THE GLOBULINS OF THE GROUND NUT

(*Arachis Hypogaea*)

I. INVESTIGATION OF ARACHIN AS A DISSOCIATION SYSTEM

by

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I. INTRODUCTION

JONES AND HORN[1] described two methods for the preparation of the arachin fraction of the ground nut globulins from a saline extract of the oil free meal;

i. dilution with water, or dilution to permanent cloudiness followed by saturation with carbon dioxide, and

ii. 40% saturation with ammonium sulphate.

It was later[2] shown, however, that these two methods of preparation did not yield the same product, a conclusion which was also confirmed in the electrophoretic analysis of FONTAINE, IRVING AND WARNER[3]. JOHNSON[4] found that the preparation (ii) of JONES AND HORN was almost homogeneous in the ultracentrifuge, containing a component of molecular weight approximately 400,000. It was further demonstrated that this component was the parent molecule (A_n) of a reversible dissociation reaction

$$A_n \rightleftharpoons nA \text{ where } \begin{cases} A_n \text{ had } s^0_{20} = 14.6\ s \\ A \text{ had } s^0_{20} = 9.5\ s \end{cases}$$

Dissociation of A_n was achieved by dilution of a salt solution of this component with water and acidification to p_H 5.0, the degree of dissociation increasing with dilution, whilst association was complete in 15% saturated (0.88 *M*) ammonium sulphate solution. Thus preparation (i) of JONES AND HORN (repeated by JOHNSON) produces a mixed fraction (A_n and A molecules) whose composition depends on the degree of dilution. Two sedimenting boundaries only were found in this fraction, one of which was identified as due to A_n molecules. It was further concluded from sedimentation and diffusion measurements that the integer n probably had the value 2 and that the dissociated molecule was in fact a half molecule of the parent. A uni-univalent salt, potassium chloride, caused only relatively slow association even in 2.8 *M* solution.

The fact that the two types of preparation ($A_2 + A$, and A_2 respectively) are chemically alike has been confirmed by an examination of their ultra violet absorption spectra[5].

The work of JOHNSON on the association-dissociation system has now been extended (Part I) and in addition a detailed study by electrophoresis at 20° C (Part II, p. 376) has been carried out.

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To ensure that measured physical constants of the A_2 and A (parent and dissociated) molecules were not mean values dependent upon the particular equilibrium position it was necessary to examine the nature of the equilibrium between them. Accordingly the results on the $A_2 \rightleftharpoons 2A$ equilibrium and the various factors involved are described before it is attempted to calculate molecular weight and to estimate molecular shape. Since the A_2 and A species sediment very differently, the identification of the two components has been performed in the following sections from sedimentation constants.

a. *Apparatus*

The determination of sedimentation constants and the examination of the distribution of the various molecular weight species was carried out with the air-driven ultracentrifuge equipped with the diagonal schlieren system described by EIRICH AND RIDEAL[6] and more recently by ALEXANDER AND JOHNSON[7]. Ultracentrifuge results are presented in the form of enlargements of actual recorded diagonal schlieren diagrams, the ordinate being proportional to dn/dx, the refractive index gradient in the cell, distances x being given by the abscissa. The area enclosed by the schlieren curve is proportional to the total refractive index change over the boundary. Movement, with time, of the schlieren peaks in such diagrams gives accurate sedimentation constants.

b. *Extraction and fractionation of the protein material*

The extraction and fractionation procedures of JOHNSON[4] have been modified slightly. Extraction of the protein from the meal was achieved with four separate volumes of 6–10% sodium chloride over a period of twenty four hours and the final saline extract was cleaned by filtration through a pad of Kieselguhr. Fractionation was as follows, the nomenclature being retained throughout this discussion

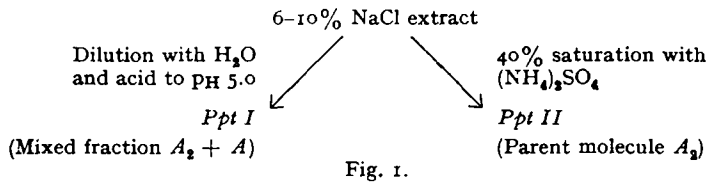

Fig. 1.

Fraction I (the original nomenclature of JOHNSON, *i.e.* fractions A and B has been changed to I and II respectively to avoid confusion with the A_2 and A molecules) was freeze dried from a small quantity of mother liquor whilst fraction II was stored for periods of several months under its mother liquor at 0°–5° C. Solutions of both fractions in the requisite buffers required prolonged dialysis (especially for electrophoretic analysis) to remove foreign electrolyte. The choice of the method of storage depends solely on convenience since it was demonstrated that samples of fraction I stored at 0°–5° C, freeze dried, or dehydrated with alcohol and ether possessed the same electrophoretic mobilities (Table I).

TABLE I

ELECTROPHORETIC MOBILITIES OF FRACTION I, IN PHOSPHATE BUFFER AT I = 0.10

Sample	pH of analysis	Δn	Mobilities · 10^{+4} ($cm^2\ sec^{-1}\ volt^{-1}$) *			
			Asc.		Desc.	
			fast	slow	fast	slow
1. Stored under supernatant at 0°–5° C	7.90	0.00111	1.13	1.04	1.09	1.00
2. Alcohol-ether dried	7.96	0.00105	1.08	0.97	1.04	0.94
3. Freeze dried	7.90	0.00105	1.12	1.00	1.10	0.94

* Only the descending fast mobility is correct when, as is usual, the conductivity of the dialysed protein solution is used in the calculation of mobilities. All other mobilities should strictly be referred to as "apparent mobilities". Error is estimated at ± 3%.

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These results also confirm the earlier observations of JOHNSON[2] who found that the materials stored according to the first two procedures gave identical sedimentation constants for their respective components. No severe change of structure therefore occurs within the molecule during these dehydration processes.

III. THE STATE OF THE PROTEIN IN THE NUT

Saline extracts possessed too high an electrical conductivity for electrophoretic analysis but could be examined in the ultracentrifuge. Thus a typical extract gave the largely one component sedimentation diagram of Fig. 2 for which $s^0_{20} = 14.4$ ($\pm$ 0.5) Svedberg units. A small quantity of very inhomogeneous low molecular weight protein (not the $s_{9.0}$ species) was also present.

