ture with 3 cc. of concd. ammonia water. The ester dissolved and the diamide separated out almost immediately. It was washed thoroughly with water and alcohol and dried. The yield was 0.27 g., 75% of the theoretical. The substance is practically insoluble in water, ethyl alcohol and ether. It commenced to discolor at 185° and gradually darkened as the temperature was raised. It melted at 209°, with effervescence.

Anal. Calcd. for $C_6H_{12}O_6N_2$: N, 13.46. Found: N, 13.50, 13.38 (Kjeldahl).

Summary

1. An improved method for the preparation of allomucic acid is described.

2. Methods for the preparation of several new derivatives of the acid are presented.

3. A comparison is made of some of the properties of allomucic and mannosaccharic acids.

PITTSBURGH, PENNSYLVANIA

[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

THE MOLECULAR WEIGHT OF EDESTIN

BY THE SVEDBERG AND ALFRED J. STAMM[1]

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The proteins that have thus far been subjected to the ultracentrifuge analysis in this Laboratory in order to determine their molecular weights as well as other physical characteristics have been water soluble[2] with the exception of serum globulin.[2f] This protein required only relatively small salt concentrations to hold it in solution. There are a number of other proteins of the globulin class that are soluble only in strong salt solutions. It was thus the authors' desire to see if the previously developed methods of study were completely applicable to the study of proteins dissolved in strong salt solutions.

The protein edestin, belonging to the vegetable globulin class, was chosen for this work so as to add another type of protein to the list of those studied. Edestin seemed especially suitable because of the ease with which it can be isolated and because of its definite crystalline structure, which is a strong indication of its homogeneity.

Preparation of Material.—Coarsely ground hemp seed (600 g.) was directly subjected to digestion and extraction with a mixture of 2000 cc. of 10% sodium chloride solution and 400 cc. of 2.1% disodium phos-

[1] Fellow of the International Education Board.

[2] (a) T. Svedberg and R. Fåhraeus, THIS JOURNAL, **48**, 430 (1926); (b) T. Svedberg and J. B. Nichols, *ibid.*, **48**, 3081 (1926); (c) T. Svedberg and J. B. Nichols, *ibid.*, **49**, 2920 (1927); (d) T. Svedberg and N. B. Lewis, *ibid.*, **50**, 525 (1928); (e) T. Svedberg and E. Chirnoaga, *ibid.*, **50**, 1399 (1928); (f) T. Svedberg and B. Sjögren, *ibid.*, **50**, 3318 (1928).

phate.[3] After standing for twelve hours at room temperature the material was heated for two hours at 40° and then filtered through a filter cloth. The filtrate was clarified in a bucket centrifuge. Three volumes of water were added to each volume of the filtrate, causing precipitation of the extracted edestin. This was allowed to stand overnight in an ice box. The supernatant liquid was removed from the precipitate in the bucket centrifuge. After washing the precipitate several times by decantation with a dilute sodium chloride solution, the precipitate was dissolved in 10% sodium chloride. The reprecipitation and solution was repeated three times. The stock edestin was kept in an ice box in a precipitated form under dilute sodium chloride. A few drops of toluene were added to the solutions in the course of the preparation and to the stock precipitate and stock solutions to prevent bacterial action.

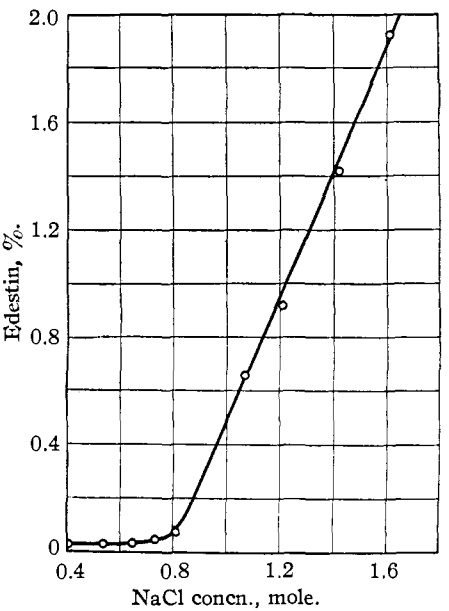

Fig. 1.

Due to the decreased solubility of edestin at low temperatures, as well as the decreased solubility of the phosphate buffers used, the stock solutions made up from the above precipitated edestin were dialyzed and kept at room temperature (about 18°). The course of the experiments showed that this had no deleterious effect.

Solubility and Isoelectric Point.—Though edestin has perhaps been studied more than any other protein of this class, practically none of its physical characteristics are known with any degree of accuracy. Two different values have been obtained for the isoelectric point. Rona and Michaelis[4] found it to be at a P_H 6.9 in phosphate buffers, and Michaelis and Mendelssohn[5] found it to be at P_H 5.6 in acetate buffers. Because of this discrepancy, it seemed advisable to determine the isoelectric point from solubility measurements under the conditions used in this research, and at the same time to get the solubility information that would be of value in connection with the ultracentrifuge analysis.

[3] The grinding of the hemp seed was kindly done for us in an experimental mill at the laboratory of Upsala Ångkvarn.

[4] Rona and Michaelis, *Biochem. Z.*, **28**, 193 (1910).

[5] Michaelis and Mendelssohn, *ibid.*, **65**, 1 (1914).

