their isoelectric points (Fontaine *et al.*, 1946a).

From the solubilization studies described above, the most effective salts for dispersing the proteins at pH values between 5 and 6 are calcium, barium, and magnesium chlorides in concentrations of 0.25 to 1.0 N. Sodium and potassium salts, with exception of flourides and acetates, are good dispersing agents at concentrations of 1.0 N. As indicated in Figure 2, more than 90% of the proteins in solvent-extracted peanut meals can be dispersed by elevating the pH of an aqueous meal slurry to 7.5; subsequent lowering of the pH to 4.5 results in virtually complete precipitation of the proteins.

Dispersion in salt solutions was used to estimate the extent of deterioration of the proteins upon prolonged storage of the peanuts (Mori, 1944a; Pickett, 1948), to isolate peanut proteins for analyses of amino acids and other components (Brown, 1942.

1944; Mori, 1944; Hellot and Macheboeuf, 1947), and to prepare "pure" proteins for food use (Perov, 1948; Smirnova-Ikonnikova and Veselova, 1951).

Relatively small amounts of "pure" peanut proteins were generally prepared by the method of Jones and Horn (1930), which involved solubilization of the meal proteins in sodium chloride solution, clarification of the slurry by filtration or centrifugation, and precipitation of various fractions by "salting out" with ammonium sulfate. Larger amounts of protein were prepared by adding sodium hydroxide to a meal slurry until the pH was elevated to 7.5, clarifying by centrifugation, and precipitating the protein by adding sulfur dioxide until the pH was lowered to 4.5 (Arthur, 1950; Arthur *et al.,* 1948; Burnett, 1946; Calvert, 1950, 1950a; Chang and Chao, 1935; Kondangekar *et al.,* 1946; McGeoch, 1947; Opper and ter Horst, 1948; Pominiski *et al.,* 1952).

A color problem arises in the production of peanut protein because some of the pigments from the red skins (testae) are extracted and precipitated along with the protein. These pigments were tentatively identified (Stansbury *et al.,* 1950), and washing the precipitated protein curd with ethanol effected some improvement in color (Hoffpauir and Guthrie, 1947). Bonotto (1937) claimed that washing leguminous materials such as peanut meal with dilute sulfur dioxide solutions removes undesirable colors, flavors, and odors. McLean (1941) reported that maintaining an aqueous slurry of peanut meal for one hour at pH 8.0 to 8.5, followed by clarification and acidification to pH 4.8 with sulfur dioxide, yielded a protein preparation having little color. Irving *et al.* (1946) proposed that proteins be extracted from peanut meal at pH 7.0 or slightly lower, and the clarified extract acidified to pH 5.9 to 6.1 to yield a pigment-free protein. Possibly the most reliable method for minimizing color in peanut proteins was to wash whole peanuts with dilute alkali before extracting the oil (Burnett 1946, 1949).

Specially prepared, aminized and phosphorylated cotton fabrics were used to prepare peanut proteins low in ash and phosphorous contents (Hoffpauir and Guthrie, 1949). The aminized fabric functioned as an anion exchange material to elevate the pH of aqueous suspensions of peanut meal to solubilize the proteins, and the phosphorylated fabric was used as a cation exchange material to lower the pH of the dispersion to the isoelectric range where the protein precipitated.

About 1962, a commercial plant was established in Plymouth, England, to manufacture a material termed "peanut lipoprotein" (Waldt *et al.,* 1963). This product was marketed as a food additive under the trade name "Lypro," and contained 6% moisture, 33% oil, 63% protein, and 1% ash. The manufacturing process started with "impulse rendering" of peanuts, utilizing mechanically generated shock waves transmitted through an alkaline medium to rupture the plant cells and release their contents (Chayen, 1960). The material from the impulse generator was separated by high-speed centrifugation into three components, a carbohydrate product, an oil emulsion, and a peanut protein-oil complex. This complex was precipitated by acidification to pH 4.9, concentrated by centrifugation, neutralized, and spray-dried to yield the commercial product. Further processing of the carbohydrate product and oil emulsion components yielded a meal and an oil, respectively.

Peanut protein, prepared by sodium hydroxide solubilization-sulfur dioxide precipitation (Arthur, 1950; Arthur *et al.,* 1948), has the composition on a moisture-free basis of: 16.23% nitrogen, 0.74% ash, and 0.06% lipid. The amino acid contents of various peanut protein preparations are presented in Table 2.

Table 2. Amino acid contents of various peanut protein preparations[a]

Amino acid	Amino acid content, %[b]			
	Protein[c]	Total protein[d]	Arachin[d]	Conarachin[d]
Glycine	4.1	5.6	1.8	----
Alanine	----	4.2	4.1	----
Valine	4.7	8.0	1.1	----
Leucine	6.7	7.0	3.9	----
Cystine	----	1.9	1.1	3.0
Aspartic acid	12.0	5.8	5.3	----
Glutamic acid	20.0	19.2	16.7	----
Tyrosine	----	4.4	5.5	2.9
Phenylalanine	5.4	5.4	2.6	----
Proline	----	----	1.4	----
Tryptophan	----	2.0	0.9	2.1
Arginine	14.0	10.6	13.5	14.6
Lysine	3.1	3.4	5.0	6.0
Histidine	2.0	2.1	1.9	1.8
Methionine	1.1	1.2	0.5	2.1
Threonine	3.4	2.9	2.6	2.0
Serine	----	----	5.2	5.0
Hydroxylysine	----	----	0.01	----
Isoleucine	4.0	4.3	----	----

[a]From Arthur, Jett C., Jr. 1953. Peanut protein: isolation, composition, and properties. p. 393-414. *In* M. L. Anson, K. Bailey, and J. T. Edsall (ed.) Advances in Protein Chemistry, Vol. 8. Academic Press, New York.
[b]Calculated to 16.0% nitrogen.
[c]Microbiological assays on sulfur-dioxide precipitated peanut protein by Murphy, Edward A., and Max S. Dunn. 1950. Nutritional value of peanut protein. Food Res. 15: 498-510.
[d]Values compiled from various sources by Guthrie, John D., Carroll L. Hoffpauir, Mack F. Stansbury, and Wilson A. Reeves. 1949. Survey of the chemical composition of cotton fibers, cottonseed, peanuts, and sweet potatoes. A literature review. U. S. Dept. Agr., Bur. Agr. Ind. Chem. AIC-61 (Revised). 116 p.

Physical Chemical Properties. Eirich and Rideal (1940) appear to have published the first report on the use of the ultracentrifuge to determine the sedimentation constants of peanut proteins. Peanut meal was extracted with a variety of salt solutions and the extracts examined. Evidence for a total of six differently sedimentating proteins was obtained, the number present in a given extract depending on the type of salt solution used. The data obtained indicated a range of molecular weights of 20,000 to 600,000 for the six proteins. No effort to fractionate these proteins was recorded.

In a general discussion of macromolecules, Campbell and Johnson (1944) published some data on the sedimentation of peanut protein, and later, Johnson (1946, 1946a) examined the fractionation of peanut proteins with the ultracentrifuge. This work was extended by Johnson, Joubert, and Shooter (1950), and by Johnson and Shooter (1950). Jones and Horn (1930) had stated that arachin could be obtained from a sodium chloride solution extract of peanut meal by dilution and acidification, or by 40% saturation with ammonium sulfate. The proteins isolated by either method were considered identical by elementary analysis and amino acid assays. The sedimentation studies, however, revealed that the protein precipitated by dilution and acidification consisted of two sedimenting species having sedimentation coefficients, S_{20}, 14.6S and

9.5S, and the protein salted out with ammonium sulfate consists of one sedimenting species having a constant of 14.6S. If the two species from dilution-acidification are redissolved in sodium chloride and salted out with ammonium sulfate, one sedimenting species with 14.6S is obtained. Furthermore, ultracentrifugal analysis of a 6% sodium chloride extract of defatted peanut meal revealed a single protein component with 14.4S and a small quantity of very low molecular weight protein. These data led to the conclusion that arachin is a dissociating system composed of a parent molecule that divided into two molecules under certain conditions:

$$A_2 \rightleftharpoons 2A$$

The molecular weight of the parent molecule was calculated to be 330,000, the dissociated molecule (A), 180,000. The globulin apparently occurs chiefly in the associated form in the nut. The equilibrium position and its rate of attainment in solution depends upon pH, salt concentration and type of salt present; lowering pH and lowering salt concentration favors increased dissociation. High concentrations of sulfate ions were especially effective in promoting almost complete association, but even in small quantities they appeared to prevent dissociation. Johnson and Joubert (1951) have examined the effects of simple ions and of long-chain compounds, e.g., sodium dodecyl sulfate, on the arachin association/dissociation system.

Goring and Johnson (1952) noted that an arachin preparation contained 6.3% of a protein having a sedimentation constant of 21.1S in addition to the main peak of the associated molecule, 13.3S. If a spherical shape is assumed, the molecular weight of the faster sedimentating component was about 660,000, twice the weight of the associated parent molecule (2A). Brand *et al.,* (1955) attempted to use arachin as a secondary molecular weight standard for light-scattering experiments but found the protein unsatisfactory because of the presence of small, but not negligible quantities of more rapidly sedimenting material, probably an aggregate of the main component. These observations indicated the presence of a protein species that is more highly associated than the parent arachin, A_2. It should be noted that Danielsson (1949) reported the sedimentation constants of the globulin components of thirty-four species in the family *Leguminosae.* The sedimentation constants for the globulins from *Arachis hypogaea* were 1.93S, 8.4S, and 13.05S. The latter two constants were in approximate agreement with those determined by Johnson (1946, 1946a).

The action of urea and guanidine hydrochloride upon arachin was examined by Johnson and Naismith (1956). Solubility, sedimentation velocity, and other methods were used for the study. Guanidine hydrochloride acted more powerfully and rapidly than urea at comparable molar concentrations, causing insolubilization that rapidly increased with time and later remained constant. Comparable urea concentrations caused less insolubilization; this increased to a maximum and later fell slowly to zero. Examination of sedimentation velocity showed that, in the presence of high urea concentrations, protein sediments as an arachin-like material, the concentration of which gradually decreases in time, being converted into slow sedimenting material of lower molecular weight (down to 20,000). Evans (1957) reported on the sedimentation of total peanut globulins in urea solutions. In 1-3M urea solutions three sedimenting species were observed; in 4-6M, two; in 7M urea, only one sedimenting species. A molecular weight of 21,000 was indicated for this last species.