Fig. 2. Sedimentation diagram of 6% NaCl extract of ground nut meal.

Since the action of 6% NaCl is insufficient to cause rapid association of any dissociated molecules (*e.g.* JOHNSON found that 21% KCl caused only slow aggregation of the $s_{9.5}$ species, taking several days) it would appear that the main part of the protein of the nut is present as the parent undissociated $s_{14.6}$ species.

IV. FACTORS NECESSARY FOR DISSOCIATION

a. *The preparation of the mixed fraction* I (A_2 and A molecules, or $s_{14.6}$ and $s_{9.0}$ species) which involves a decrease in both salt and protein concentration and a p_H lowering to ca. 5.0, causes partial dissociation of the parent $s_{14.6}$ species. It is of interest to enquire into the relative importance of these factors. The starting point for each of the following experiments was an aliquot volume of an approx. 1% solution of the undissociated protein, fraction II, in 6% NaCl at p_H 6.0. The final precipitates from these procedures were dissolved in phosphate buffer ($I = 0.10$; p_H 7.98) to a final p_H of approx. 7.60 and examined in the ultracentrifuge within one or two hours of solution. These latter conditions were chosen because it was shown that no change in the composition of either fraction I or II occurred during several hours in the phosphate buffer, $I = 0.10$ at p_H's near 8.0. Sedimentation velocity analysis of the final solutions therefore provided an exact analysis of the respective precipitates. The three procedures are now listed, the sedimentation diagrams, corrected sedimentation constants and experimental details for the final precipitates being given in Fig. 3.

1. Precipitation by decrease of both salt and protein concentration was achieved by diluting the starting solution tenfold with distilled water (final conc. 0.1 *M* NaCl, final p_H 6.0).

2. Precipitation at p_H 5.0 with a decrease in protein concentration only, involved a preliminary dilution with 9 volumes of the 6% NaCl before acidification.

3. Precipitation at p_H 5.0 with a decrease in salt concentration only. Here the protein concentration was maintained constant (at least until precipitation) by dialysing the original solution in a completely filled cellophane bag against distilled water whose p_H was constantly adjusted to 5.0.

Little dissociation occurred in procedures 1 and 2. In a similar experiment JOHNSON found no dissociation when an identical starting solution was precipitated at p_H 5.0 without preliminary dilution. It is now evident that a decrease in salt or protein concentration (or both) without acidification to p_H 5.0 or a decrease in protein concentration

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and a p_H lowering to 5.0 are insufficient to cause dissociation. Thus both the conditions of procedure 3, a low salt concentration and a p_H in the neighbourhood of 5.0 are essential factors in dissociating the parent species.

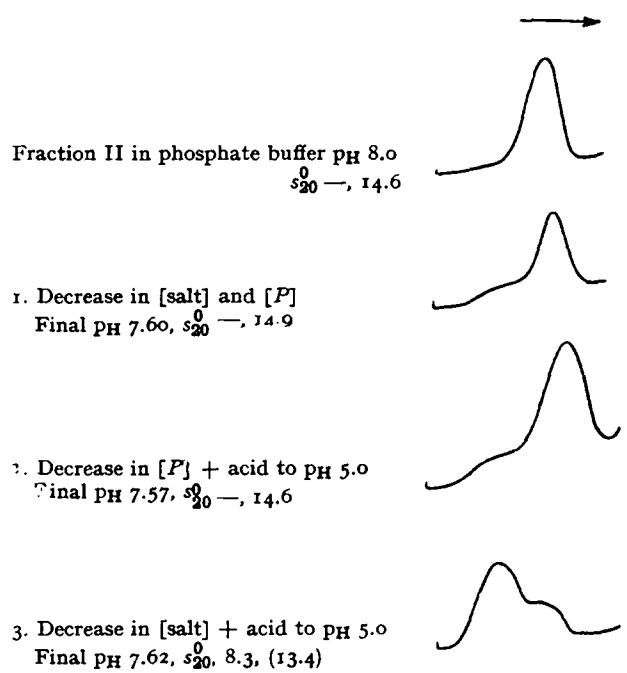

Fig. 3. Precipitation procedures on solution of fraction II in 6% NaCl at pH 6.0

Note. Sedimentation diagrams enlarged × 4.74 from original plate

To explain the need for the combination of these two factors the equilibrium system has been examined over a range of p_H values and salt concentrations in phosphate, barbiturate and to a smaller extent in borate buffers. The term barbiturate is used throughout as an abbreviation for diethylbarbiturate.

b. *Solutions in phosphate buffer at p_H's lower than 8.0*

The rates of the reactions

$$A_2 \rightleftharpoons 2A$$

are very slow in 0.05 *M* phosphate solutions at p_H 8.0 containing small quantities (≈ 1% saturation) of ammonium sulphate[4]. The minimum p_H's to which approximately 0.6% solutions of the fractions I and II in phosphate buffer at I = 0.10 may be taken without precipitation are 6.90 and 7.10 respectively. Solutions near these p_H's were therefore examined immediately after attaining these conditions and after standing for many hours. For example a solution of fraction I dialysed at p_H 7.90 (in phosphate buffer, I = 0.10) for 48 hours contained a slight excess of the lower molecular weight species (Fig. 4a).