Three sets of solubility measurements were made. In each case the concentrations were determined by evaporating 2 cc. of the solvent and 2 cc. of the solution, and drying to constant weight in an oven at 105°. The solutions were allowed to stand in contact with an excess of edestin for several hours at room temperature (about 18°) and they were then clarified in a bucket centrifuge before pipetting off the samples for analysis. Figure 1 gives the solubility of edestin in different concentrations of sodium chloride solution. The solubility is small up to 0.8 *M* sodium chloride, but above this concentration the solubility increases rapidly. Figure 2 gives the changes in solubility of edestin in phosphate buffer solutions with the change in *P*H of the solvent. Curve A is for the phosphate buffer alone and Curve B for the phosphate buffer together with sodium chloride. Curve A gives a minimum solubility isoelectric point at *P*H 5.5 and B gives it at *P*H 5.4. A solution of edestin, in a previously boiled sodium chloride solution (*P*H 5.8), after twelve days' dialysis against the solvent, gave a *P*H of 5.5. As the sodium chloride has practically a negligible buffering action, the *P*H of the solution should be practically at the isoelectric point. These three values are in very good agreement and also check the value of the isoelectric point given by Michaelis and Mendelssohn.

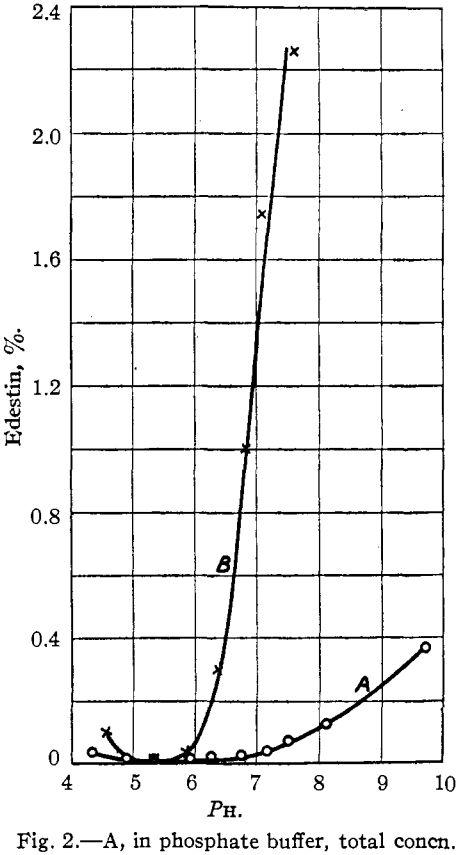

Fig. 2.—A, in phosphate buffer, total concn. 0.15 *M*; B, in phosphate buffer, total concn., 0.15 *M* + 0.735 *M* NaCl.

The solubility curves show that the solubility of edestin in buffered solutions is very small near the isoelectric point. The measurements to follow, that were made at the isoelectric point, have hence been made on unbuffered sodium chloride solutions. Measurements on solutions containing no sodium chloride could only be made at the higher *P*H values.

Specific Volume.—The partial specific volume of the protein was determined pycnometrically at 19.8°. Measurements were made on three

different solutions of edestin of different P_H value and different concentration, as shown in Table I. Each of the solutions was dialyzed against

TABLE I

PARTIAL SPECIFIC VOLUME OF EDESTIN IN DIFFERENT SOLVENTS AT 19.8°

Edestin concn., %	0.56	1.19	0.58
Solvent	1.24 *M* NaCl	0.735 *M* NaCl	0.62 *M* NaCl
		0.15 *M* Na_2HPO_4	0.0375 *M* Na_2HPO_4
		0.06 *M* KH_2PO_4	0.025 *M* NaOH
P_H of soln.	5.5	6.7	11.3
Part. sp. vol.	0.744	0.743	0.745

the solvent for twelve days at room temperature (16 to 18°). The outer liquid was changed twice a day. The dialysis was conducted under a bell jar to minimize evaporation from the solvent. The table shows that the partial specific volume is not affected by the P_H of the solution over the range tested. These values agree, within the range of experimental error, with the partial specific volume of egg albumin, hemoglobin, serum albumin, serum globulin, phycocyan and phycoerythrin, as determined in this Laboratory.

Light Absorption.—The light absorption of edestin solutions was determined with the Judd–Lewis spectrophotometer. The specific extinction coefficient, $\epsilon/c = 1/cd \log I_0/I$ (where c is the concentration in per cent., d the thickness of the solution, I_0 the intensity of the light beam after passing through the solvent and I the intensity of light after passing through the same thickness of solution), is plotted against the wave length of the light (see Fig. 3). Measurements were made upon 0.110 and 0.055% solutions of edestin in 1.24 *M* NaCl, P_H 5.5; 0.198, 0.099 and 0.0495% solutions of edestin 0.735 *M* in NaCl, 0.15 *M* in Na_2HPO_4 and 0.06 *M* in KH_2PO_4, P_H 6.7; a 0.090% solution of edestin 0.15 *M* in Na_2HPO_4, 0.0036 *M* in KH_2PO_4 and 0.0052 *M* in NaOH, P_H 9.7; a fresh 0.053% solution of edestin 0.62 *M* in NaCl, 0.0375 *M* in Na_2HPO_4 and 0.025 *M* in NaOH, P_H 11.3; a similar solution to the latter made up from a solution five days old containing originally 0.58% of edestin; and a 0.089% solution of edestin 0.206 *M* in NaCl and 0.0167 *M* in HCl, P_H 1.8. All of these solutions gave practically