The conarachin fraction of peanut globulins was examined by Johnson and Naismith (1953). They showed by ultracentrifugation and light-scattering that the dissociated species, having a molecular weight of about 190,000, was favored by high ionic strength and high pH. With lowered ionic strength at constant pH or lowered pH at constant ionic strength, the degree of association increased, and at least four new species, with molecular weights as high as 2 x 10^6, appeared.

When dissolved in aqueous solutions, proteins are generally electrically charged, and hence will move when an electrical field is applied across the solution. The electrophoretic mobility (cm.^{2} sec.$^{-1}$ volt^{-1}) of the migrating protein depends upon the nature of the protein (type of amino acid residues, shape, etc.), on the temperature, and on the nature of the medium (pH, ionic strength, viscosity, presence of specific ions, etc.). Electrophoretic techniques were employed to examine peanut meal extract, arachin, and conarachin in ammonia buffer, pH 9.26, ionic strength 0.1 (Irving *et al.*, 1945). One major component and two minor components occurred in the meal extract, which contains about 98% of the meal proteins. Arachin, representing about 63% of the total protein, contained two components in a ratio of about 3 to 1. Conarachin, about 33% of the total protein, contained two components in a ratio of about 4 to 1. Table 3 summarizes these data. These same authors (Fontaine *et al.*, 1945)

Table 3. Mobilities and estimates of the relative amounts of the protein components in the peanut meal extract, arachin, and conarachin[a]

	Protein Component					
	A		B		C	
Fraction	Mobility[b]	%[c]	Mobility	%	Mobility	%
Buffer extract of peanut meal	-6.3	76	-5.1	11	-3.7	13
Arachin, twice reprecipitated[d]	-6.0	76	-5.3	24	-----	0
Conarachin, once precipitated	-6.0	80	-----	0	-3.5	20
Conarachin, once reprecipitated	-6.0	78	-----	0	-3.4	22

[a]From Irving, George W., Jr., Thomas D. Fontaine, and Robert C. Warner. 1945. Electrophoretic investigation of peanut protein. I. Peanut meal extract, arachin, and conarachin. Arch. Biochem. 7: 475-489.
[b]Mobility in cm.^{2}volt^{-1}sec^{-1} X 10^5 reduced to 0°C.
[c]Calculated from descending pattern.
[d]The estimated amounts of components A and B in arachin are open to question due to the asymmetry of the electrophoretic patterns in this instance.

described the electrophoretic analysis of several protein fractions obtained by simple precipitation from slightly alkaline extracts of peanut meal.

The same electrophoretic patterns were obtained when peanut meal was extracted with borate, veronal, ammonia and glycine buffers, ionic strength 0.1, over a pH range from 8.3 to 10.0. The major components migrated as a single entity under most conditions. If, however, the protein was separated from a meal which had been washed with water adjusted to pH 5 to remove soluble sugars and phytin, the major components separated into two almost equal fractions (Karon *et al.*, 1950).

Johnson *et al.* (1950) have studied the properties of arachin by electrophoresis using phosphate and barbiturate buffers as solvents. The S_{20} 14.6S and 9.0S species exhibited surprisingly similar mobilities and no complete separation of the two species

was achieved at any pH value. Examination of the 14.6S species indicated slow dissociation of this parent molecule, and estimates of the rate of dissociation at pH 7 yielded a half-life of about 50 hours, and at pH 8, a half-life of greater than 100 hours. The general picture of arachin dissociation (Johnson and Shooter, 1950) was confirmed for phosphate and barbiturate buffers.

The conarachin fraction of peanut globulins was examined by electrophoresis and by other means (Johnson and Naismith, 1954). Electrophoresis failed completely to detect the different states of aggregation occurring with change in ionic strength or pH as indicated by ultracentrifugation and light-scattering (Johnson and Naismith, 1953). Two conarachin fractions, I (precipitated in range 40-65% saturation with ammonium sulfate) and II (precipitated in range 65-85% saturation with ammonium sulfate), were examined. Conarachin I consisted of typical conarachin protein contaminated with arachin, and conarachin II was well-defined and indistinguishable electrophoretically from arachin. It alone is involved in the association/dissociation system. pH-Mobility curves for both conarachin components at different ionic strengths were obtained, and the isoionic points were 7.1 $\pm$ 0.2 (conarachin I), and 5.5 $\pm$ 0.1 (conarachin II). The unsuitability of electrophoresis as a method of investigation when changes in states of aggregation are suspected was pointed out.

The utilization of light scattering to study conarachin has already been mentioned (Johnson and Naismith, 1953). Goring and Johnson (1952) examined an arachin solution (93.7% S_{20} 13.3S and 6.3% S_{20} 21.1S) in phosphate buffer by use of a light-scattering apparatus. An apparent molecular weight of 17,000,000 was obtained. After further clarification by ultrafiltration, the molecular weight was 333,000, in agreement with the accepted molecular weight 330,000 calculated from sedimentation and diffusion data. This report revealed the great importance of proper clarification for valid light-scattering estimates of molecular weights.

Dean (1949) studied the ultraviolet absorption spectra of peanut proteins at pH 7.3 and 11.0, demonstrating the identity of the spectrum of the "mixed protein" (total globulins) with the spectrum of arachin. This agreed with sedimentation data indicating that these two preparations were equilibrium states of the same molecule. Variations of pH in the experimental range caused reversible change in the adsorption. This was due to the ionization of the tyrosine groups of the proteins. The absorption of conarachin at pH 7.3 was explained in terms of the tyrosine, tryptophan, and cystine present, but at pH 11.0 there was no agreement. At pH 7.3 the spectra of conarachin and arachin are similar, but not identical, while at pH 11.0 the spectra have little resemblance.

The preparation of aqueous peanut protein dispersions of high viscosity by use of sodium hydroxide was described by Dickson and Sever (1944). Burnett *et al.* (1945) published a more thorough study of methods for preparing and determining the viscosity of vegetable protein solutions at useful concentrations. Solutions containing more than 26% protein became thixotropic at pH 8.5. At pH values below 10.0 and protein concentrations above 28%, the solutions either gelled or developed very high viscosities. Latin square designed experiments on the rheological conditions of the system, peanut protein — caustic soda — water, were published by Thomson and Traill (1945), and Thomson and Johnston (1947). The production of viscous aqueous solutions of peanut proteins was described by Thomson and Swift (1947), and by Thomas and Traill (1949). Millidge and Knight (1949) claimed that small amounts of cyanide compounds stabilize such viscous peanut protein solutions. Arthur and Many

(1952) have shown that alkaline solutions of peanut proteins may be classified as viscoelastic solutions, since their viscosities are relatively large at low shear rates and decrease with increasing rates of shear.

The viscosities of aqueous solutions of peanut proteins, solubilized with sodium hydroxide or with urea, increase with time to a maximum value. Astbury *et al.* (1935) claimed that this is due to the gradual "unfolding" of the globular peanut protein molecules when exposed to these reagents. The axial ratio of the molecules increased resulting in increased interaction between the molecules and increased viscosity.

The intrinsic viscosity of peanut protein in 10M urea was increased by reacting the protein with the bifunctional acid chloride, terephthalyl dichloride. Reaction of the protein with equivalent amounts of benzoyl chloride did not increase the intrinsic vicosity (Mann, 1953).

Chemical Reactions. Peanut proteins were subjected to a variety of chemical reagents with the objective of cross-linking or tanning them to improve their physical properties. The general subject of cross linkages in proteins was reviewed by Bjorksten (1951). Traill (1945) described treatment of peanut protein fibers with formaldehyde, sulfur dioxide, carbonyl chloride, thionyl chloride, and sulfur chloride. Peanut protein was treated with phenyl isocyanate (Olcott and Fraenkel-Conrat, 1946) and with palmitoyl chloride (Gordon *et al.,* 1946) to reduce water retention and to impart plastic properties. Tetlow (1950) published a brief study of the reaction of peanut protein with formaldehyde, and treatment with terephthalyl dichloride has already been described (Mann, 1953). The use of other reagents including polyvalent metals such as aluminum, chromium, zinc, cadmium, and mercury to improve fibers and bristles was documented by Arthur (1953). Little or no theoretical work on the mechanisms of these reactions with peanut proteins has been published.

Nonfood Uses. Just prior to the outbreak of World War II in Europe, a woollike fiber made from peanut protein was developed in England. It was known as "Ardil," the registered trade name of Imperial Chemical Industries. The product appeared promising and was ready for commercial production, but the war intervened. The peanuts normally imported into England from India and West Africa, were needed for food (Sherman and Sherman, 1946).

A similar fiber, called "Sarelon," was developed by the U. S. Department of Agriculture at the Southern Regional Research Laboratory (Merrifield and Pomes, 1946; Arthur, 1949; Arthur and Many, 1950). The fiber was light cream colored in its natural state and had a soft hand and a warmth similar to that of wool. It possessed an affinity for dyes normally used on protein fibers and could be dyed with vat and direct cotton dyes. Its major weakness, like that of all synthetic protein fibers, was its low strength.

The field of synthetic protein fibers was reviewed by Lundgren (1949), and the copious literature on peanut protein fibers was cited by Arthur (1949, 1953).

The use of peanut meal in the preparation of plywood adhesives was reported (Laucks, 1934; Corwin and Dunham, 1940; Narayanamurti and Singh, 1942). Burnett and Parker (1946) described the specifications for peanut meal suitable for plywood glue, the effect of meal processing conditions on the characteristics of the glue, and the comparison of peanut meal glue with other glues. Joint tests indicated that peanut meal glue met the requirements established for casein and casein-type glues. Glues suitable for making gummed tape and paper were prepared from isolated peanut protein (Burnett, Parker, and Roberts, 1945). Peanut proteins from hydraulic-pressed meals yielded glues

having adhesive strengths of about 65-78% of the strengths of animal glues, and for proteins from solvent-extracted meals, the glues had strengths 12-85% of animal glue strengths.