Sufficient *N*/10 HCl was then added to decrease the p_H to 7.02 and the solution immediately re-examined (Fig. 4b). No visible change in the relative concentrations of the parent and dissociated molecules was detected. However, a second volume of the original solution at p_H 7.90 dialysed over a period of 88 hours to a p_H of 7.08 (spending the last

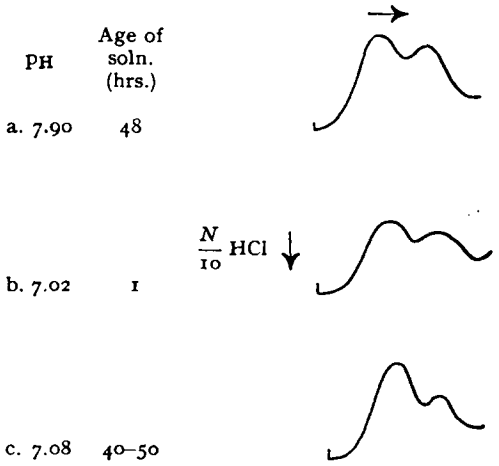

Fig. 4. Sedimentation diagrams of fraction I in phosphate buffer; I = 0.10

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40–50 hours at this final p_H) (Fig. 4c) showed some decrease in the concentration of the faster sedimenting parent species. (Control experiments with the same dialysis tubing showed that the final p_H in this type of dialysis was attained in about 40 hours provided the initial p_H was not considerably greater than 8.5). A similar result is obtained for solutions of fraction II (*i.e.* parent $s_{14.6}$ species only) which provide the better system since

a. the rate of dissociation is greater the higher the concentration of the undissociated molecule,

b. the appearance of even small traces of the $s_{9.0}$ species in diagrams originally without this component is readily detected.

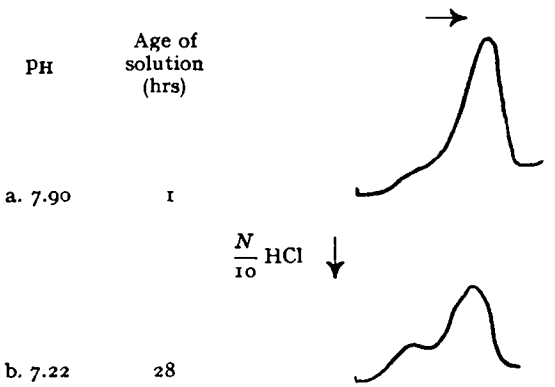

Fig. 5. Sedimentation diagrams of fraction II in phosphate buffer; I = 0.10

Thus an undialysed solution of this fraction at p_H 7.90 was practically homogeneous with respect to the $s_{14.6}$ component as in Fig. 5a, whilst the same solution acidified to p_H 7.22 and stood for 28 hours before examination contained some 20% of the dissociated form (Fig. 5b).

Reaction or dissociation rates do therefore increase at the lower p_H's in phosphate buffers.

The original values of the sedimentation constants of the A_2 and A molecules[4] were 14.6 and 9.5 *s* respectively, but it now appears from many determinations that the mean value for the dissociated A molecule is 9.0 ± 0.2 *s*. The dissociated component is now referred to in the following sections as the $s_{9.0}$ species, the parent component as before —the $s_{14.6}$ species.

c. *The equilibrium in sodium diethylbarbiturate buffers*

Although originally chosen for electrophoretic properties, the results on the equilibrium positions of the dissociation-association process and its rates of reaction in barbiturate buffer as determined by the ultracentrifuge are more extensive than in the very limited phosphate buffer system. (The former buffer covers the range in which the solubility of the protein is sufficiently large). A range of barbiturate buffers from p_H 7.0 to 9.4 was used at a constant ionic strength (assuming complete dissociation of the sodium barbiturate) of 0.04. This is the maximum value which can be obtained at p_H 7.00 without the addition of neutral salt—the low solubility of the barbituric acid being the limiting factor. The same procedure was adopted as in the phosphate experiments; solutions were usually examined immediately after making up and then after standing for specific periods.

1. *The higher p_H's 8.0 and 9.0*

The composition of fresh solutions at these p_H's showed no change from the composition of the solid material as determined in phosphate buffer at p_H 8.0 and I = 0.10. Thus fraction I at p_H 8.47 and fraction II at p_H 9.18 after solution for one hour (one hour is approx. the time between preparing the solution and half sedimentation) gave

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sedimentation diagrams (Fig. 6b) very similar to those obtained in phosphate buffer at p_H 8.0.

These solutions when stored for considerable periods developed a yellow colour and very slight precipitation occurred; this would, however, in no way account for the changes in composition which were noted. A solution, for example, of the fraction I in barbiturate buffer at p_H 8.95 stored with a little toluene at room temperature (18° C) for 162 hours showed a decrease in the concentration of the more rapidly sedimenting boundary, the $s_{14.6}$ species (Fig. 6c). This composition probably represents the equilibrium composition at this p_H for a similar solution stored for 200 hours gave an identical sedimentation diagram (Fig. 6d)—the dissociation is not therefore complete at p_H 8.95. On the other

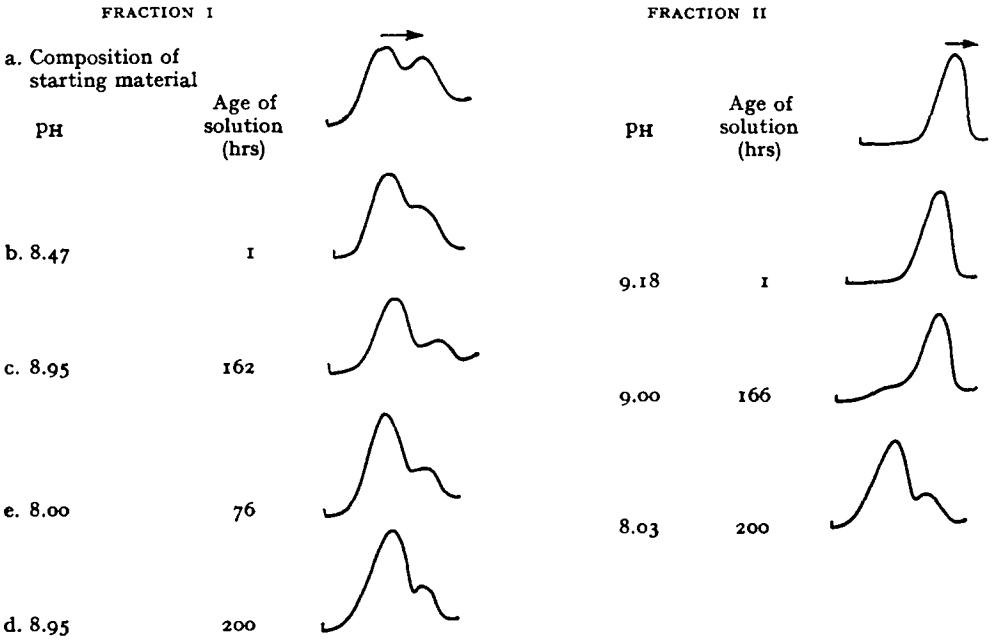

Fig. 6. Sedimentation diagrams of fractions I and II in barbiturate buffers; I = 0.04, of high p_H