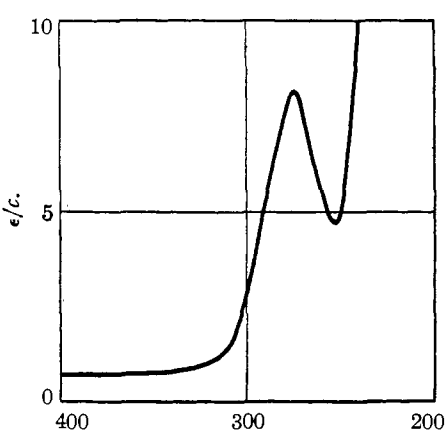

Fig. 3.

identical curves so only the average value is plotted. The absorption showed a maximum at 276$\mu\mu$, and a minimum at 254$\mu\mu$.

Determination of the Molecular Weight

Sedimentation Velocity Method.—The apparatus and the method have been so completely described in previous publications[2c,d,e,f,6] that no attempt will be made to repeat the description here. The modifications described by Svedberg and Sjögren[2f] were effective in this work. As the maximum light absorption of edestin is similar to that for the other proteins studied, the same optical system and the same chlorine and bromine filters were used.

The molecular weight is given by the relation

$$M = \frac{RTs}{D(1 - V\rho)}$$

where R is the gas constant, T the absolute temperature, s the specific sedimentation velocity or $1/\omega^2 x \cdot dx/dt$, D the diffusion constant, V the partial specific volume of the protein, ρ the density of the solvent, x the distance from the axis of rotation, ω the angular velocity and t the time.

Table II gives the results of a typical run made with an edestin solution at the isoelectric point. The constancy of the values for the specific

TABLE II

RESULTS OF A TYPICAL SEDIMENTATION VELOCITY RUN MADE UPON AN ISOELECTRIC SOLUTION OF EDESTIN IN 1.24 *M* SODIUM CHLORIDE SOLUTION

Concentration, 0.56%; P_H of solution, 5.5; $V = 0.744$; $\rho = 1.0479$; rel. vis. of solvent, 1.0690; length of column of solution, 1.38 cm.; thickness of column, 0.60 cm.; average speed, 23,900 r.p.m.; aperture of objective, F:36; time of exposure, 80 sec.; time interval between exposures, 35 min.; temperature, 21.0°.

Sedimentation				Diffusion		
ΔX, cm.	Mean X, cm.	Centrif. force, $\omega^2 X \times 10^{-7}$	s_{20} cm./sec. per cm./sec.^{2} $\times 10^{13}$	Time, sec.	Mean Z, cm.	D_{20} cm./sec. $\times 10^7$
0.066	4.653	2.93	10.48	2,520	0.033	4.61
.067	4.719	2.98	10.43	4,620	.045	4.67
.067	4.786	3.05	10.19	6,720	.052	4.29
.065	4.853	3.09	9.75	8,820	.065	5.11
.065	4.917	3.09	9.75	10,920	.073	5.20
.070	4.985	3.07	10.57	13,020	.079	5.11
.070	5.055	3.06	10.60	15,120	.086	5.22
.067	5.123	3.16	9.85	17,220	.098	5.95
		Av.	10.2×10^{-13}			5.02×10^{-7}

Av. molecular weight, 225,000.

"Mean Z" is the mean of the distances on the photometer curves from the point where the concentration is 50% to the points where the concentrations are 25 and 75%, respectively, the concentration in the unchanged part of the solution being taken as 100%.[2c]

[6] The Svedberg, "Colloid Chemistry," 2nd ed., Chemical Catalog Co., Inc., New York, 1928.

sedimentation velocity s and the diffusion constant D indicates that the edestin is mono-disperse and that the solvent shows no time function effect upon the systems. The edestin further settled so as to give clear buffer at the top of the cell, thus showing that there was no non-centrifugible material present with a light absorption between 250 and 290$\mu\mu$, the wave lengths of the light entering the cell.

Figure 4 gives the concentration curves from which the data of Table II were obtained. The curves have been corrected for the sector shape of the cell and the variation of the centrifugal force with distance from the center of rotation. The dotted curves for each represent the theoretical diffusion curves of a substance of only one molecular species when subjected to the same experimental conditions. The deviations obtained are well within the range of experimental error.

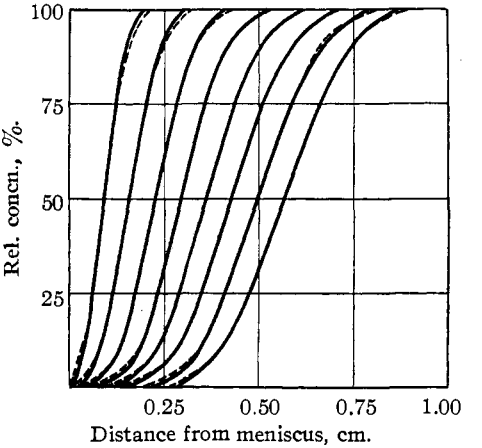

Fig. 4.