Paper coatings containing peanut proteins were developed and compared with coating containing other proteins (Arthur *et al.,* 1948). Coatings containing peanut proteins performed as well as coatings containing soybean alpha-protein and casein. Cotton muslin sized on a laboratory scale with peanut protein solutions has the tensile and flexibility characteristics similar to commercially sized samples using animal glues. Peanut protein was applicable as a sizing material for use in window shade manufacture and similar applications (Arthur and Cheng, 1949).

Levin (1946) claimed that a fire extinguishing liquid can be made by suspending peanut cake in aqueous calcium hydroxide, heating for two hours at 95°C., filtering, neutralizing, and concentrating to 30-40% solids.

A water-resistant molding powder was prepared by mixing finely ground defatted peanut meal with synthetic resins, such as condensation products of phenols and formaldehyde (Quintin-Toronelli, 1939).

The U. S. Department of Agriculture (1951) published a very thorough study of the marketing potential for oilseed protein materials in industrial (nonfood) uses. It was reported to be difficult for processors to obtain adequate supplies of peanuts that are suitable for making an undenatured meal. The high cost and limited supply reflect the fact that production of peanut oil and meal is the marginal part of the peanut industry, food uses being far more important.

"Until larger quantities of peanuts are regularly available for industrial processing at lower and more stable prices, any development of solvent extraction and isolate production on a scale sufficient to supply an industrial market seems unlikely." In recent years the appearance of growing worldwide protein hunger has focused research activities on utilization of the peanut proteins for food, rather than nonfood, applications.

Current Work

In 1958, under the auspices of the U. S. Department of Agriculture, Southern Utilization Research and Development Division, the Seed Protein Pioneering Research Laboratory was established to acquire fundamental information leading to better characterization of plant proteins and associated materials, and to investigate the nature and biological function of seed proteins. To this end a comprehensive plan was developed for studying seed protein purification and chemistry, protein metabolism, ultrastructure of seeds, and the nature of subcellular particles in the developing, quiescent, and germinating seedling, using modern and up-to-date techniques. Included in these investigations was research on the peanut. The peanut was chosen because, from the standpoint of composition and properties, it is ideally suited for food, feed, and industrial uses.

Chromatography with DEAE-cellulose. Since proteins are extremely sensitive to heat, acids, bases, and organic solvents, they are usually isolated by methods different from those used to isolate other organic compounds. In the past few decades, several chromatographic methods have been developed for fractionation of protein mixtures. These were based on the relative strengths of adsorption of proteins on certain media, or on the relative size and/or structure of proteins and of the particular adsorbent material used to achieve the separation. One of the most useful techniques for separating and identifying proteins is chromatography on diethylaminoethyl-cellulose, a weakly basic ion exchanger, in which the hydroxyl groups of cellulose are linked by ether

bonds to the hydroxyl groups of diethylaminoethyl alcohol. Fractionation of protein mixtures is dependent upon the affinity of the proteins for DEAE-cellulose which, in turn, is governed by the mutual attraction of charged groups of opposite sign, hydrogen bonding, and interaction by van der Waal's forces (attraction at the atomic level). In a mixture of proteins, the one with the lowest affinity for the solid phase (i.e., the least tightly held) will move through the column fastest, followed by the next protein, and so on. Since the electrostatic attraction between ionized groups of the exchange resin and charged groups of the opposite sign in the protein can be lowered by salts, proteins that are strongly held to the solid phase can be eluted by increasing the salt concentration of the eluting solvent.

About 90% of the proteins of peanut cotyledons are soluble in dilute neutral buffer or 10% sodium chloride solution. They can be easily separated into two major globulin fractions by ammonium sulfate precipitation. The fraction precipitating at 40% saturation is called "arachin", and the protein fraction precipitating in the range of 40 to 85% saturation is "conarachin." Neither of these fractions, however, is a pure protein. The complexity of the arachin and conarachin fractions has been shown by a number of investigators. Recently, the work of Cherry *et al.* (1972), who characterized peanut proteins and enzymes by employing DEAE-cellulose, gel-electrophoreses, and immunochemical techniques, has reconfirmed that these proteins are very complex and great care should be taken when interpreting data obtained from different means of "purification."

Conarachin. Dechary *et al.* (1961) chromatographed these two globulin fractions on DEAE-cellulose and eluted the proteins with a sodium chloride gradient (Figure 3A). The two fractions were separated into four groups of protein: the first group was not adsorbed on the cellulose, and was referred to as "the break-through fraction," or Group I; the second was eluted by salt concentrations up to 0.14 M, Group II; the third was eluted in the range of 0.14 to 0.23 M, Group III, and the fourth fraction, by concentrations of salt above 0.23 M, Group IV. The arachin fraction, represented in the figure as a solid line, consists mostly of Group IV, while conarachin comprises Groups I, II, and III. The conarachin fraction was further fractionated and the major constituent comprising Group III was isolated and named "alpha-conarachin." The chromatographic profile of alpha-conarachin on DEAE-cellulose, as seen in Figure 3D, shows only one component. The yield of alpha-conarachin was 30% of the original conarachin fraction.

Alpha-conarachin, containing 15% nitrogen, has about 2% mannose, which is firmly bound and can be liberated by mild treatment with acid and detected by paper chromatography (Dechary, 1963). The amino acid composition of alpha-conarachin (Table 4) is that of a typical seed protein, i.e., high in glutamic acid, aspartic acid, and arginine. The two dicarboxylic acids probably exist as the amide form *in situ.* Interestingly, alpha-conarachin was the first peanut globulin isolated in such a high state of purity and among the first globulins of any seeds isolated by this manner.

The chromatographic pattern of the total globulins extracted from peanut cotyledons is depicted in Figure 3B. Using the same chromatographic techniques, observations were made of changes in the total protein profile after the peanut cotyledons were germinated for three days at 28°C. An examination of these results, illustrated in Figure 3C, shows that the proteins of Group III disappeared almost entirely. Since these data demonstrated that alpha-conarachin is the first globulin to disappear on germination, then alpha-conarachin may be called a storage or reserve protein, i.e., one that supplies nitrogen- and carbon-containing materials to the growing seedlings during the early

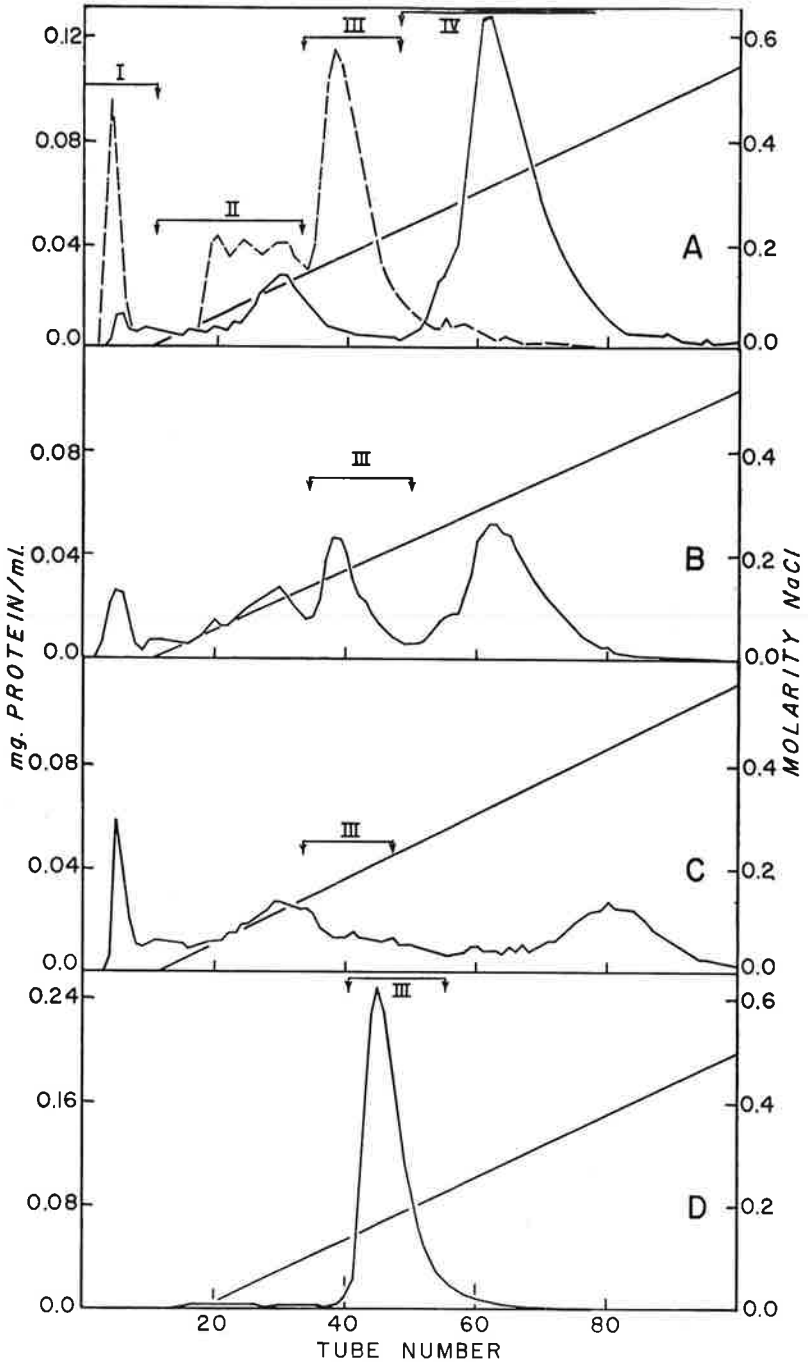


Figure 3. Chromatography of peanut protein fractions on DEAE-cellulose. 10 mgm. of each sample were put on 2 gm. of cellulose. The straight line indicates the sodium chloride gradient as measured on the eluate. A, Full line represents arachin fraction; broken line, conarachin fraction; B, extract of proteins from peanut cotyledons with buffer, pH 7.9, $\mu = 0.06$; C, extract of proteins from cotyledons of germinated peanuts with same buffer; D, α-conarachin. (From Dechary, *et al.* 1961. α-Conarachin. Nature 190:1125-1126.)