hand the corresponding undialysed solution of fraction II (containing therefore residual traces of $(NH_4)_2SO_4$) stored under identical conditions at p_H 9.00 for a period of 166 hours had not shown a similar decrease in the $s_{14.6}$ component (Fig. 6c). It would therefore appear that small amounts of ammonium sulphate can either reduce the rates of the equilibrium reaction or significantly alter the equilibrium position (see below).

Solutions of the two fractions when *dialysed* for 76 and 200 hours at p_H's 8.00 and 8.03 respectively gave identical sedimentation diagrams (Fig. 6e) both showing a decrease (very considerable for fraction II) in the concentration of the $s_{14.6}$ component. Indeed these two solutions differing in original composition had after some long but arbitrary time reached an identical composition which would therefore appear to be the true equilibrium position of the reaction at this p_H. The half life of the reaction at this p_H can further be estimated at less than 200 hours since in this time the decrease in the concentration of the $s_{14.6}$ component in the solution of fraction II was very

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considerable (> 50%). Removal of final traces of ammonium sulphate by dialysis thus allows the reaction to proceed to its normal equilibrium position.

2. *Analysis at p_H's around 7.0*

A solution of fraction I dialysed for 70 hours from an original p_H of 7.63 to a final p_H of 7.00 gave a sedimentation diagram which although not greatly different from that of the original solid (Fig. 7b) showed an increase in the $s_{14.6}$ component when compared with the equilibrium composition at p_H 8.0 (Fig. 7c).

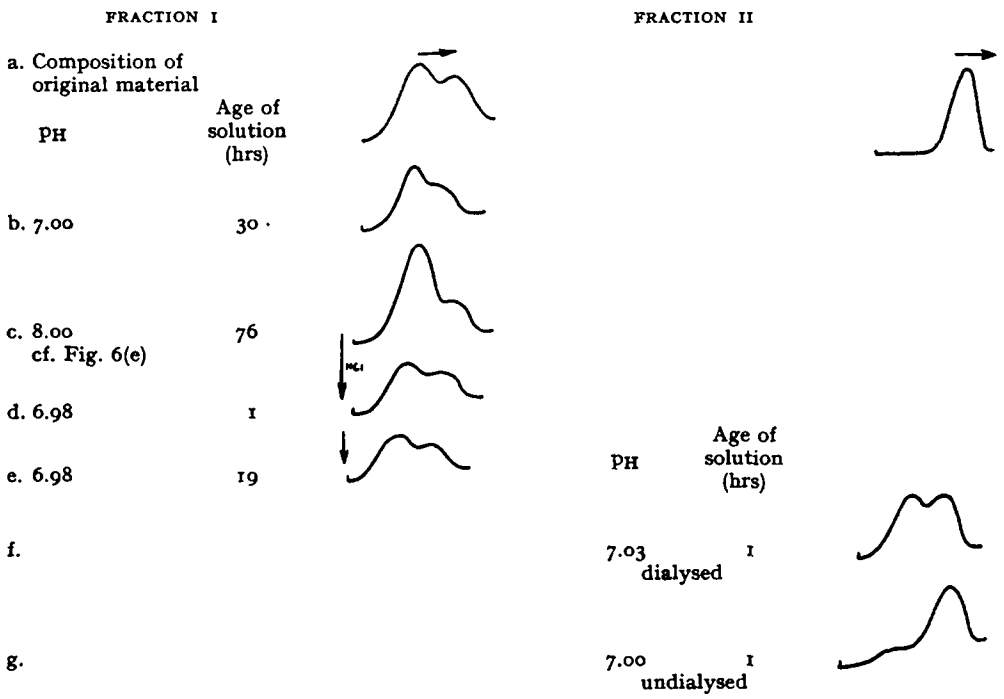

Fig. 7. Sedimentation diagrams of fractions I and II in barbiturate buffers; I = 0.04, p_H's around 7.0

To a further solution in equilibrium at p_H 8.0 (Fig. 7c) *N*/10 HCl was added to give a final p_H of 6.98. This solution was examined immediately (Fig. 7d) and after standing for 19 hours (Fig. 7e). Although these diagrams do not differ greatly a reaction during standing seems to be occurring, for which a half life time of 10–20 hours would seem probable (see also p. 368).

Excess $(NH_4)_2SO_4$ was removed from a solution of fraction II by dialysis at p_H 8.0 for 50 hours (involving some dissociation) and acid was subsequently added to p_H 7.03 —the composition of the final solution is given by the diagram of Fig. 7f (no precipitation occurred) and its similarity with the equilibrium diagram at p_H 7.0 achieved from fraction I is apparent. Some considerable increase in the rate of the dissociation (and therefore association) reaction at p_H's around 7.0 as compared with higher p_H's is clearly indicated.

An identical undialysed solution containing up to 1% $(NH_4)_2SO_4$ was treated in a similar manner, *i.e.* storage at p_H 8.0 for 50 hours followed by addition of acid to a

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final p_H of 7.00, and immediately examined (Fig. 7g). Relatively little dissociation had occurred, very much less than in the corresponding dialysed solution. As already suggested ammonium sulphate seems to be an effective agent for preventing dissociation either by reducing the rates of the equilibrium reactions or changing the equilibrium position.