Table III gives a summary of all of the sedimentation velocity runs. The effect of varying only the edestin concentration was determined for two different solvents. The specific sedimentation velocities and the diffusion constants show no regular shift between the concentrations of 1.16 and 0.193% in 1.24 M NaCl, P_H 5.5. The resulting molecular weight values are constant within experimental error over this range. The same is true in the solutions at P_H 6.7 from a concentration of 1.38% edestin to a concentration of 0.155%. At the concentration of 0.108 there is an appreciable change in the specific sedimentation velocity and diffusion constant, indicating a change in the molecular dispersion of the edestin. Dissociation has very likely taken place in this dilute solution. The abnormal specific sedimentation velocity and the continuous shift in the apparent diffusion constant from 3.1 at the start of the run to 11.3 at the end of the run indicate the heterogeneity of the system. The molecules of different molecular weight settling at different speeds caused a spreading or blurring at the sedimentation boundary, which according to the methods of calculation is included with the true diffusion. Though such runs as this cannot be quantitatively analyzed and the true molecular weights calculated, they show very nicely in a qualitative way what has happened to the solution.

The molecular weights calculated from the data of all of the runs at

TABLE III

SUMMARY OF RESULTS BY THE SEDIMENTATION VELOCITY METHOD

No.	NaCl	Solvent, M Na_2HPO_4	KH_2PO_4	P_H soln.	Density of soln.	Rel. vis. of soln.	Concn. of edestin, %
1	1.24	..	..	5.5	1.0479	1.069	1.16
2	1.24	..	..	5.5	1.0479	1.069	0.560
3	1.24	..	..	5.5	1.0479	1.069	.290
4	1.24	..	..	5.5	1.0479	1.069	.193
5	0.735	0.15	0.06	6.7	1.0529	1.120	1.38
6	.735	.15	.06	6.7	1.0529	1.120	0.890
7	.735	.15	.06	6.7	1.0529	1.120	.445
8	.735	.15	.06	6.7	1.0529	1.120	.222
9	.735	.15	.06	6.7	1.0529	1.120	.155
10	.735	.15	.06	6.7	1.0529	1.120	.108
11	Like 5–10 with 55.7% of water added			6.7	1.0225	1.055	.296
12	..	.0715	.0017	7.9	1.0073	1.031	.150
13	.0052[a]	.15	.0036	9.7	1.0171	1.075	.300
14[b]	.62	.075	.0018	9.7	1.0325	1.072	.580
15	.62	.0375	.025[a]	11.3	1.0295	1.035	.580
16	.62	.0375	.025[a]	11.3	1.0295	1.035	.580
17	.206	.00217[c]	..	3.1	1.0079	1.011	.193
18	.206	.0167[c]	..	1.8	1.0079	1.011	.193

[a] Sodium hydroxide. [b] Sodium hydroxide, 0.0026 M, added. [c] Hydrochloric acid.

No.	Av. r.p.m. of centr.	Non-centrif. material, %	s_{20} cm./sec. per cm./sec.^{2} $\times 10^{13}$	D_{20} cm.^{2}/sec. $\times 10^7$	Water basis $s_{20} \times 10^{13}$	Water basis $D_{20} \times 10^7$	Mol. wt.
1	23,900	1	10.8	5.63	13.4	6.02	213,000
2	23,900	0	10.2	5.02	12.7	5.37	225,000
3	23,800	0	10.4	5.49	12.9	5.88	209,000
4	24,600	2	10.0	5.00	12.4	5.35	221,000
5	23,500	7	9.46	4.83	12.5	5.40	219,000
6	43,800	..	9.45	..	12.5	..	.....
7	23,700	7	9.54	5.11	12.6	5.72	209,000
8	23,600	9	9.70	4.98	12.8	5.58	218,000
9	24,300	11	9.32	4.99	12.6	5.59	209,000
10	23,800	14	[8.70]	[7.48]	..	..	.....
					Av 12.77	Av. 5.62	Av. 215,000
11	23,400	7	11.8	5.63	13.3	5.94	213,000
12	24,600	..	12.5	5.67	13.1	5.85	214,000
13	23,700	4	12.1	5.93	13.7	6.37	204,000
14	23,800	0	10.75	5.44	12.7	5.83	208,000
15	23,700	16	4.82	5.7[a]	5.47	5.90	.....
16	42,500	6	4.90	6.1[a]	5.55	6.32	.....
17	23,700	28	12.2	..	12.7	..	.....
18	24,500	50	9.16	..	9.48	..	.....

[a] Extrapolated values (see Fig. 5).

P_H 5.5 and P_H 6.7, with the exception of the one above cited where dissociation took place, are in very good agreement. The average molecu-

lar weight of 215,000 is almost exactly the same as that of phycoerythrin obtained by Svedberg and Lewis. This molecular weight is considerably larger than any of the estimates that have been made from analytical data; Cohn[7] gives 29,000 as the minimal molecular weight of edestin. Osborne[8] gives 7250 as the minimal molecular weight from the acid combining capacity and chemical composition. Estimates by the osmotic pressure methods cannot be made as in the case of several of the other proteins, because high electrolyte concentrations are necessary to hold the edestin in solution and they would exert a far greater pressure than the edestin itself.

