decreases, the error involved in substituting them for $s_{20,w}^0$ and $D_{20,w}^0$ in the calculation of a molecular weight will be even lower. Unfortunately, molecular weights calculated from the combined data of sedimentation and diffusion constants are dependent to a considerable extent on the value of the partial specific volume, $\bar{V}$, an error of 1% in $\bar{V}$ causing an error of about 3% in the final result. The values of 4.6 for $s_{20,w}^0$ and 6.1 for $D_{20,w}^0$, often quoted (e.g. Oncley *et al.* 1947), give a result of 68 500, but the present work gives 61 500 if we assume the same partial specific volume, 0.733.

SUMMARY

1. The albumin fractions of a number of normal human sera have been isolated electrophoretically.
2. Sedimentation constants for these preparations in the concentration range 0.2–0.3% are con-

siderably lower than the hitherto accepted values. The reasons for this have been thoroughly explored.

3. Diffusion measurements in the Gouy diffusometer show small, statistically significant variations from one preparation to another.

The author thanks Prof. E. C. Dodds, F.R.S., for the help, advice and encouragement which he has given during this work. He is indebted to Dr J. M. Creeth for many stimulating discussions, particularly in regard to the diffusion measurements. Thanks are due to Dr R. A. Kekwick for making possible the ultracentrifuge experiment at the Lister Institute, to Dr A. G. Ogston for the β -lactoglobulin solution, and to Drs D. N. Baron and J. M. Johnstone for providing the sera. The earlier part of this research was carried out whilst the author was in receipt of a personal grant from the Medical Research Council, the final phases being completed during the tenure of an I.C.I. Research Fellowship. The work described in this paper formed part of a thesis approved for the degree of Ph.D. in the University of London.

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The Proteins of *Arachis hypogaea* and Fibre Formation

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(Received 10 May 1951)

In the course of development of a textile fibre from the proteins of the groundnut (*Arachis hypogaea*) these proteins have recently been the subject of considerable study. Included in this has been the laboratory fractionation of the proteins and an examination of the fractions as fibre-forming agents.

The protein of the groundnut appears to have been first investigated with other vegetable protein

systems by Ritthausen (1880), who extracted the proteins from the oil-free groundnut meal with aqueous sodium chloride and weakly basic solutions, and precipitated them by acidification. Ritthausen considered the solids so obtained to be identical. The investigations of Johns & Jones (1916) have indicated that the total protein of the nut consists of globulins and a very small amount of heat-coagulable albumin. They found it was possible, by

means of ammonium sulphate fractionation of a sodium chloride extract of groundnut meal, to separate the globulins into two fractions—'arachin' and 'conarachin'—which have been found to differ in optical rotation and in the content of sulphur, basic nitrogen, lysine, methionine, cystine, threonine, tryptophan and tyrosine. Later Jones & Horn (1930) stated that arachin could be prepared from 10% sodium chloride extract by dilution until the extract became cloudy, followed by saturation with carbon dioxide, or by the addition of 2 vol. of saturated ammonium sulphate to 3 vol. of the extract. Arachin could also be obtained by dilution alone. Conarachin, which was the more soluble fraction, could not be isolated by dilution, but could be precipitated by dialysis from the filtrate after precipitation of the arachin fraction, or by complete saturation with ammonium sulphate. Conarachin can be coagulated at a lower temperature than arachin. The fractional precipitation of protein mixtures is known to be an arbitrary procedure (cf. Cohn *et al.* 1940) and it is not easy to decide whether the arachin and conarachin are single proteins or mixtures in more or less constant proportions. In order to determine whether these protein fractions were homogeneous, Irving, Fontaine & Warner (1945) conducted electrophoretic analyses on groundnut meal, arachin and conarachin. Their results indicated that the meal contained at least three and probably four components, and that the arachin and conarachin fractions each consisted of mixtures of at least two components. Johnson (1946) investigated the groundnut protein fractions by the ultracentrifuge. He found that the arachin obtained by dilution and addition of carbon dioxide consisted of at least two sedimenting species of protein, while arachin obtained from ammonium sulphate fractionation had only one major constituent. Danielsson (1949) has made a study of seed globulins in the ultracentrifuge and showed the globulins of many leguminous plants, including *A. hypogaea*, to contain 'vicilin' and 'legumin' with molecular weights of 186 000 and 331 000 respectively. He made no attempt to relate his fractions to arachin and conarachin. Karon, Adams & Altschul (1950) have shown by electrophoretic measurements that groundnut protein consists of two major and several minor components. If the protein was separated from a meal which had been washed with water and adjusted to pH 5 to remove soluble sugar and phytin, the major component separated into two almost equal fractions. There is evidence of the close relationship between the protein components of the groundnut in the interconversion from one component to the other which takes place in alkaline buffer solutions.