The actual contours of the sedimentation diagrams and values of sedimentation constants are of importance in estimating reaction rates. Thus the appearance of discrete peaks at the higher p_H's (*e.g.* p_H 8.95, Fig. 6c) suggests that the two components are relatively stable and the reaction constants k_1 and k_2 in the system

$$A_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} 2A$$

are indeed small. These diagrams may be compared with the boundaries obtained in phosphate solutions at p_H 8.0 (Fig. 4a) where the establishment of the equilibrium takes longer and the system behaves as a non-interacting two component type. It has now been demonstrated that equilibrium is established more rapidly at the lower p_H's. The sedimentation diagrams at p_H 7.0, however, still show two sedimenting species, if admittedly the schlieren peaks are less discrete than at the higher p_H's. It must therefore be concluded (*e.g.* LONGSWORTH[8]) that the half lives of the two opposing reactions at p_H 7.0 are considerably longer than the duration of an experiment which is ca. 1 hour. This view is confirmed by the absence of noticeable convergence of the two sedimentation constants (14.6 and 9.0) at p_H 7.0 (Table II).

TABLE II

SEDIMENTATION CONSTANTS OF FRACTIONS I AND II IN BARBITURATE BUFFER; I = 0.04, p_H 7.0

Solution	Sed. diagram	p_H	Age of solution (hrs)	s^0_{20}	
Fractions I and II in phosphate; p_H 8.0	Fig. 7a			8.5	14.6
Fraction I	b.	7.00	30	9.1	(17.2)
	d.	6.98	1	8.3	15.5
	e.	7.07	19	8.4	15.5
Fraction II	f.	7.03	1	8.7	14.8
		Mean 8.6 ± 0.3, 15.0 ± 0.2			

If the half lives were of the order or less than the duration of an experiment a very blurred (probably single) boundary would arise whose sedimentation constant would be intermediate between the values for the separate species.

The various equilibrium positions in the barbiturate buffer system together with the estimates of the rates of reaction are collected together in Fig. 8.

The experiment at p_H 6.63 outside the buffer range proper (0.03 *M* barbituric acid, 0.001 *M* sodium barbiturate and 0.039 *M* NaCl) was achieved only by using a low concentration of fraction I ($\approx$ 0.3%) but, even so, some precipitation occurred. The

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resultant solution after a short time (probably sufficient if the results at p_H 7.0 are recalled) for equilibration gave the sedimentation diagram in Fig. 8, the concentration of the parent $s_{14.6}$ component being greater than that of its dissociation product, $s_{9.0}$ species. The general trend therefore in this barbiturate system is for the relative concentration of the parent $s_{14.6}$ species to increase with decreasing p_H; it has already been noted that reaction rates increase with decreasing p_H.

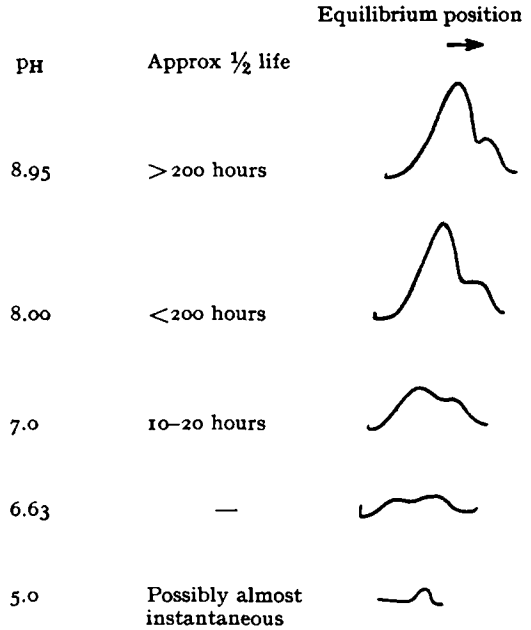

Fig. 8. Sedimentation diagrams giving the equilibrium positions of the association-dissociation processes in barbiturate buffers; I = 0.04

It may be recalled that the equilibrium position in phosphate buffer (I = 0.10) at p_H 7.08 contains a much smaller concentration of the $s_{14.6}$ than $s_{9.0}$ species (cf. Fig. 4c—this solution has probably not reached complete equilibrium). The barbiturate system is evidently displaced relative to that in the phosphate and, in general, the equilibrium position and the rate of its attainment at a particular p_H will be expected to vary with the nature and concentration of the buffer ions.

Whilst borate buffer systems have not been studied in detail it would appear that in 0.05 *M* solution at p_H 7.9 an equilibrium mixture contains about 60% of dissociated material, the half life being of the order of 200 hours.

3. *Effect of barbiturate buffer concentration*

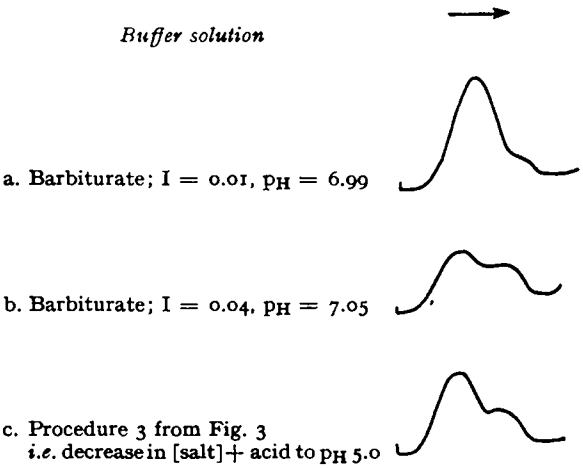

Fig. 9. Effect of salt concentration on the dissociation

A solution of fraction I in barbiturate buffer p_H 7.98 and ionic strength 0.04 was dialysed over a period of 120 hours to p_H 6.99 and a lower ionic strength of 0.01 (*i.e.* the solution remained at p_H 6.99 for ≈ 90 hours). Upon examination the resultant solution (some precipitation occurred) contained only a very small quantity, 5–10% of the $s_{14.6}$ component (Fig. 9a) and showed a very marked decrease in this component when compared with a similar solution in equilibrium at this p_H and an ionic strength of 0.04, (Fig. 9b) or prepared according to procedure 3, page 363 (Fig. 9c).