In order to compare the specific sedimentation velocity and the diffusion constant in the different solvents, the experimentally determined values have been converted to a basis of sedimentation and diffusion in pure water. The specific sedimentation velocity depends inversely upon the viscosity of the solvent and directly upon the difference in density between the particle and the solvent. The experimental values have thus been multiplied by the relative viscosity of the solvent and the ratio between the density differences in water and in the solvent. As the diffusion depends inversely upon the viscosity, it has been multiplied by the relative viscosity. The values of s on the water basis for the solutions at P_H 5.5 and 6.7 show a maximum deviation of 5%, and a mean deviation of 2% from the average value. The values of D show a maximum deviation of 7% and a mean deviation of 4% from the average.

The percentage of non-centrifugible normal light-absorbing material from each run is also included in Table III. These values were obtained from the deviation of the light absorption of the liquid at the top of the cell from that of the pure buffer. For all of the solutions at P_H 5.5 it was very small. In the case of the solution of P_H 6.7 it was somewhat larger and showed a slight increase with a decreased edestin concentration, as has been noted with other proteins.[2f] The fact that the solutions at P_H 6.7 were considerably older at the time the runs were made than those at P_H 5.5 may account for the slight decomposition of the former. In none of these cases, however, is the percentage of non-centrifugible material present sufficient to interfere with the determinations.

The effect of the dilution of the solvent with water is shown by Run 11, Table III. The values of the specific sedimentation velocity, diffusion constant and molecular weight are quite normal. The fact that the concentration of the solvent and its saline composition can be varied over a considerable range without affecting the results indicates the applicability of the sedimentation velocity method to the study of the molecular weights of proteins in strong salt solutions.

[7] Cohn, *Physiol. Rev.*, **5**, 360 (1925).

[8] Osborne, THIS JOURNAL, **24**, 39 (1902).

Table III also shows the effect of varying the P_H of the solution. The results show that edestin is stable over a considerable range of P_H on the alkaline side of the isoelectric point. Run 13 shows the results for a sample that was made up three months before the measurements were made. Not only is the molecular weight normal, but the sedimentation curves further show that only 4% of non-centrifugible material of normal light absorption can be present. This is even less than the amount present in the case of several of the runs at P_H 6.7.

A further increase in the P_H above P_H 9.7 shows a definite decomposition or dissociation. Runs 15 and 16 were at a P_H of 11.3. The former was run at a speed of 23,900 r.p.m. and the latter at 42,500 r.p.m. The specific sedimentation velocities are in good agreement but they are less than half of the normal value. The diffusion constants, which are apparent rather than real because of the heterogeneity of the system, are plotted in Fig. 5 for the different times of sedimentation. In the case of the high-speed run the change of the apparent diffusion with time is greater than for the low-speed run, because of the greater separation of the sedimentation boundaries of the different molecular species present. It is of interest to note that the ratio of the slopes of the two curves after a considerable time of centrifuging approaches the square of the ratio of the centrifugal forces, that is, the Z displacements per unit of time approach proportionality to the centrifugal forces applied. This means that with increased time the proportion of the apparent diffusion constant that is caused by true diffusion becomes less and less, and that the spreading of the sedimentation boundaries predominates. When the apparent diffusion curves are extrapolated to zero time the converse should be true for the resulting values. There should be no spreading of the boundaries and the diffusion values should be real, and a sort of average value for the diffusion constants of the various constituents present in the mixture. It will be shown later that this is the case.

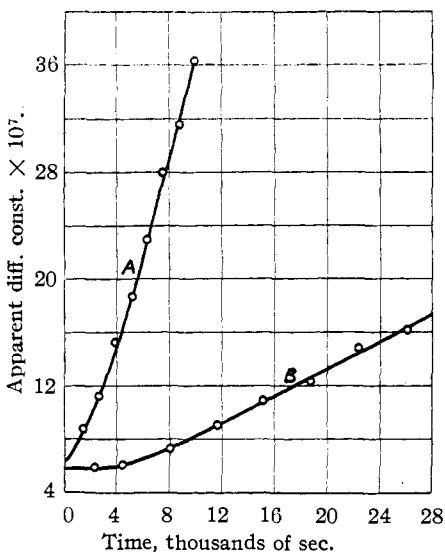

Fig. 5.—A, 42,500 r.p.m.; B, 23,900 r.p.m.

A further analysis of the data of Run 15 is given in Fig. 6. The experimental sedimentation curve E corrected for the sector shape of the cell and the variation of the centrifugal force with distance from the

center of rotation is given for the longest time of centrifuging (corresponding to the highest point on Curve A (Fig. 5). The dotted curves A, B and C are the theoretical sedimentation curves for normal edestin, a protein of one-half the normal molecular weight and one of one-third the normal molecular weight, respectively, each of which is subjected to the actual experimental conditions. The specific sedimentation velocities and diffusion constants used for the fractional molecular weight calculations were the actual experimental values obtained for the proteins that have these molecular weights.[2c,d,f] The dotted curve, D, is for the theoretical mixture of 15% A, 30% B and 55% C, respectively. This curve