Johnson, Joubert & Shooter (1950), in more recent work, have investigated reversible dissociation of the arachin fraction. They have shown that the

parent molecule of this fraction with a molecular weight of about 400 000 dissociates under appropriate conditions into two sub-units of molecular weight 200 000. The parent and sub-molecules are very easily distinguishable with the ultracentrifuge.

From the literature it appeared that the most practical method for preparing protein fractions in experimental quantities was fractionation by differential solubilities. Differential solubility in various concentrations of sodium chloride, indicated by Jones and his co-workers and Johnson and his co-workers, seemed the simplest procedure. The possibility of fractionation using salts other than sodium chloride and even aqueous solvents other than salt solutions has not been overlooked, but the work reported here has been restricted to the use of sodium chloride. Methods of fractionation based on electrophoresis or ultracentrifugation appeared to offer no opportunities on the scale necessary.

EXPERIMENTAL

In the literature the principal method of extraction of the protein from groundnut meal is by extraction with salt solution. In the extraction of protein for fibre manufacture by the 'Ardil' process described by Traill (1945) dilute aqueous caustic soda (0.1 g. NaOH/l. of water) is employed, and the protein is precipitated from this solution by adjusting to the pH of lowest solubility, which is 5. Protein extracted and precipitated in this way may be washed and spray-dried. This spray-dried product is the starting point for the investigation described in the present paper.

The work on fractionation of spray-dried groundnut protein divided itself into three main sections:

(a) The investigation of the solubility of spray-dried protein from nuts from various sources in aqueous solutions of NaCl.

(b) The study of the fractions present by a chromatographic technique.

(c) Isolation of suitable fractions in sufficient quantity for tests of fibre-forming properties.

The investigation of the solubility of spray-dried groundnut protein from nuts from various sources in aqueous solutions of sodium chloride

The separation of groundnut protein into two fractions, one of which was soluble in both 10 and 2% (w/v) aqueous NaCl in slightly acid solution, and one which was soluble in 10%, but insoluble in 2% aqueous NaCl under the same conditions, may be a considerable simplification of complex solubility phenomena. It was decided to study in more detail the effects of varying salt concentration and pH value over a limited range. This range was from 0 to 10% NaCl concentration and pH 1–7, and within this range the protein solubilities were determined. Spray-dried protein (4 g.) was shaken in 100 ml. aqueous salt solution of the stated concentration, the pH was adjusted with NaOH or HCl (dilution due to this was kept negligibly small) until equilibrium was attained at the required value and the mixture was filtered. The residue was washed with large volumes of methylated spirit to remove the water and at least some of the salts

and then the methylated spirit was washed out with acetone. The residue was oven-dried and weighed. By this method the protein which remains behind on the filter paper will be contaminated with NaCl. The errors caused by this salt were less than 2% of the weight of residual protein. Difficulties were encountered because in each pH range at certain salt concentrations the whole of the protein residue swelled and became jelly-like. Under these conditions the

Fig. 1. Fractional solubility of groundnut protein (batch 1) in aqueous sodium chloride. For details see text. Δ , pH 7; $\square$, pH 5; $\times$, pH 3; $\circ$, pH 1.