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Thus not only does a lowering of the salt concentration favour dissociation but under the conditions of the above experiment this dissociation becomes almost complete. It is now possible to explain why the two factors (1) p_H in the neighbourhood of 5.0 and (2) a low salt concentration are essential for the dissociation of the parent species and the preparation of the mixed fraction I. Low salt concentrations cause a shift of the equilibrium in favour of the dissociation products whilst low p_H's make possible the rapid attainment of this equilibrium state.

V. THE DISSOCIATION PROCESS

In the last precipitation procedure (3), the dialysis at p_H 5.0, on account of the higher mobility of the hydrogen and hydroxyl ions compared with that of sodium and chloride, the p_H of the solution must fall more rapidly than its salt concentration. Since precipitation can be effected by p_H lowering alone, considerable precipitation therefore occurs before the salt concentration is appreciably lowered. Such material is either unchanged initial protein or is incompletely equilibrated. Whilst below p_H 6, equilibrium is probably almost instantaneous, the protein solubility is so low that the equilibrated material now precipitated is only a small fraction of the whole. Further, this p_H is undoubtedly reached before the salt concentration has fallen to its lowest value so that even under these favourable conditions dissociation cannot be complete. As a whole therefore, this procedure could not approach complete dissociation.

However if in the above dialysis the p_H were maintained for a considerable time in the region of 7.0 instead of 5.0 the dissociation at low salt concentration though slower would not be interrupted by precipitation and would proceed nearer completion. This is confirmed by the experiment already given (page 369) when after dialysis at p_H 7.0 and the low ionic strength of 0.01 the residual solution contained some 90–95% of the dissociated $s_{9.0}$ species.

VI. THE SIZE AND SHAPE OF THE PARENT AND DISSOCIATED MOLECULES

a. The calculation of molecular weight and frictional ratio from the usual equations (SVEDBERG, 1940)

$$M = \frac{RT\,s}{D(1-\bar{v}\varrho)} \text{ and } f/f_o = 10^{-8}\left(\frac{1-\bar{v}\varrho}{D^2 s\,\bar{v}}\right)^{1/3}$$

involves a knowledge of the partial specific volume ($\bar{v}$) and the diffusion coefficient (D).

JOHNSON[4] quotes the value 0.72 ± 0.005 for the partial specific volume of the parent $s_{14.6}$ species and this value is used in the present calculation. COHN AND EDSALL[9] have, moreover, shown that partial specific volumes of proteins may be fairly accurately calculated from the volumes of the constituent groups and atoms (the calculated value for the $s_{14.6}$ species is 0.70). Since the $s_{9.0}$ species is a dissociation product of the $s_{14.6}$ species, its amino acid content must be very similar and the error introduced by using the value 0.72 for the $s_{9.0}$ species also, will not therefore be serious.

Diffusion coefficients have been determined by the free boundary method using the TISELIUS U-tube for boundary formation. An electrolytic form of compensator has been devised[10] to move the boundary between protein and buffer solutions into the observation channel without disturbance. The boundary was observed over two or three

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days and photographic recordings were made regularly. A typical set of schlieren peaks of a diffusing boundary ($s_{14.6}$ species in phosphate buffer against phosphate buffer) is given in Fig. 10.

ALBERTY, ANDERSON AND WILLIAMS[11] have shown that some variation in diffusion coefficient is found if the photographic recordings are reversed by inverting the diagonal edge, *i.e.* a white peak on a black background, or if the exposure times are altered. These variations arise from errors in recording and locating the correct plot of $\frac{dn}{dx}$ across the boundary. To reduce them to a minimum the edge angle, the setting of the base line and the exposure time have been kept constant throughout all the diffusion experiments.

Of the various methods available for the analysis of the schlieren curves (and therefore for the determination of D) the statistical method has been preferred. The calculation of the second moment (μ_2^0) of the schlieren curves is a standard technique but it should be noted that μ_2^0 is often determined using a class breadth of a finite width on a set of *continuously* varying observations. The correction needed to overcome this factor was determined by SHEPPARD (see *e.g.* WHITTAKER AND ROBINSON[12]) and it is necessary to subtract the quantity $\frac{1}{12} w^2$ (w = class breadth) from the second moment of the curve to obtain its correct value. Table III indicates the order of the SHEPPARD correction in some experimental curves used.

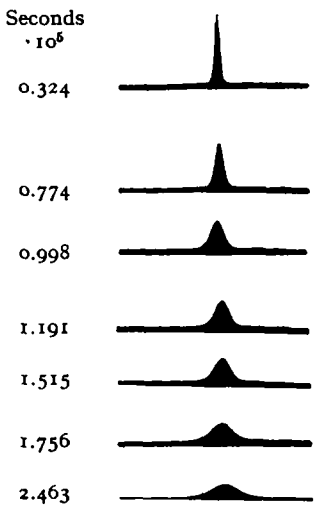

Fig. 10. Diffusion of a boundary formed between fraction II (≈0.65%) in phosphate buffer (p_H 7.88; I = 0.10) and the same buffer. The figures opposite each peak denote the time from the formation of the boundary

TABLE III

SHEPPARD CORRECTIONS IN CALCULATION OF SECOND MOMENTS

Class breadth w (graph units)	$\frac{1}{12} w^2$	μ_2^0 (graph units)	Corrected by method of SHEPPARD μ_2^0 corr. (graph units)
0.20	0.0033	1.3500	1.3467
0.40	0.0133	3.0500	3.0367
0.80	0.0533	5.4740	5.4207

b. *Experimental data*

The diffusion coefficient of the A_2 molecule was determined in a phosphate solution of fraction II at a p_H near 8.0 and an ionic strength of 0.10. This solution was dialysed for 40 hours against the above buffer and it is now evident from the recent work of JOHNSON AND JOUBERT[13] that a small amount of dissociated material (5–10% of $s_{9.0}$ species) is found under these conditions.

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It has also been noted that the dissociation reaction proceeds in barbiturate buffers, I = 0.04 at high p_H (page 365). Thus a solution of fraction I in the barbiturate buffer at p_H 8.89 which had an original composition identical to the solid material, Fig. 11a, changed gradually over a period of some 200 hours until it contained only ca. 15% of the parent $s_{14.6}$ species (Fig. 11b).