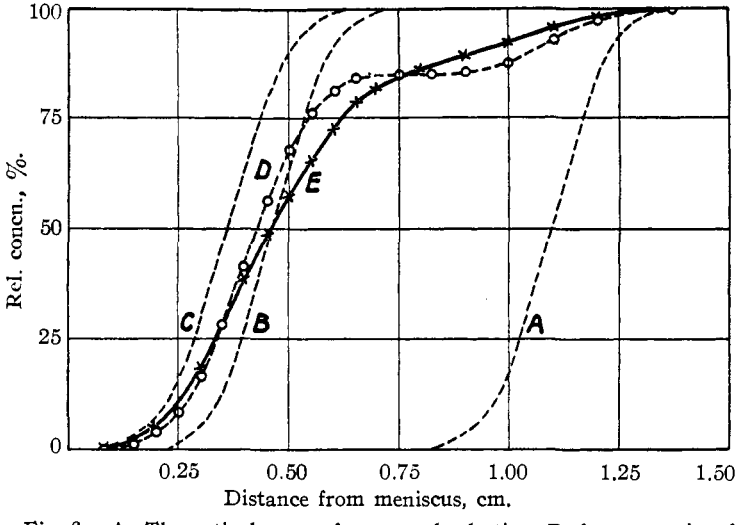

Fig. 6.—A, Theoretical curve for normal edestin; B, for a protein of $M=106{,}000$; C, $M=68{,}000$; D, 15% A, 30% B, and 55% C; E, actual experimental curve for edestin at $P_H=11.3$.

shows a fair agreement with the actual experimental curve, E. It is especially good at the ends, the deviation being almost entirely near the inflection point. Similar curves for shorter times of sedimentation showed far better agreement between the observed and the theoretical curves. Such an analysis as this of course cannot prove the molecular constitution of the mixture, for perhaps other combinations will satisfy the experimental conditions as well as this. It serves, however, to show the general nature of the mixture. In the last section of this paper the constitution of this mixture will be shown quite definitely to be that represented here.

Edestin is far less stable on the acid side of the isoelectric point than on the alkaline side. Decomposition takes place both at P_H 3.1 and 1.8. Though the specific sedimentation velocity is almost normal, showing the presence of normal edestin, the diffusion constant is definitely of the

apparent diffusion type pictured above. The decomposition, however, is quite different from that on the alkaline side of the isoelectric point, as the decomposition is mostly into material of such a low molecular weight that no sedimentation will take place. This can be seen readily by comparing the percentages of non-centrifugible material present for each of the runs listed in Table III. Slight irregularities in the sedimentation curves for both of these acid runs indicate that there are some centrifugible decomposition products present, as is required by the abnormal diffusion constant. Though these cannot be identified definitely, it seems probable from the curves that a material of one-half of the normal molecular weight is present.

Sedimentation Equilibrium Method.—A few runs were made by the sedimentation equilibrium method as previously described.[2a,b,d,e] The molecular weight is given by the relation

$$M = \frac{2RT \ln (c_2/c_1)}{(1 - V\rho)\omega^2(x_2^2 - x_1^2)}$$

c_1 and c_2 are the concentrations at the distances x_1 and x_2 from the center of rotation and the other symbols have the same significance as previously.

Table IV gives the results of a typical run showing that there is no regular change of molecular weight with height. Table V gives a summary of all of the equilibrium runs. The molecular weights obtained by this method agree quite well with the values obtained by the sedimentation velocity method. An unsuccessful attempt was made to determine the molecular weight of the protein at P_H 11.3 by this method.

TABLE IV

RESULTS OF A TYPICAL SEDIMENTATION EQUILIBRIUM RUN MADE UPON AN ISOELECTRIC SOLUTION OF EDESTIN IN 1.24 *M* SODIUM CHLORIDE SOLUTION

Concentration, 0.58%; P_H of solution, 5.5; V = 0.744; ρ = 1.0479; length of column of solution, 0.59 cm.; thickness of column, 0.2 cm.; distance of outer end of solution from axis of rotation, 5.95 cm.; average speed, 5280 r.p.m.; aperture of lens, F:25; time of exposures 1, 2 and 3 min.; exposures made after 30, 35 and 45 hours of centrifuging.

Distances, cm.		Mean concn. (orig. soln. = 1.00)		No. of exposures	Mol. wt.
x_2	x_1	c_2	c_1		
5.86	5.81	2.09	1.76	6	222,000
5.81	5.76	1.76	1.48	9	223,000
5.76	5.71	1.48	1.29	12	179,000
5.71	5.66	1.29	1.12	12	185,000
5.66	5.61	1.12	0.95	12	216,000
5.61	5.56	0.95	.81	12	212,000
5.56	5.51	.81	.68	12	238,000
5.51	5.46	.68	.58	9	217,000
5.46	5.41	.58	.49	9	234,000
5.41	5.36	.49	.42	9	214,000
				Mean	214,000

The results showed definitely that the edestin had decomposed or dissociated into several molecular species of the order of molecular weight of those predicted by the sedimentation velocity method, but no quantitative results could be obtained because of the complexity of the system and the long time required to obtain equilibrium during which further changes in the protein may have resulted.