Table 4. Amino acid composition of α-conarachin (gm./100 gm. of protein)

Amino Acid	α-Conarachin
Asp	10.5
Thr	2.2
Ser	4.1
Glu	16.0
Pro	2.7
Ileu	3.7
Leu	5.6
Tyr	1.1
Phe	4.6
Lys	4.9
Gly	2.8
Ala	3.0
CyS	0.7
Val	4.5
Met	1.1
His	2.4
Ammonia	1.3
Arg	8.6
Try	1.0
Total recovery, %	83.0

From Altschul, A. M. 1964. Seed proteins, pp. 295-313. *In* H. W. Shultz and A. F. Anglemier (ed.) Symposium on foods: Proteins and their reactions.

period of development. Surely, these chromatographic patterns point out that seed proteins are as complex as those found in any other biological tissue.

Although alpha-conarachin was shown to be a homogeneous species by DEAE-cellulose chromatography (Dechary *et al.*, 1961), sedimentation analyses by ultracentrifugation (Evans *et al.*, 1962) showed that alpha-conarachin is comprised of at least two components when dissolved in pH 7.8 phosphate buffer, whether at low or high ionic strength. In each instance (Figures 4C and 4D), there was only one major component and a trace of a second, lighter component. In low ionic strength buffer (Figure 4C) the sedimentation coefficient of the major component was 13.1S (corresponding to a molecular weight of 295,000) and 2.0S for the minor component. If the ionic strength was increased to 0.2 (Figure 4D), the sedimentation coefficient of the major component was altered to 10.3S, and at ionic strength of 0.5, pH 7.9, the sedimentation constant was 7.8S, which corresponds to a molecular weight of 142,000 (Dechary *et al.*, 1961). In essence, the molecular weight of alpha-conarachin varies from ca. 140,000 to 295,000 depending on the ionic strength of the buffer and on the pH. The molecular weight is highest at the lower ionic strength. When the protein is dialyzed against mild acid, an interesting rearrangement is observed. Two species now appear; one with the original molecular weight of ca. 280,000 and another one, approximately 30% of the total, with the molecular weight doubled. This dimerization is induced by mild acid treatment and is not reversible. Evans *et al.*, (1962) interpreted this as indicative of an associa-

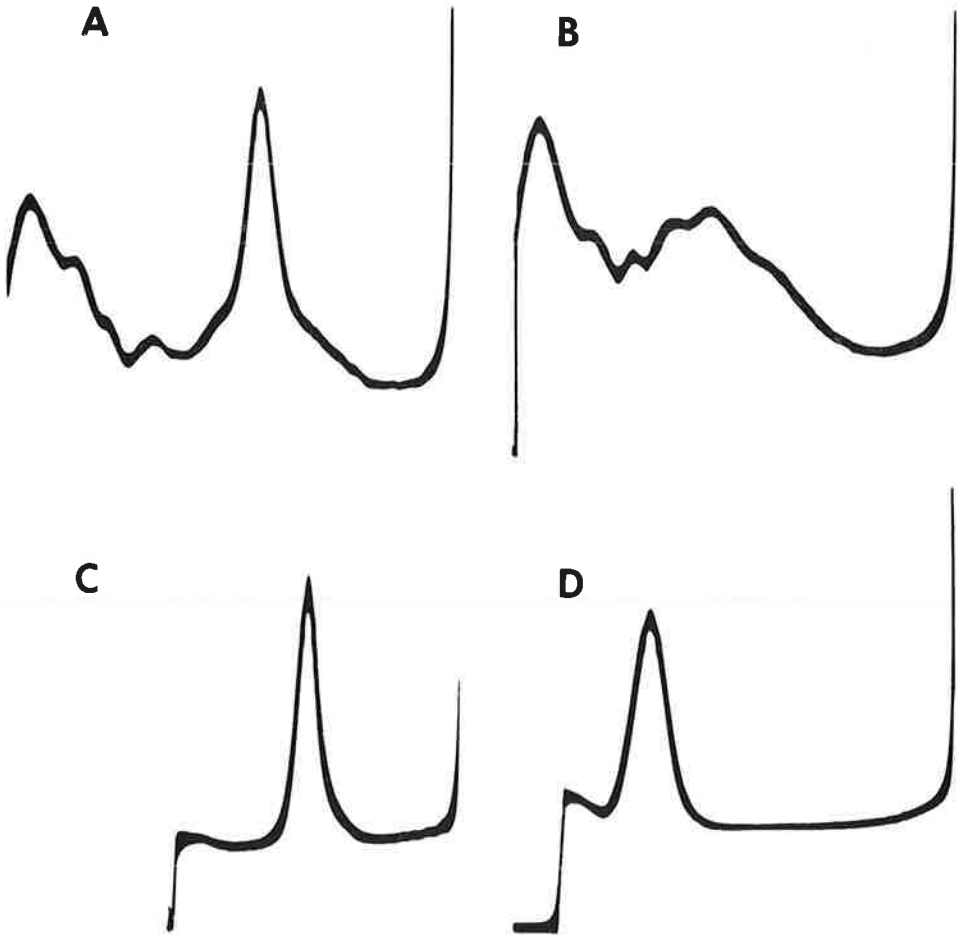


Figure 4. Sedimentation patterns of conarachin and α-conarachin. R.p.m. 59,780; bar angle 60°. Conarachin: A,μ = 0.03; B.μ = 0.2; protein concentration 3 g/100 ml.; pictures taken: A, 20 min. and B, 26 min. after top speed. α-Conarachin: C,μ = 0.03; D,μ = 0.2; protein concentration 1.5 g./100 ml.; pictures taken: C, 15 min. and D, 14 min. after top speed. (From Evans, *et al.*, 1962. Zone electrophoresis of conarachin, α-conarachin, and bovine serum albumin on polyacrylamide gel. Arch. Biochem. and Biophys. 96:233-239.)

tion-dissociation system which was previously described for conarachin by Johnson and Naismith (1953). This phenomenon was later confirmed in a subsequent report (Evans *et al.*, 1963) on the acid-induced transformation of alpha-conarachin, and was later observed even with peanut globulins isolated in sucrose solution (Neucere and St. Angelo, 1972). The heterogeneity of conarachin is illustrated by Figures 4A and 4B, which show that the conarachin fraction in pH 7.8 phosphate buffer of low ionic strength, had at least eight components, and at high ionic strength, six components were detected.

Using a more discriminating method to study the heterogeneity of conarachin, zone electrophoresis with polyacrylamide gel, Evans *et al.* (1962) identified approximately 20 separate components.

When this method was applied to alpha-conarachin, they were able to resolve but three closely situated components.

Alpha-conarachin was examined by optical rotatory dispersion (Jirgensons, 1963), which showed that it belongs to the "nonhelical" globular proteins since its Moffit constant, b_o value, was near zero. Upon treatment of alpha-conarachin with anionic detergents, the protein changed its conformation in a manner similar to that of the γ-globulins and Bence-Jones proteins, which are typical globular proteins. The negative shift of b_o was interpreted as a conformational transition in which a partial α-helix formation occurs, as suggested previously for γ-globulin and soybean trypsin inhibitor.

Manganin. While arachin and conarachin, the principal reserve proteins in the peanut, have been the subject of many investigations, Dieckert and Rozacky (1969) have recently reported on the isolation and characterization of a manganoprotein, called manganin, from peanut seeds. There are a few manganoproteins reported from animal sources; however, this particular type of protein had not been hitherto reported in higher plants. Manganin, extracted from the conarachin fraction, was homogeneous by ultracentrifugal analysis and cellulose polyacetate strip electrophoresis. It has a molecular weight of 56,300 based on the manganese content, which is one atom of manganese per molecule of protein. The amino acid content, as seen in Table 5, is different from that reported for arachin and conarachin. For example, Tombs (1965) reported arachin to contain approximately 7 mol percent for glycine; manganin has 20 mol percent. In comparing the amino acid composition of manganin with that for conarachin as reported by Hoffpauir (1953), Dieckert and Rozacky maintained that manganin contains less half-cystine, tryptophan, and arginine, and more threonine and lysine than conarachin.

Table 5. Amino acid composition of manganin*

Amino Acid	μ Moles[a]	Mol %	Residues/Molecule
Lys	0.375	5.19 (5.32)[b]	29
His	0.124	1.72 (1.56)	10
Arg	0.314	4.34 (4.37)	25
Asp	0.762	10.54 (10.07)	59
Ser	0.555	7.68 (7.55)	43
Thr	0.319	4.41 (5.55)	25
Glu	0.738	10.21 (10.47)	57
Pro	0.401	5.55 (4.57)	31
Gly	1.447	20.02 (19.99)	112
Ala	0.452	6.25 (6.31)	35
½-Cyst	0.048	0.66 (0.92)	4
Val	0.438	6.06 (5.93)	34
Met	0.073	1.01 (0.99)	6
Leu	0.425	5.88 (5.84)	33
Iso	0.299	4.14 (3.99)	23
Tyr	0.200	2.77 (2.94)	15
Phe	0.233	3.22 (3.26)	18
Try	0.025	0.35	

a The sample was 0.85 mg.
b Values in Parentheses are averages of duplicate analyses of a second batch of manganin.
* From Dieckert, J. W., and E. Rozacky. 1969. Isolation and partial characterization of manganin, a new manganoprotein from peanut seeds. Arch. Biochem. Biophys. 134: 473-477.

Arachin. Arachin, the major peanut protein, has a molecular weight of about 330,000 (Johnson and Shooter, 1950) and the ability to dissociate reversibly to the monomeric form of molecular weight 180,000. When the classically prepared arachin fraction was chromatographed on DEAE-cellulose by Dechary *et al.* (1961), the profile showed one major component and two minor constituents (see solid line of Figure 3A). The main component, Group IV, was eluted at a salt concentration of 0.23M.