Fig. 2. Fractional solubility of groundnut protein (batch 2) in aqueous sodium chloride. For details see text. Δ , pH 7; $\square$, pH 5; $\times$, pH 3; $\circ$, pH 1.

distinction between soluble and insoluble was far from clear-cut. Figs. 1 and 2 show the solubilities of protein from two samples of groundnuts (from two different areas in Africa) in aqueous NaCl solutions (0–10%, w/v) in the pH range 1–7. Such data are best considered as a solid diagram, and such solids can be visualized from Figs. 1 and 2, the curves being cross-sections. In Fig. 1 the following may be observed: (i) the point of minimum solubility (pH 5 in water) becomes more acid as salt concentration increases; (ii) at pH 7 the solubility is high at all salt concentrations; (iii) there is a fall in solubility between salt concentrations of 9 and 6% at pH 5. This last appears to be due to the insolubility of Jones's arachin at low salt concentration. In Fig. 2 it may be seen that the fall in solubility described in (iii) is not present. The protein of Fig. 2 appears to be deficient in arachin. It may be seen that separation into the two solubility forms of Jones (both soluble in 10% salt but only one soluble in 2% salt) can be carried out at pH 5. This pH value corresponds reasonably with that attained by Jones on the addition of CO_2 and exactly with the pH value used by Johnson. The spray-dried protein from both sources contains a fraction which is insoluble in 10% salt; this at pH 7 amounts to about 30% of the whole protein and at pH 5 to about 50%.

The study of fractions present by a chromatographic technique

At this stage in the work the need for a rapid method for identifying arachin and conarachin was felt. A chromatographic technique was evolved which showed considerable promise.

If a 10% (w/v) aqueous solution of NaCl is allowed to diffuse from a reservoir across a filter paper already wet by water, the diffusion front will not be sharp but will consist of a boundary area with salt concentrations varying from 0 to 10%. The movement of this boundary area will be accelerated by permitting free evaporation of water from the filter

Fig. 3. Method of making protein chromatogram. Protein solution is applied over hatched area.

paper. It has been found that if the first of the diffusing salt solution also had groundnut protein in solution, separation into two forms, arachin and conarachin, occurred at the diffusion front. This was most easily observed by dyeing the protein on the paper by the method of Jones & Michael (1950). A study has not yet been made of the mechanism of this separation, but it seems likely that displacement chromatography or salting-in chromatography may occur (Shepard & Tiselius, 1949; Swingle & Tiselius, 1951; Tiselius, 1948). The practical details of the development of the chromatogram are illustrated by reference to Fig. 3. A filter paper is cut into the mushroom shape shown and is soaked with distilled water. Any surplus is removed by blotting. One drop of a solution of groundnut protein in 10% aqueous NaCl is placed in the area shaded. This forms a barrier above the wick which dips into a vessel containing 10% aqueous NaCl. As the water from the paper evaporates the salt solution spreads upwards and outwards. After about 15 min. the paper is ready for dyeing.

Using this method the arachin and conarachin separated quite clearly into lines with a separation of 2–3 mm. (Fig. 4). The front line, being in the more dilute part of the zone, was identified as conarachin and the rear line as arachin. The identity of these lines was tested in several ways. According to Jones & Horn (1930) conarachin is precipitated by heating. A solution of the protein in 10% NaCl solution which gave two lines originally, gave only one

after boiling and filtering off the precipitate (Fig. 5). This one line was presumably the arachin line. If arachin is precipitated from the original 'two-line' solution by dilution and filtered off, the filtrate should consist of conarachin alone. When such a filtrate was fortified with salt to 10% again the resulting figure showed one line only, the conarachin line. This solution, mixed with the arachin filtrate from the previous experiment, gave a two-line diagram.

Fig. 4. Chromatogram of groundnut protein in aqueous sodium chloride. Conarachin line outside arachin line.

Similar confirmation was obtained by using the dilution precipitate as the source of arachin and the 2% salt extract of the whole protein as the source of conarachin.

Two routes are available for the preparation of arachin and conarachin from spray-dried groundnut protein at pH 5.