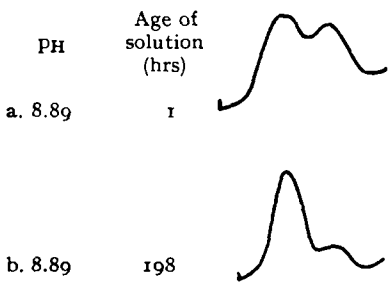

Fig. 11. Sedimentation diagrams of fraction I in barbiturate buffer at I = 0.04 — the preparation of the $s_{9.0}$ species

This solution was used in the present diffusion work to obtain a value of D for the dissociated molecule. Although neither of the above solutions contain only parent or dissociated molecules they represent the closest approach to stable homogeneous solutions suitable for diffusion work that have so far been prepared. In both cases the proportion of minor component was obtained from the ultracentrifuge and it was thus possible from the weight average diffusion coefficients measured to calculate the values for the major components.

The plots of the second moment ($\sigma^2 = \mu_2^0$) against $2t$ and the comparisons of the normalised and ideal Gaussian curves for the above two solutions are given in Figs. 12 and 13. A lower degree of accuracy is claimed for the value of D for the mainly dissociated material ($s_{9.0}$ species) because of the increased scatter of the points in Fig. 13. This

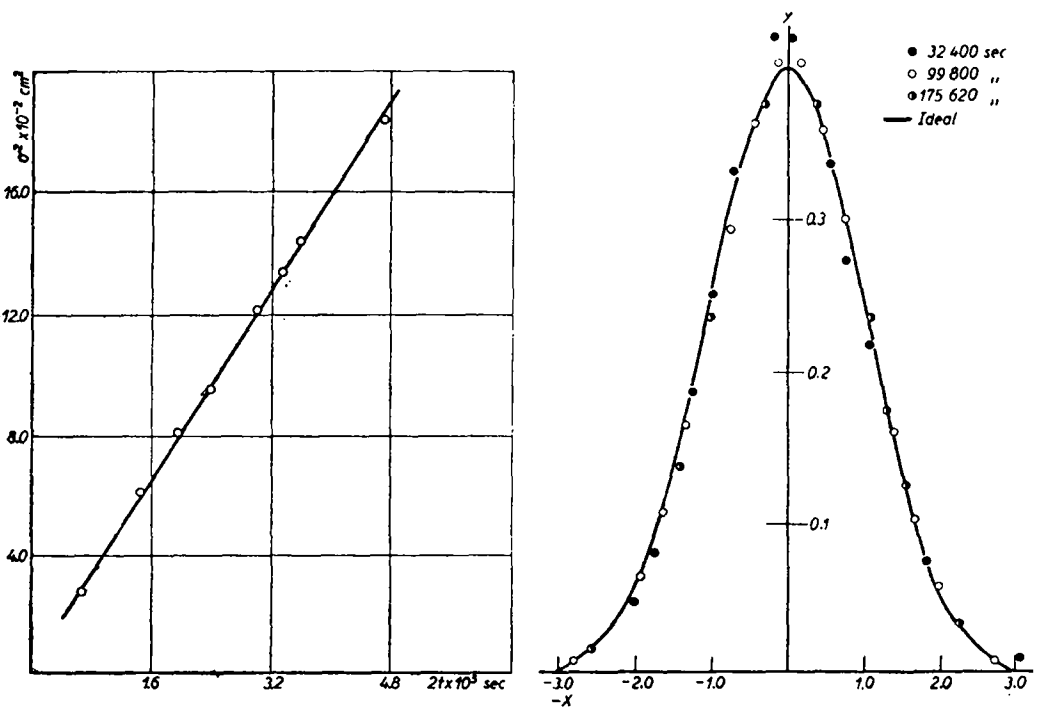

Fig. 12. a. σ^2 *v* $2t$ for the parent $s_{14.6}$ species b. Ideal and normalised distributions for $s_{14.6}$ species

References p. 375.

arises from the lower concentration of the solution in the barbiturate buffer, the decrease in the area of the diffusing schlieren peaks and the consequent uncertainly in peak contours. It must be further emphasised that values of diffusion coefficients are derived from a single experiment in each case and additional values would be desirable. The normalised curves of the two diffusing boundaries both coincide well with the ideal Gaussian distribution. Such agreement cannot therefore be used as a sensitive test for homogeneity (see *e.g.* PEARSON[14]).

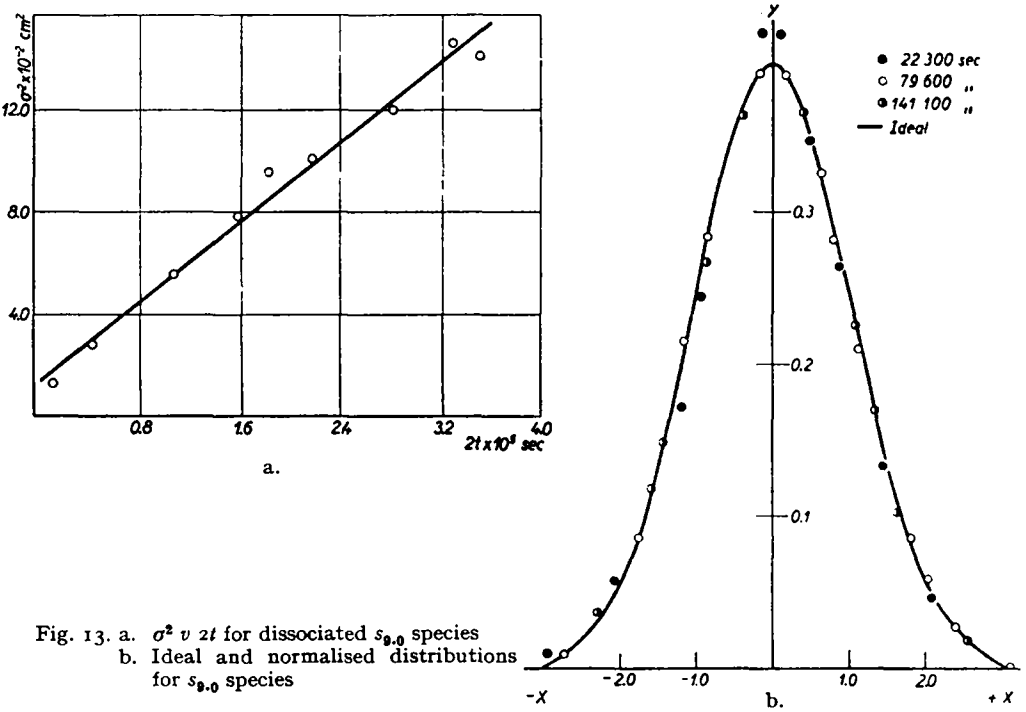

Fig. 13. a. σ^2 *v* $2t$ for dissociated $s_{9.0}$ species
b. Ideal and normalised distributions for $s_{9.0}$ species