Tombs (1965) chromatographed the classically obtained arachin fraction on a DEAE-Sephadex column to remove minor contaminants, and then separated it into two closely related forms by electophoresis on polyacrylamide gel. He showed that these polymorphic species of arachin, whose contents may even differ from peanut to peanut, are made up of complex structures involving three or four kinds of peptide chains. He further suggested that the first polymorphic form of arachin, termed "Arachin A," contained four different kinds of peptide chains: 4α, 4β, 2γ, and 2δ chains. The B form of arachin was presumed to be 8β, 2γ, and 2δ chains, but it did not contain any α chains. The α and β chains have molecular weights of 35,000 while the γ and δ chains have molecular weights of only 10,000 each. Both A and B forms of arachin have a total molecular weight of 330,000. The amino acid composition of each form, presented in Table 6, shows them to be very similar in that respect. These data were in agreement with the partial analysis of arachin by Tristram (1953).

Table 6. Amino acid analysis of arachins A and B. Composition (residues/330, 000g of protein)

Time of hydrolysis (hr.)	24	
Amino Acid	A	B
Aspartate	345.3	347.0
Threonine	74.2	73.8
Serine	167.9	170.0
Glutamate	480.6	474.0
Proline	140.4	140.7
Glycine	189.7	202.4
Alanine	162.2	174.3
Cystine*	12.7	7.8
Valine	129.2	121.5
Methionine*	11.2	6.9
Isoleucine	91.9	93.2
Leucine	194.2	202.7
Tyrosine	102.0	98.2
Phenylalanine	131.5	130.8
Lysine	57.7	57.8
Histidine	57.3	50.0
Arginine	253.0	262.0
Tryptophan*	35	35

Methionine was partly converted into sulphoxides and these were corrected for. Tryptophan was estimated from the u.v. absorption of an AB arachin sample. Traces of cysteic acid were not corrected for. The totals exclude amino acids marked.

From Tombs, M. P. 1964. An electrophoretic investigation of groundnut proteins: the structure of arachins A and B. Biochem. J. 96:110-133.

Using osmometry to determine the subunits of arachin, Tombs and Lowe (1967) found that the B form of arachin had the structure $\beta_4\gamma\delta$, and the A form, $\alpha_2\beta_2\gamma\delta$. They identified the N-terminal amino acids of arachin as glycine, valine and (iso) leucine in the proportions 4:1:1. They discovered a third form of arachin, isolated

from an African groundnut variety, which migrated more rapidly than Arachin A. This new form, called "A-1," had the structure $\alpha_4\gamma\delta$. All three forms of arachin had a molecular weight of 170,000. Further experiments by gel electrophoresis showed that arachin dissociated into fragments when exposed to 8M urea. These fragments appeared to be dimers of the type α-S-S-β, and β-S-S-β, held together by disulphide bonds.

Recent studies of peanut proteins by Neucere (1969) and Daussant *et al.* (1969, 1969a) described the identification, isolation, and characterization of a highly purified protein component, named "α-arachin," from the classical arachin fraction. Neucere (1969) isolated α-arachin from peanut cotyledons by two different procedures, precipitation at low temperature and chromatography on DEAE-cellulose.

In the first method, cryoprecipitation, peanut cotyledons were homogenized in phosphate buffer and centrifuged to remove all undissolved material (Figure 5). The supernatant was allowed to stand at 2°C. for 17 hours, then centrifuged. The subsequent pellet was dissolved in the same buffer at 25°C. and precipitated again at the low temperature. This precipitate, P_3, when dissolved in buffer and chromatographed on DEAE-cellulose (Figure 6C) exhibited one main peak with a shoulder, which

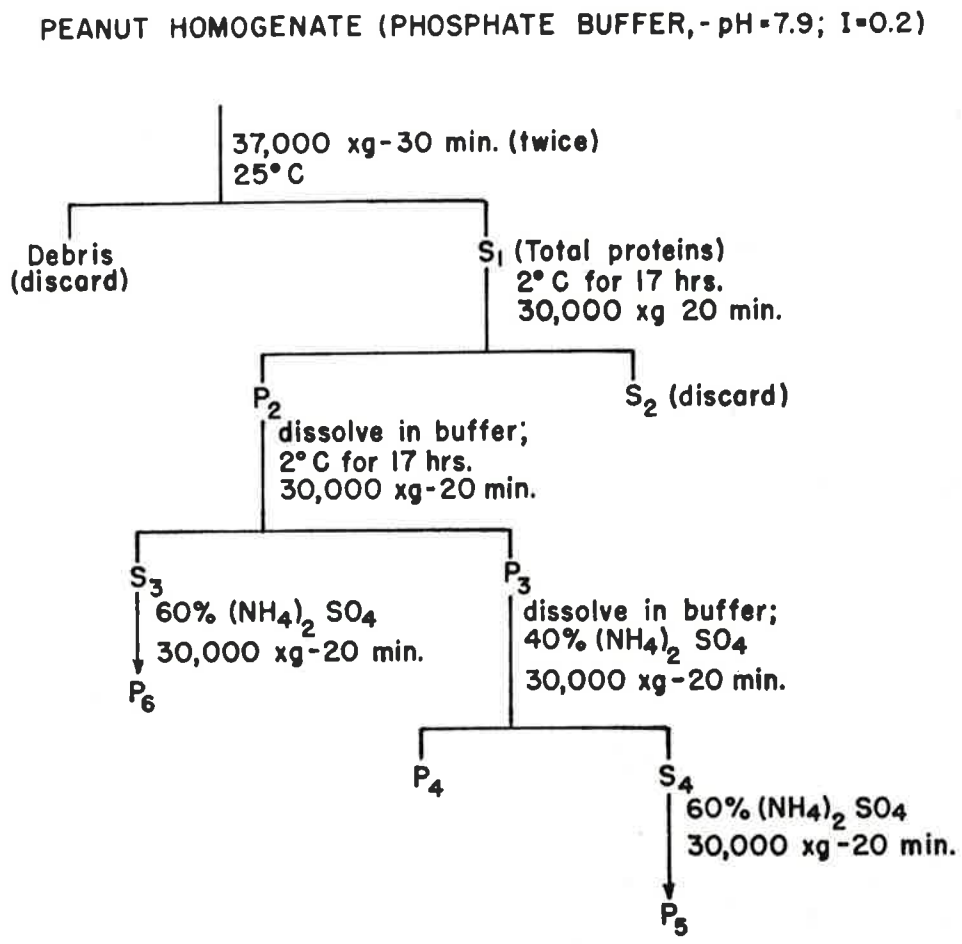


Figure 5. Purification scheme of α-arachin by precipitation at 2°C. for 17 hr., followed by ammonium sulfate fractionation. (From Neucere, N. J. 1969. Isolation of α-Arachin, the major peanut globulin. Anal. Biochem. 27:15-24.)

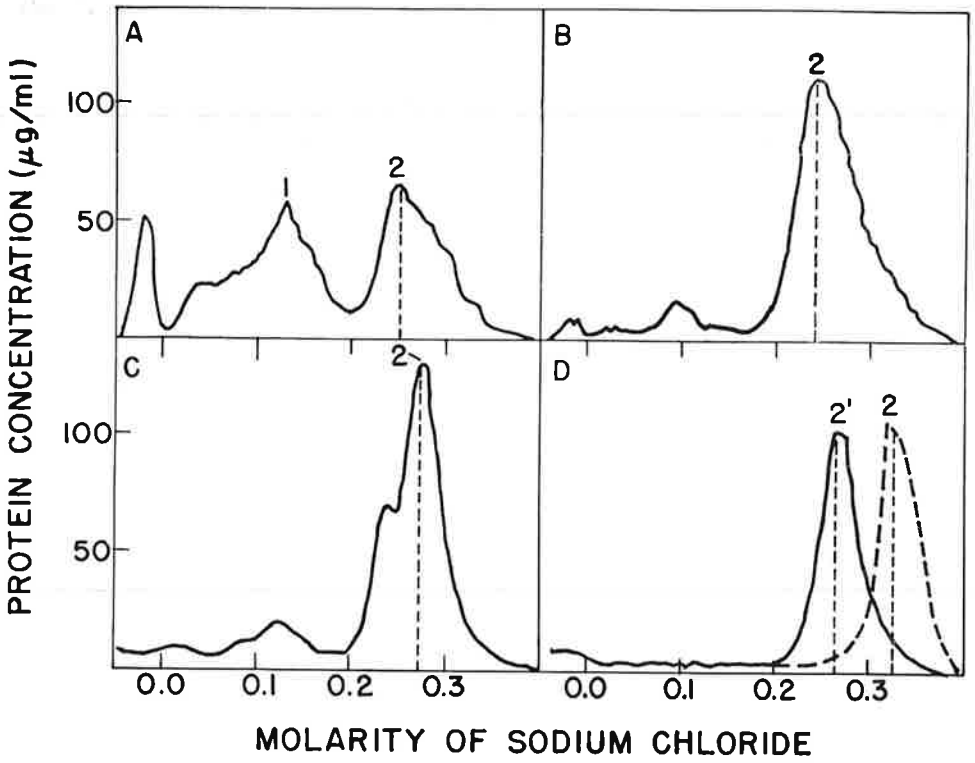


Figure 6. DEAE-cellulose chromatography of peanut proteins: (1) α-conarachin; (2′,2) α-arachin; (A) total cotyledonary extract (S_1 of Figure 5); (B) classic arachin fraction (0/40); (C) precipitate from A at 2°C. for 17 hr. (P_3 of Figure 5); (D) 2′, α-arachin obtained by 40 % ammonium sulfate precipitation of C (P_4 of Figure 5), and 2, α-arachin obtained by chromatography of B on DEAE-cellulose. 10 mg of each sample was absorbed on 2 gm of cellulose in each case. Samples were eluted with 500 ml of a 0 to 0.6M NaCl gradient in phosphate buffer, pH 7.9, I = 0.03, in 5 ml fractions. (From Neucere, N. J. 1969. Isolation of α-Arachin, the major peanut globulin. Anal. Biochem. 27:15-24.)

indicated a heterogeneous substance. This minor constituent was removed by further fractionation with ammonium sulfate. However, further precipitation of P_3 with ammonium sulfate yielded only one component (see 2′ of Figure 6D).