In a study of these routes whole protein was extracted first with 2% (w/v) aqueous NaCl to give a solution of con-

arachin. The residue was extracted with 10% (w/v) aqueous NaCl to give a solution of arachin and a residue of the fraction insoluble in 10% (w/v) NaCl. Alternatively, whole protein was extracted first with 10% (w/v) aqueous NaCl to give a solution of arachin and conarachin, and a residue insoluble in 10% (w/v) NaCl. The solution was diluted with four times its volume of water to precipitate the arachin. Conarachin remained in solution.

Fig. 5. Chromatogram of groundnut protein boiled in aqueous sodium chloride. Arachin line showing.

Fig. 6 shows a full investigation of the two routes using the chromatographic technique. From this there is no evidence of a form insoluble in 10% (w/v) aqueous NaCl and soluble in 2% (w/v) NaCl.

It would also appear that the chromatographic technique separates the proteins into the same fractions as solubility

Fig. 6. Chromatographic examination of fractions of groundnut protein.

Table 1. *Fibre-forming properties of fractionated groundnut protein*

(A = arachin; C = conarachin; X = insoluble in 10 % aqueous NaCl at pH 5.)

Fraction	Constituents	Extruded solution (g./100 g. water)		Fibre-forming properties	Remarks
		NaOH	Protein		
Whole protein	ACX	1.1	26	Good	Discrete filaments, easily stretched
Insoluble in 10% (w/v) NaCl	X	0.8	19	Good	
Insoluble in 2% (w/v) NaCl	AX	1.0	21	Good	
Arachin	A	0.9	24	Good	
Soluble in 10% (w/v) NaCl	AC	0.7	18	Bad	Stuck filaments, difficult to stretch
Conarachin	C	0.8	15	Unspinnable	Filaments, when formed, too weak to remove from coagulating bath

methods and shows no sign of the complexities in the solubility fractions shown by ultracentrifuge and electrophoresis work. No examination of the present preparations was made by the latter two methods, and, until this is done, it must be supposed that the chromatographic separation is as arbitrary as Cohn *et al.* (1940) consider fractional precipitation to be.

Isolation of suitable fractions for fibre-formation tests

The primary object of this research is the development of a protein fibre made from groundnut protein. Experience has shown that variations occur in the fibre-forming properties of different lots of protein. An accurate method of measurement of these fibre-forming properties has yet to be developed, but in filament extrusion in the laboratory, in accordance with the 'Ardil' process, there is no difficulty in assessing fibre-forming properties as good, bad or unspinnable. This assessment is based on the ability, on extrusion from a many-holed spinneret, to form discrete filaments which, during coagulation, may be stretched to several times the extruded length and may be wound out of the coagulating bath. Where these conditions are fulfilled, fibre formation may be regarded as good. Where the first two conditions are not fulfilled, but the third is, the fibre formation is bad. Where all three are unfulfilled the material is unspinnable.

With this as a basis, extrusion experiments were performed on whole groundnut protein and on suitable fractions of the same lot of protein. The protein fractions were prepared for extrusion by dissolving them in dilute aqueous caustic soda in accordance with the directions of Traill (1945). After maturing for 20 hr. at 20° solutions of suitable viscosity were obtained. These solutions were extruded through viscose spinnerets into a coagulating bath at 30° composed of 11 ml. H₂SO₄ and 250 g. Na₂SO₄ dissolved in 1 l. of water. Table 1 shows the fractions used, the composition of the solution extruded and the results obtained.

The mixed fraction which is insoluble in 2% (w/v) NaCl (Table 1) contained arachin and insoluble fractions in the same proportions as they occurred in the parent protein, and similarly for the arachin and conarachin in the mixed fraction which was soluble in 10% (w/v) sodium chloride. Both arachin and insoluble fractions were good fibre-formers and conarachin was not. A mixture of arachin and the insoluble fraction was also a good fibre-former. Conarachin could be tolerated in the proportion in which it

occurs in the whole protein, but when the proportion was increased by removing the insoluble fraction, fibre formation deteriorated.

No made-up mixtures of the fractions were studied in these experiments, but it is proposed to continue the work with such studies.