TABLE V

SUMMARY OF THE RESULTS BY THE SEDIMENTATION EQUILIBRIUM METHOD

No.	1	2	3
Solvent, NaCl	1.24 *M*	0.735 *M*	0.735 *M*
Solvent, Na_2HPO_4	...	.15 *M*	.15 *M*
Solvent, KH_2PO_4	...	.06 *M*	.06 *M*
*P*H of solution	5.5	6.7	6.7
Concn. of edestin, %	0.580	0.595	0.595
Mean speed, r.p.m.	5280	4970	4900
Exposures after hours	30, 35, 45	29, 38	30, 36, 44
Molecular weight	214,000	209,000	202,000 Mean, 208,000

Sedimentation Velocity Method as Modified by Lamm.—Ole Lamm, working in this Laboratory, has developed a new method for determining the changes in concentration in a sedimenting system based on the measurement of the changes in the refractive index of the system. The theory of the method has been described by Lamm.[9] For a complete description of the method the reader is refered to the above publication and subsequent publications of Lamm to follow. It suffices to state here that the measurements yield directly a function which is proportional to the change in the concentration with changes in height, that is, a first derivative curve. As the results will show, such a function gives the abnormalities due to the presence of several molecular species in the system much more definitely than the simple concentration–height relationship obtained by the old method. A run made by the new method on a 0.487% solution of edestin in 1.24 *M* sodium chloride (average speed, 24,300 r.p.m.) gave a molecular weight of 222,000, a specific sedimentation velocity of 12.4×10^{-13} and a diffusion constant of 5.30×10^{-7} on a water basis. These values all agree well with the values given in Table III.

In Fig. 7 is plotted the function of the change in concentration with height against the height for a 0.487% solution of edestin at *P*H 11.3 after one and one-half hours' sedimentation. Instead of obtaining the simple one-maximum curve that would result if only one molecular species were present, there are three definite maxima. The three dotted curves

[9] Lamm, *Z. physik. Chem.*, **138**, 313 (1928). The application of the method to the ultracentrifuge was not as yet published at the time of the preparation of this manuscript, but should appear in print soon. The authors are grateful to Lamm for granting them permission to publish their results obtained by the new method before his complete description of the method has appeared in print.

represent the three simple curves whose summation is the experimental curve. From the position and the shape of these three curves the specific sedimentation velocities and the diffusion constants of each of the constituents can be calculated according to the method which Lamm will describe. Table VI gives a summary of the results of this run, obtained from the curves of Fig. 7, as well as from similar curves for different times of centrifuging. Because of the high centrifugal force used the normal edestin is so completely removed from the system in two hours that only two values for the specific sedimentation velocity could be obtained from the data. The small percentage of the normal edestin present, as is shown by the relatively low maxima, made it practically impossible to obtain diffusion data for this constituent. The specific sedimentation velocity for Constituent 1, however, shows definitely that it represents the original normal edestin. Both the specific sedimentation velocities and the diffusion constants for Constituent 2 are very close to the values for serum, globulin and phycocyan,[2d,f] and those for Constituent 3 are very close to the values for hemoglobin and serum albumin.[2e,f] The data thus definitely show that edestin at a P_H of 11.3 dissociates into particles having one-half and one-third of the original molecular weight, and that these dissociation products have the same specific sedimentation velocity and diffusion constant as the other proteins that have the same molecular weights. This run by the new method further shows that the proportions of the three constituents present are of the same order of magnitude as in the theoretical calculation of Fig. 6.

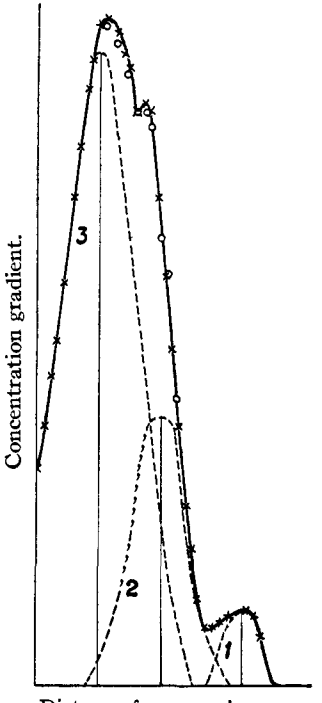

Fig. 7.

This one illustration shows very nicely the possibilities of applying the new method to the study of mixed proteins. Further work along this line is now under way at this Laboratory and should yield more important data regarding the dissociation of proteins.

Discussion of Results

This research has shown quite definitely the applicability without modifications of both the sedimentation velocity and sedimentation equilibrium methods to the study of proteins dissolved in strong salt solutions.

TABLE VI

SUMMARY OF THE RESULTS OBTAINED ON EDESTIN AT P_H 11.3 BY THE NEW METHOD DEVELOPED BY OLE LAMM

Edestin concentration, 0.487%; solvent 0.62 *M* in NaCl; 0.0375 *M* in Na_2HPO_4; 0.025 *M* in NaOH; P_H of solution, 11.3; density of solvent, 1.0295; relative viscosity of solvent, 1.0350; thickness of column of solution, 0.60 cm.; average speed, 42,500 r.p.m.; average temperature, 21.2°

	Specific sedimentation velocities at 20°		
Time, hours	Constituent 1	Constituent 2	Constituent 3
1–1.5	10.44×10^{-13}	5.73×10^{-13}	3.29×10^{-13}
1.5–2	10.59×10^{-13}	5.22×10^{-13}	3.40×10^{-13}
2–2.5	.....	4.78×10^{-13}	3.16×10^{-13}
2.5–3	.....	5.28×10^{-13}	3.76×10^{-13}
3–3.5	.....	5.23×10^{-13}	3.77×10^{-13}
Av.	10.52×10^{-13}	5.25×10^{-13}	3.47×10^{-13}
Water basis values	11.9×10^{-13}	5.94×10^{-13}	3.94×10^{-13}