The second procedure used to obtain pure α-arachin was to twice chromatograph classically prepared arachin on DEAE-cellulose. The profiles of α-arachin prepared by both methods are shown in Figure 6D. It is of interest to note that α-arachin prepared by the second method (Figure 6D-2) is eluted at a higher salt concentration than α-arachin prepared by the cryoprecipitation method (Figure 6D-2′). This decrease in solubility after chromatography on DEAE-cellulose was also noted by Dechary (1963) for the classically prepared arachin fraction.

The high degree of purity of α-arachin prepared by both methods was confirmed by zone electrophoresis, immunoelectrophoresis, and sedimentation coefficient studies. Photographs of electrophoretic patterns on polyacrylamide gel are shown in Figure 7. The α-arachin protein is represented as the B-2 band in Figure 7; the total proteins of the peanut extract are A-1; the classically prepared arachin and conarachin fractions are A-2 and A-4, respectively. Sedimentation patterns at two different ionic strength solutions of α-arachin prepared by the two methods and the cryoprecipitate, P-3, are

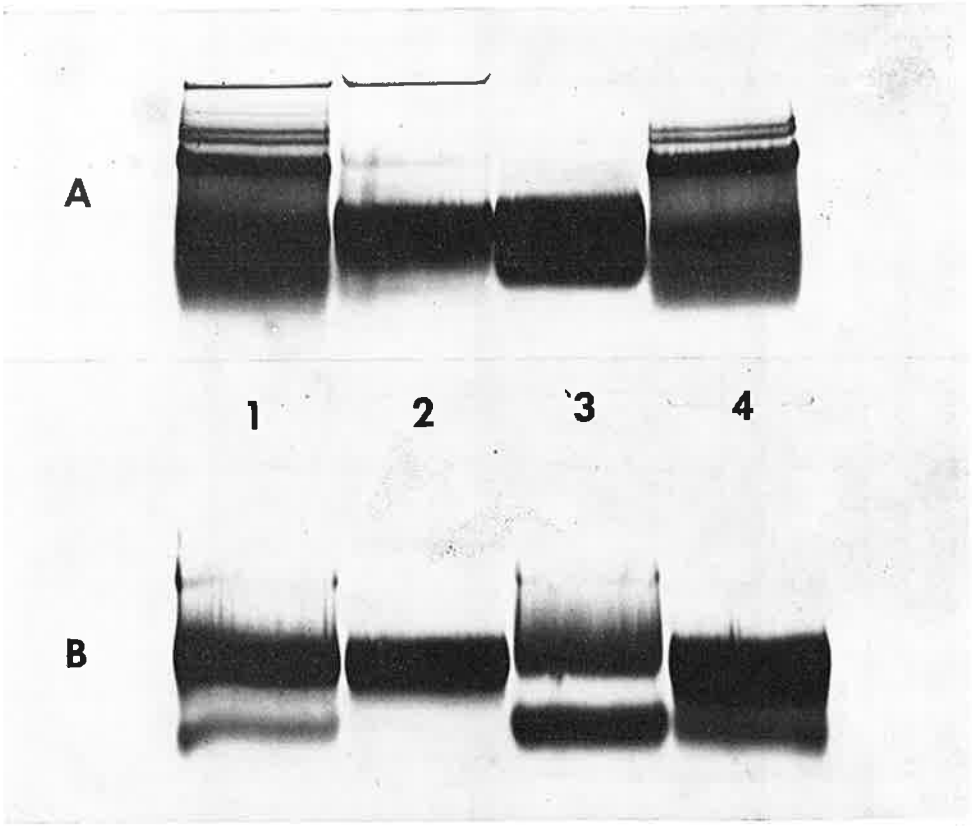


Figure 7. Zone electrophoresis of crude and purified protein fractions (two separate experiments); (A) 1—total proteins (A of Figure 6 or S_1 of Figure 5), 2—classic arachin fraction (B of Figure 6), 3—C of Figure 6 or P_3 of Figure 5, 4—classic conarachin (40/85); (B) 1—P_3 of Figure 5, 2—P_4 of Figure 5 (α-arachin), 3—P_5 of Figure 5, 4—P_6 of Figure 5. All samples approximately 0.1 mg protein in each case) were dialized against phosphate buffer pH 7.9, I = 0.03, before electrophoresis; separation was conducted in this buffer. (From Neucere, N. J. 1969. Isolation of α-Arachin, the major peanut globulin. Anal. Biochem. 27:15-24.)

shown in Figure 8. The cryoprecipitate, P-3, which exhibited one peak and a shoulder by chromatography on DEAE-cellulose (Figure 6C) and which showed two components by gel electrophoresis (Figure 7-B1), now appeared as two components of different sedimentation coefficients, 9.9S and 15.5S as seen in Figure 8-1. α-Arachin isolated by the cryoprecipitation method exhibited only one major component by analytical ultracentrifugation. Both methods showed only one major component having a sedimentation coefficient of approximately 15.5S.

The amino acid analysis of α-arachin prepared by cryoprecipitation, shown in Table 7, is comparable to the amino acid composition of arachin reported by Tombs (1965), Table 6.

Phytohemagglutinin. Proteins from seeds of certain plants are able to strongly agglutinate human and other red blood cells (erythrocytes). Phytohemagglutinins are of special interest to immunologists since these compounds are easily accessible and are an inexpensive source of blood grouping reagents. Phytohemagglutinins was extensively reviewed by Dechary (1968).

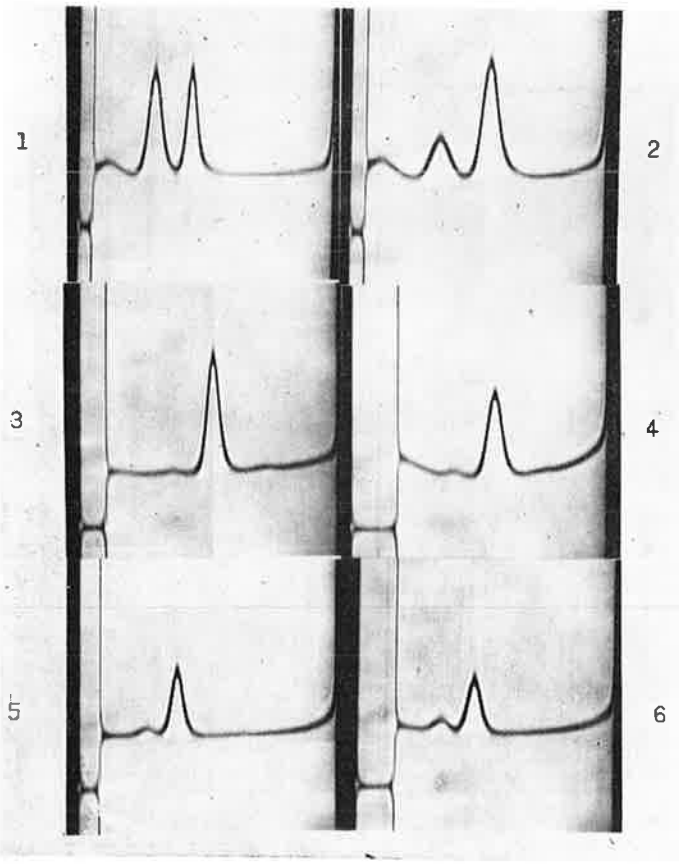


Figure 8. Sedimentation patterns of crude and purified arachin at two different ionic strengths (migration is from left to right: (1,2) P_3 of Figure 5, (3,4) α-arachin or P_4 of Figure 5, (5,6) α-arachin from DEAE-cellulose or D-2 of Figure 6, (1,3,5) phosphate buffer, pH 7.9, I =0.03, (2,4,6) phosphate buffer, pH 7.9, I = 0.2 Sedimentation coefficients (major peaks from left to right): (1) 9.9S, 15.5S, (2) 8.8S, 159S, (3) 15.3S, (4) 15.8S, (5) 15.6S, (6) 15.4S. The photographs were taken 28 min. after top speed (59,780 rpm). No corrections were made to reduce sedimentation coefficients relative to the viscosity and density of water at 20°C. and for zero concentration; S refers to the Svedberg unit. (From Neucere, N. J. 1969. Isolation of α-Arachin, the major peanut globulin. Anal. Biochem. 27:15-24.)

In the late fifties, Boyd and associates (1959) reported a hemagglutinin found in crude peanut extracts. A decade later, Dechary *et al.* (1970) partially purified the peanut hemagglutinin by salt extractions and gel permeation. The sedimentation pattern of the hemagglutinin indicated a major protein component of molecular weight ca. 20,000 and a minor component of unknown molecular weight. A comparison of the amino acid composition of the peanut hemagglutinin showed a remarkable similarity to a nonspecific hemagglutinin, concanavalin, which is found in jack bean meal. Agglutination of erythrocytes by peanut hemagglutinin indicated that the hemagglutinin was nonspecific also.

Subcellular Distribution. As a seed develops, it synthesizes and stores food in the form of proteins, carbohydrates, and lipids. When the seed approaches maturity, these processes diminish and the seed becomes a food storage entity. Under the proper conditions for germination, the stored food is consumed and a seedling begins to grow into a mature plant.

Table 7. Amino acid composition of α-arachin analyses are single values after 24 hr. acid hydrolysis (percent nitrogen = 14.3; acidic:basic = 2.32:1).

Amino acid	Grams/100 gm sample
1. Cysteic acid	—
2. Aspartic acid	12.07
3. Methionine sulfone	—
4. Threonine	2.47
5. Serine	4.11
6. Glutamic acid	19.51
7. Proline	2.38
8. Glycine	3.49
9. Alanine	3.79
10. Cystine/2	0
11. Valine	4.03
12. Methionine	0.07
13. Isoleucine	3.31
14. Leucine	6.23
15. Tyrosine	3.30
16. Phenylalanine	5.34
17. Lysine	2.14
18. Histidine	2.00
19. Ammonia	1.36
20. Arginine	9.42
TOTAL	85.00

From Neucere, N. J. 1969. Isolation of α-arachin, the major peanut globulin. Anal. Biochem. 27: 15-24.

Protein, one of the components of "stored food" in seeds, probably serves a dual purpose: first, as enzymes and other metabolic components, and secondly, as inactive storage proteins. These latter are the reserve proteins, which vary in physicochemical properties according to the species. In dicotyledonous plants, such as the peanut, globulins are usually dominant, and in many instances this particular class of proteins has been experimentally obtained in rather purified form, e.g., α-conarachin and α-arachin.