DISCUSSION

The protein of the groundnut, whether extracted by aqueous sodium chloride or by aqueous caustic soda is of some complexity. The position is not greatly clarified by considering the differences in the components shown by the solubility evidence, the ultracentrifugal evidence and the electrophoretic evidence. From the point of view of commercial fibre formation the presence of a considerable fraction showing no fibre-forming properties whatever is interesting. There is immediate need for a study of the interaction of three selected fractions (arachin, conarachin and insoluble fraction) on the properties of the finished fibre. The fraction which is insoluble in 10% (w/v) aqueous sodium chloride at pH 5 requires further study. Some of this fraction is soluble in 10% sodium chloride at pH 7 while more of it is not. It may well consist of a less soluble derivative of either arachin or conarachin, but no experimental evidence is presented in support of this view.

SUMMARY

1. The solubility has been determined of groundnut protein in aqueous solutions of sodium chloride at concentrations up to 10% (w/v) and over the pH range 1-7.

2. A paper-chromatographic technique has been developed which demonstrates the presence or absence of arachin and conarachin.

3. Arachin, conarachin and a third fraction which is insoluble in 10% aqueous sodium chloride have been studied as fibre-forming agents.

The author wishes to thank Mr J. E. L. Thomas for helpful discussion, and Mr W. Hart for much of the experimental work.

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A Technique for the Identification and Separation of Enzymes by Paper Chromatography

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(Received 19 June 1951)

Paper partition chromatography, first described by Consden, Gordon & Martin (1944), has proved an effective method for separating the simpler chemical compounds from complex mixtures such as protein hydrolysates and for identifying them on a micro-scale. So far, paper partition chromatography has been used chiefly in the analysis of amino-acids, carbohydrates, purines, nucleic acids, organic acids, vitamins and other substances of biological importance. Very little is recorded in the literature on the application of this technique or of adsorption chromatography on paper to the study of enzymes, except the recently published reports by Franklin & Quastel (1949), Mitchell, Gordon & Haskins (1949) and Reid (1950). Franklin & Quastel (1949) have reported their preliminary investigations on the enzyme urease. They studied the movement of this enzyme on paper, using a cysteine-glycine solution as the developing solvent and determined the activity of the enzyme on paper, manometrically. They showed that the movement of the enzyme on paper can be followed by this technique, without the enzyme losing its activity during the experiment. However, they did not attempt to separate enzymes by this technique. Mitchell *et al.* (1949) made use of the 'chromatopile' (a pile of filter-paper disks) for the separation of the constituents of takadiastase preparations, by placing the enzyme mixture near the top of the filter-paper pile and fractionating by a process involving solubility in a concentration gradient. They report that some separation of adenosine deaminase from amylase was obtained by using aqueous ammonium sulphate as the solvent. In these methods the enzymes are located by carrying out a series of determinations of the activity of the enzymes

present in different parts of the paper. This procedure is very cumbersome and time-consuming for carrying out preliminary investigations on the movement of enzymes on paper. As a consequence, we have employed a simpler technique for locating the enzymes on paper, using agar plate containing the substrate on which the enzyme acts and suitable reagents for the detection of the hydrolysed products (cf. Goodall & Levi, 1947). The ease with which the enzymes on paper can be located by means of this technique prompted us to investigate the movement of enzymes on paper, with a view to separating enzymes by paper chromatography. Reid (1950) has given a preliminary account of similar studies, chiefly with fungal enzymes. The essential feature is the use of precipitating solvents such as aqueous acetone or alcohol or salt solutions as the moving phase (for references see Swingle & Tiselius, 1951). A preliminary account of this technique has already been given (Giri & Prasad, 1951).

It is intended in the present paper to demonstrate the usefulness and potentialities of this technique in the study of the chromatographic behaviour of enzymes and to describe some examples of the separation of enzymes, which have so far been achieved in preliminary investigations of some important enzyme systems. The study of other enzymes is in progress.

MATERIAL

The various enzyme preparations used in the present investigations were either isolated as dry powder by precipitation or obtained as aqueous extracts from natural sources.