The experimental values of diffusion coefficients for the phosphate (chiefly A_2 molecules) and barbiturate (chiefly A molecules) solutions were 3.89 and $4.16 \cdot 10^{-7}$ cm^2 sec^{-1}. Correcting these weight average values for the presence of the minor component we have

$$D_{A_2} = 3.89 - 0.10\ (4.16 - 3.89) = 3.86 \text{ cm}^2 \text{ sec}^{-1}$$

Similarly

$$D_A = 4.20 \text{ cm}^2 \text{ sec}^{-1}$$

The values of diffusion coefficients, molecular weights, frictional and axial ratios are collected in Table IV.

It is evident from Table IV that the molecular weight of the dissociated molecule ($s_{8.0}$ species) is within experimental error one half the molecular weight of the parent molecule ($s_{14.6}$ species) and the dissociation equilibrium may be strictly written as

$$A_2 \rightleftharpoons 2A$$

On the other hand the frictional ratio for the $s_{9.0}$ species is considerably higher than

References p. 375.

TABLE IV

PROPERTIES OF THE PARENT AND DISSOCIATED MOLECULE

Property		Parent molecule	Dissociated molecule
D^0_{20} ($cm^2\ sec^{-1}$)		$3.86\ (\pm\ 0.10)\cdot 10^{-7}$	$4.20\ (\pm\ 0.20)\cdot 10^{-7}$
s^0_{20} (Svedberg units)		14.6	9.0
M		330,000	180,000
f/f_0		1.216	1.352
a/b for prolate ellipsoid	assuming 30% hydration	2.6	4.6
a/b for oblate ellipsoid	assuming 30% hydration	0.37	0.20

for the $s_{14.6}$ species. It is reasonable to assume (see *e.g.* ALEXANDER AND JOHNSON[15]) that the hydration of the protein molecules may be of the order of 30%. Thus, with this assumed value, it was possible, using the curves of ONCLEY[16] based on the equations of PERRIN[17], to obtain the axial ratios of the parent and dissociated molecules. With prolate and oblate ellipsoidal models the values of a/b for the dissociated A molecule are, respectively, approximately twice and one half a/b for the parent A_2 molecule. It is not therefore possible to choose between the models but it is of interest that whatever the model the A molecule is of greater asymmetry than the parent. All values of D for the A molecule which lie in the given experimental range (4.00–4.40) lead also to a greater asymmetry for this molecule.

The increased asymmetry of the dissociated molecule is not sufficient, however, to give a large dependence of the sedimentation constant on concentration in the range of concentration which could be studied (0.1–0.6%). The values of s^0_{20} for this molecule varied randomly about the mean value of $9.0\cdot 10^{-13}$. In addition no pronounced trend of sedimentation constant of the A_2 molecule with decreasing protein concentration was observed.

SUMMARY

It has been confirmed that the globulin protein of the ground nut (*Arachis hypogaea*) forms a reversibly dissociating system in solution of the type

$$A_2 \rightleftharpoons 2A$$

the globulin occurring chiefly in the nut in the associated form. The equilibrium position and its rate of attainment in solution depends upon the p_H, salt concentration and type of salt present, lowered p_H tending to give more association and more rapid reaction rates and lowered salt concentration increased dissociation. High concentrations of sulphate ion are especially effective in promoting almost complete association but even in small amounts they appear to prevent dissociation. From sedimentation and diffusion measurements it appears that the dissociation products are of greater molecular asymmetry than the parent molecule.

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RÉSUMÉ

Nous avons confirmé que la globuline des arachides (*Arachis hypogaea*) forme en solution un système à dissociation réversible du type

$$A_2 \rightleftharpoons 2A$$

et que la globuline se trouve dans les noix surtout sous sa forme associée. La position de l'équilibre et sa vitesse d'établissement en solution dépendent du p_H, de la concentration en sels et du type de sels présents; un abaissement du p_H augmente l'association et la vitesse de réaction et une diminution de la concentration en sels augmente la dissociation. Des concentrations élevées en ions sulfates stimulent une association à peu près complète mais, même en faibles quantités, ces ions empêchent la dissociation. Il découle de mesures de sédimentation et de diffusion que les produits de dissociation ont des molécules moins symétriques que la forme associée.

ZUSAMMENFASSUNG

Es wurde bestätigt, dass das Globulin der Erdnuss (*Arachis hypogaea*) in Lösung ein reversibel dissoziierendes System von der Form

$$A_2 \rightleftharpoons 2A$$

bildet und dass das Globulin in der Nuss hauptsächlich im assoziierten Zustand vorkommt. Die Lage des Gleichgewichtes in Lösung, und die Zeit in der sich dieses Gleichgewicht einstellt hängen vom p_H, von der Salzkonzentration und der Art der anwesenden Salze ab; nimmt das p_H ab, so nehmen Assoziation und Reaktionsgeschwindigkeit zu, nimmt die Salzkonzentration ab, so schreitet die Dissoziation fort. Sulphationen in hohen Konzentrationen befördern die Assoziation nahezu vollständig, scheinen aber auch in geringen Mengen die Dissoziation zu verhindern. Aus Sedimentations- und Diffusionsmessungen ergibt sich, dass die Moleküle der Dissoziationsprodukte asymetrischer sind als die der assozierten Form.

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