The fact that seed proteins are in specific locations in the seed has been known for over a century. In 1855, Hartig isolated subcellular particles from various oilseeds by a nonaqueous technique and showed that they contained a high concentration of globular protein. Hence the name "protein body" or "aleurone grain" (from the Greek "aleuron" meaning flour) evolved. Aleurone grains, or protein bodies, are spherical organelles that vary from 2 to 20 microns in diameter and, generally vary in population density according to their protein content. The isolation of these small particles during their early history proved difficult. Therefore, techinques were developed for removing proteins from the aleurone grains by solvent extraction methods.

That protein does exist in the aleurone grain has been demonstrated in a number of different seeds; e.g., soybean (Bils and Howell, 1963), peanut (Dieckert *et al.,* 1962), cottonseed (Yatsu and Altschul, 1963; Yatsu and Jacks, 1968), corn (Duvick, 1961), pea (Varner and Schidlovsky, 1963), wheat (Graham *et al.,* 1962), rice (Mitsuda *et al.,* 1967), and hempseed (St. Angelo *et al.,* 1968). In 1963, protein bodies were the main subject of a conference held on seed proteins (Talluto, 1963). Later the protein body was one of the topics covered in two review articles (Altschul *et al.,* 1966, Dechary and Altschul, 1966). Furthermore, Altschul and coworkers (1964) offered a novel means of classifying seed proteins by suggesting that the reserve proteins contained within the aleurone grains be designated as "aleurins" to distinguish them from those proteins in the cytoplasm.

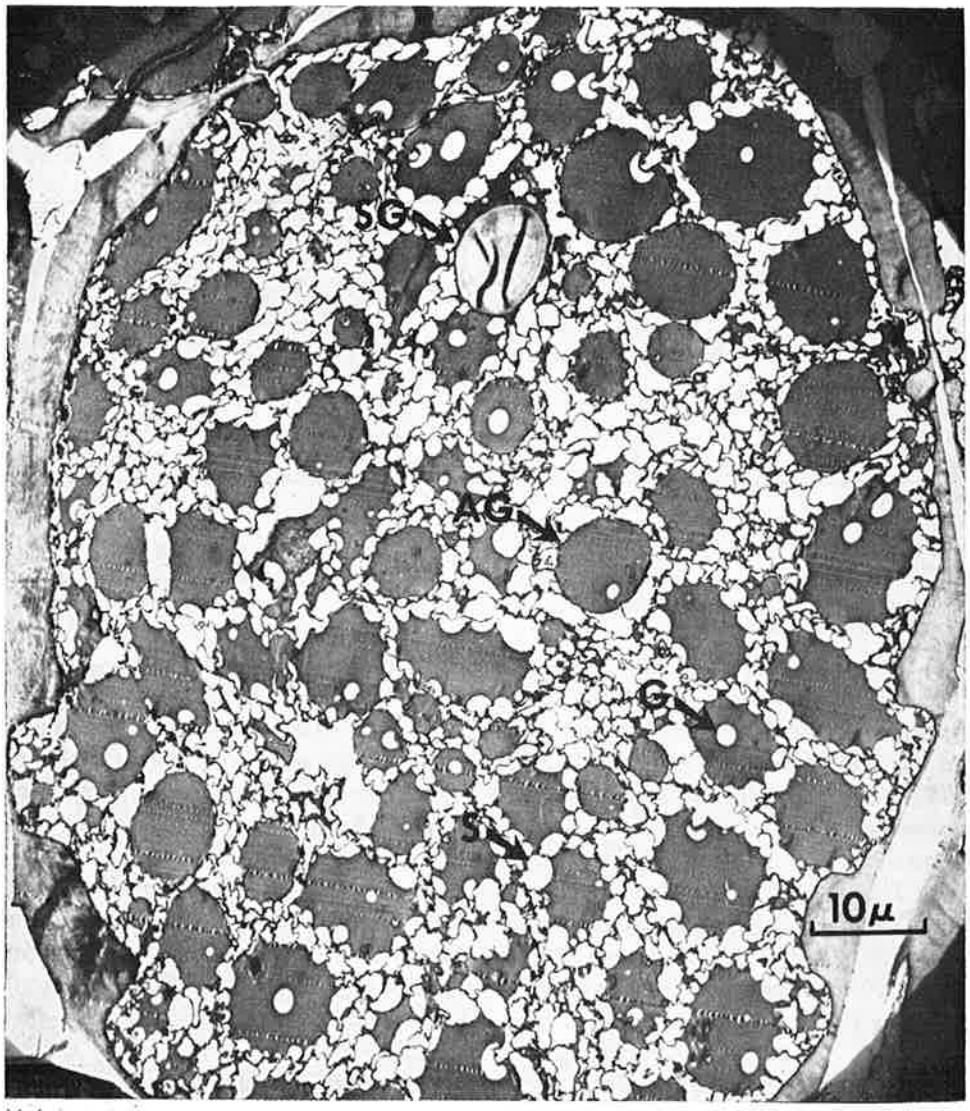


Figure 9. Electron micrograph of a cross section of peanut cotyledon. S, spherosomes; G, globoids; SG, starch granule; AG, aleurone grain. Courtesy of Dr. L. Y. Yatsu.

Upon germination of seeds there is an ordered series of events leading to the degradation of storage protein in the protein body. As germination progresses, the protein bodies swell and seemingly develop internal cavities. Eventually these swollen bodies disintegrate into many fragments that are digested and finally disappear. This observation has been reported in a number of seeds: peanut (Bagley *et al.*, 1963); yucca (Horner and Arnott, 1965); pea (Bain and Mercer, 1966); French bean (Opik, 1966); and soybean (Tombs, 1967).

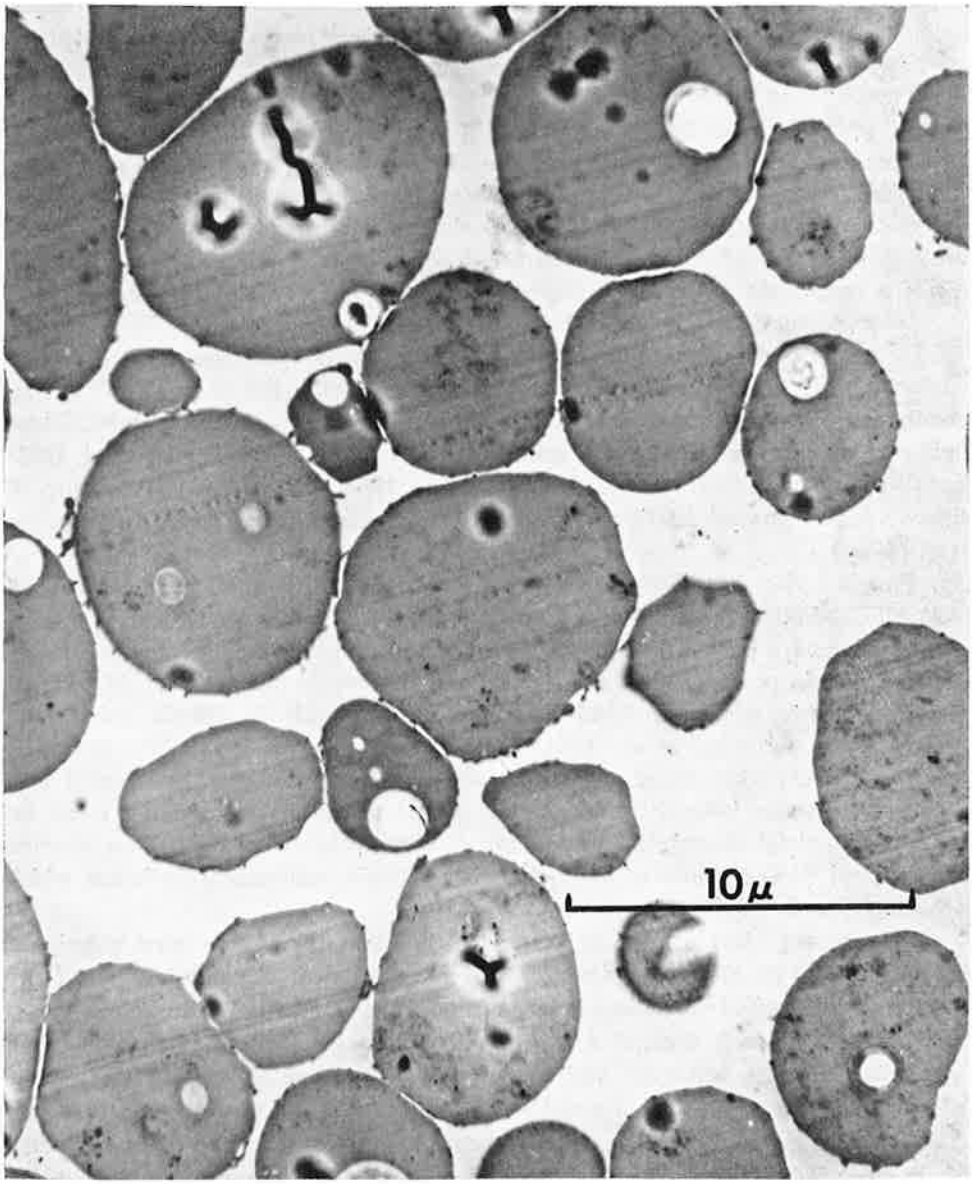


Figure 10. Electron micrograph of a cross section of isolated aleurone grains. (From Daussant, *et al.*, 1969. Immunochemical studies on *Arachis hypogaea* proteins with particular reference to the reserve proteins. I. Characterization, distribution, and properties of α-arachin and α-conarachin. Plant Physiol. 44:471-479.)

With the increase in our knowledge of the ultrastructure of cells over the past few decades and the introduction of electron microscopy, numerous subcellular organelles have been identified and studied. Consequently, there has been a renewed interest in aleurone grains.

Dieckert *et al.* (1960) isolated intact aleurone grains from peanut cotyledons using a nonaqueous density gradient technique comprised of carbon tetrachloride and cottonseed oil. These isolated, protein-rich fractions, which appeared similar under the electron microscope to the protein bodies seen in tissue sections, contained 11 and 13% nitrogen, respectively. Both fractions had similar protein profiles when examined by chromatography on DEAE-cellulose, but one of them contained practically all of the phytic acid, the major storage form of phosphorus in plant seeds.

Figures 9 and 10 are electron micrographs of a cross section of a peanut cotyledon cell and isolated protein bodies, respectively. Tightly packed inside the cell (Figure 9) a number of subcellular particles are easily distinguishable. For example, the most easily recognized organelles are the large, potassium permanganate stained aleurone grains (protein bodies) marked "AG." The small electron-transparent particles found inside the aleurone grains are the globoids, marked "G," which were shown (Lui and Altschul, 1967) to contain the phytic acid in cottonseed. A starch grain (SG) is also observable.

Since peanut is an oilseed, and the oil in kernels ranges from 44-56% (Eckey, 1954), then one would expect to see a very large volume of spherosomes, the lipid-containing subcellular particles found in seeds. These particles, indicated by an "S" in Figure 9, were not potassium permanganate positive as were the aleurone grains. These lipid storage bodies were recently described in the literature (Yatsu and Altschul, 1963; Frey-Wyssling and Mühlethaler, 1965; Jacks *et al.*, 1967; Ory *et al.*, 1968). Figure 10 shows a highly purified fraction of aleurone grains isolated and reported by Daussant *et al.* (1969).

Protein bodies have also been isolated from peanuts by other methods. Altschul *et al.* (1961) isolated them from peanut cotyledons with a high molecular weight osmotic agent, Carbowax, a polyethylene glycol. They demonstrated that 75% of the total soluble proteins were in protein bodies, and showed that they would dissolve in 0.5M buffered sucrose. However, when unbuffered 0.25M sucrose was used, the protein bodies were isolated intact (Altschul *et al.*, 1964). The protein obtained from these organelles was shown by chromatographic and sedimentation coefficient studies to be identical with the arachin fraction isolated by the classical procedure. Conarachin was not found associated with these aleurone grains and was thought to be cytoplasmic. Thus, arachin, the principal storage protein of the peanut, is an aleurin, and conarachin is not, which was recently confirmed by Neucere and Ory (1970).

In a thorough investigation on peanut proteins using immunochemical techniques, Daussant *et al.* (1969) detected fourteen antigenic constituents in the total cotyledonary extract. They identified the major proteins of the classic arachin and conarachin fractions and found that arachin contains four antigens (the major one called α-arachin) and conarachin contains two (α_1- and α_2-conarachin) as shown in Figure 11. When the effect of temperature on the antigenicity of peanut proteins was investigated (Neucere *et al.*, 1969; Ory *et al.* 1970), α-arachin remained antigenically active after being heated for one hour at 145°C. All of the other antigenic constituents were destroyed by that treatment.

Daussant *et al.* (1969) isolated two types of protein bodies, one very large in diameter and the other much smaller. Both of these appeared similar in their qualitative anti-

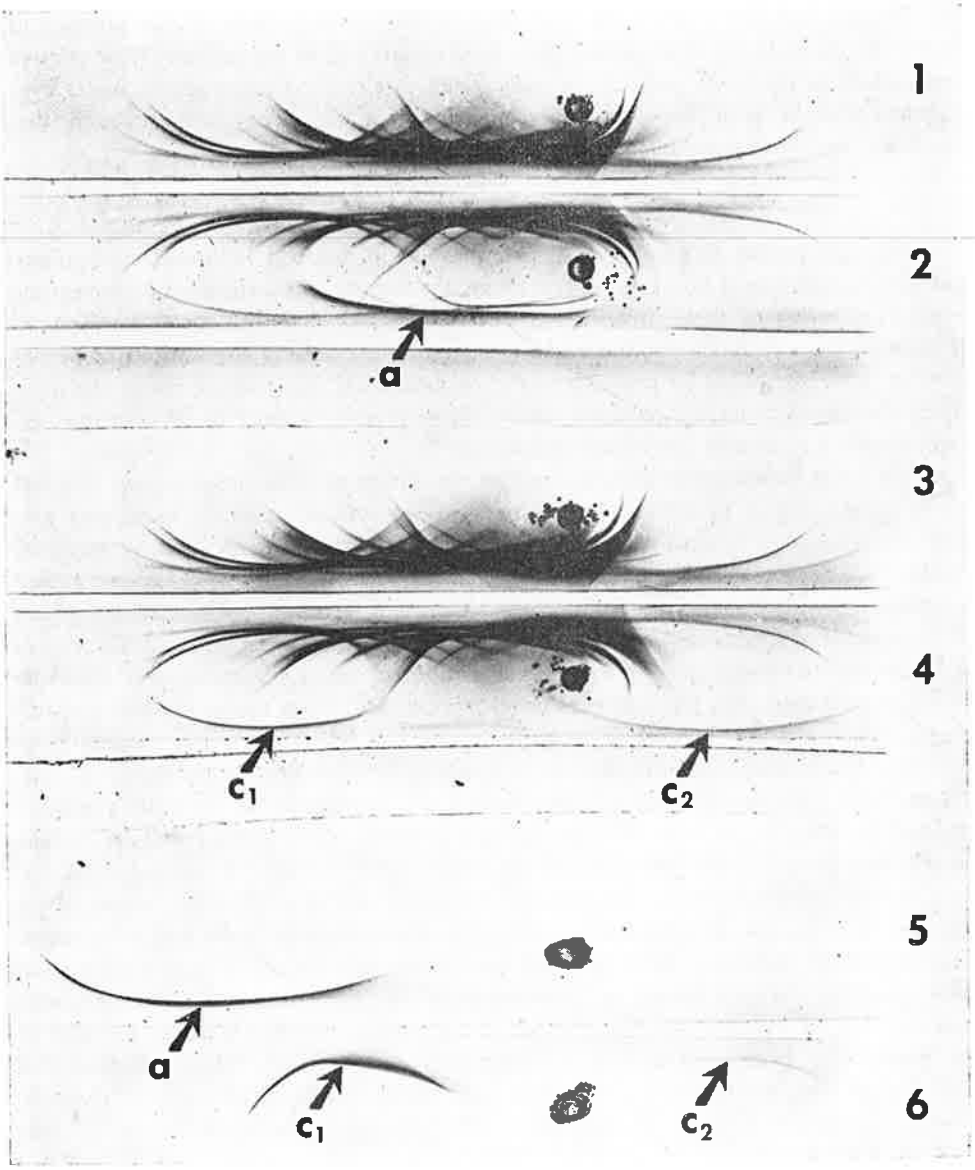


Figure 11. Immunoelectrophoretic analysis of proteins of the peanut. 1,2,3,4) Total cotyledonary extract; 5) Arachin; 6) α-Conarachin; Immune serum anti-cotyledon in trough below 1, 3, and 5; Immune serum anti-arachin in trough below 2; Immune serum anti-α-conarachin in trough below 4. a α-arachin; C_1 α_1-conarachin; C_2 α_2-conarachin. (From Daussant, *et al.*, 1969. Immunochemical studies on *Arachis hypogaea* proteins with particular reference to the reserve proteins. I. Characterization, distribution and properties of α-arachin and α-conarachin. Plant physiol. 44:471-479.

genic compositions. Extensive analysis of the protein within revealed that α-arachin was in both the large and the small aleurone grains, but α_1-conarachin was not associated with either particle. The subcellular distribution of α_2-conarachin was less definite since it was found in all fractions, both large and small protein bodies and in the cotyledons, but constituted a larger proportion of the proteins in the soluble cytoplasmic fraction than in either the large or small protein body fractions.

Neucere and Ory (1970), in describing parallel investigations on the proteins in peanut cotyledonary and axial tissues, their localizations within the cell, and their relative distributions in these two parts of the seed, found that α_2-conarachin predominates over α_1-conarachin in both the axial and cotyledonary tissue. They also reported that α-arachin and α_2-conarachin predominate in the cotyledonary tissue.

Summary

Although peanut proteins have shown promise in nonfood industrial applications, including production of fibers, glues, and sizing, the current world situation of impending protein utilization of these proteins has directed research activities towards utilization of these proteins in foods and feeds. Also, many of the nonfood applications of peanut proteins have been filled by products of the petrochemical industries. Even in the more highly developed countries, nonfood uses of these proteins appear to be uneconomical applications of a valuable nutritional commodity.

The many fundamental investigations on the nature of these proteins have revealed an increasing degree of complexity with increasing sophistication of techniques employed. Ritthausen's original globulin was fractionated into "arachin" and "conarachin" by the "salting out" technique. Applications of ultracentrifugation and light-scattering techniques revealed that these proteins were actually association-dissociation systems whose state of aggregation depended upon the nature of their environments. The failure of electrophoretic investigations to detect the different states of aggregation occurring with environmental changes is of theoretical interest, indicating the unsuitability of this method when changes in state of aggregation are suspected. The more modern techniques of low-temperature precipitation (cryoprecipitation) and chromatographic fractionation on diethylaminoethyl-cellulose verified the complexity of these proteins, and resulted in isolation of two partially purified fractions, α-conarachin and α-arachin. The former proved to be heterogeneous by zone electrophoresis and sedimentation criteria and exhibited association-dissociation phenomena, but α-arachin was homogeneous by these criteria, and by immunoelectrophoresis. Electron microscopy and nonaqueous density gradient techniques have indicated that reserve proteins of the peanut are packed into subcellular particles known as "protein bodies" or "aleurone grains." These particles contain 75% of the total soluble proteins of the peanut. Other subcellular particles of the peanut also have been described. Immunochemical techniques have revealed that the classical arachin contains four antigens (the major one being α-arachin) and conarachin contains two (α_1- and α_2-conarachin). The α_1-conarachin is apparently nonparticulate while α_2-conarachin is particle-bound.

Thus, the state of knowledge concerning these complex proteins has been advanced considerably. Much, however, remains to be done, especially larger-scale preparations of these homogeneous proteins to permit more detailed evaluations of their compositions and properties. It is expected that this research will lead to optimal utilization of the peanut in its evident main role of contributing to alleviation of protein hunger throughout the world.

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