	Diffusion constants at 20°		
Time, sec.	Constituent 1	Constituent 2	Constituent 3
4,020	.....	4.78×10^{-7}	5.43×10^{-7}
5,820	.....	4.68×10^{-7}	6.02×10^{-7}
7,620	.....	5.25×10^{-7}	5.75×10^{-7}
9,420	.....	5.91×10^{-7}	5.35×10^{-7}
11,220	.....	.....	6.52×10^{-7}
13,020	.....	.....	5.98×10^{-7}
	Av.	5.15×10^{-7}	5.84×10^{-7}
Water basis values	.....	5.33×10^{-7}	6.05×10^{-7}
Molecular weight	.....	106,000	62,200

The specific sedimentation velocity values obtained in different solvents can be converted to a basis of sedimentation in water merely by multiplying by the relative viscosity of the solvent and the ratio of the difference in densities between the particle and the solvent, in water and in the solvent under consideration. The diffusion constant values can be converted to a basis of diffusion in pure water by merely multiplying by the relative viscosity of the solvent. The results in Table III show very nicely the constancy of both the specific sedimentation velocity and diffusion constant when corrected to a water basis for all of the solvents except those in which dissociation took place.

The molecular weight of normal edestin is practically the same as that of phycoerythrin. The specific sedimentation velocity and diffusion constant are likewise the same within experimental error. Calculation of the radius of the particle by applying both Stoke's law and Einstein's law shows that the edestin molecules are practically spherical as in the case with phycoerythrin.[2d]

This research gives considerable further evidence in favor of the senior author's theory that the proteins all have molecular weights that are integral multiples of the molecular weight of egg albumin, namely, 34,500.

Normal edestin has a molecular weight very close to six times this value. Every one of the proteins thus far studied in this Laboratory has a molecular weight of either 1, 2, 3 or 6 times that of the egg albumin with the exception of the two hemocyanins, which are such large multiples that it is impossible to determine whether they are integral multiples.

The nature of the dissociation products of edestin at P_H 11.3, obtained by the new method of Lamm, gives perhaps the best single piece of evidence obtained to date of the validity of the theory. Not only are the molecular weights of the dissociation products even multiples, but they are the same multiples as other known proteins and have the same specific sedimentation velocities and diffusion constants. No protein with a molecular weight 4 times that of egg albumin has ever been found. It is of interest to note that there was no evidence of any such multiple molecular weight dissociation product either, or a multiple of 1.5 as might be expected if the dissociation took place in two steps. A more extended research upon the dissociation products of edestin as well as of other proteins outside of their normal P_H stability range will undoubtedly settle this theoretical consideration definitely.

The expenses connected with these experiments have been defrayed by grants from the Nobel Fund of Chemistry and from the foundation "Therese och Johan Anderssons Minne."

Summary

1. The previously developed ultracentrifuge methods for determining the molecular weights of proteins have been shown to be completely applicable to the study of proteins dissolved in strong salt solutions.

2. The normal molecular weight of edestin was found to be 212,000 ± 10,000, the average specific sedimentation velocity 12.8×10^{-13} cm./sec. and the average diffusion constant 5.6×10^{-7} cm.^{2}/sec. both on a water basis at 20°.

3. The edestin concentration showed no effect upon the resulting molecular weight, above a concentration of 0.1%. Below this value definite dissociation or decomposition took place.

4. Edestin was shown to be quite stable and to give a normal molecular weight on the alkaline side of the isoelectric point (P_H 5.5 by solubility measurements) as high as a P_H of 9.7. At a P_H of 11.3 definite dissociation took place. Edestin was found to be quite unstable on the acid side of the isoelectric point, definite dissociation taking place at P_H 3.1.

5. Employing a new method developed by Ole Lamm at this Laboratory for determining the concentration gradients in the sedimentation systems, the molecular constitution of dissociated edestin at a P_H of 11.3 was definitely determined. Under the conditions of the experiment given,

molecules of one-half and one-third of the normal molecular weight are present together with the normal.

UPSALA, SWEDEN

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE COLLEGE OF THE CITY OF NEW YORK]

THE FATTY ACIDS ASSOCIATED WITH RICE STARCH

BY LEO LEHRMAN

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It has been shown that in several naturally occurring starches fatty acids are combined with carbohydrate.[1] In the case of corn and rice the fatty acids constitute part of the molecule of one of the components, namely, the α-amylose.[2] The fatty acids liberated by the hydrolysis of corn starch free from extraneous material have been identified and their amounts determined.[1b,3] From a review of the "fat by hydrolysis,"[1b] acid number and iodine number of the fatty acids liberated from corn and rice starch free from extraneous material,[1b] a difference in amounts and possibly in kind is apparent. Therefore, it is important that these fatty acids which are in the rice starch molecule[1] (α-amylose portion) should be determined both qualitatively and quantitatively. This would enable a comparison of these two starches and furnish additional evidence in the understanding of the different behavior of the starches.

In the course of the investigation the probable absence of sterols or other substances that might occur in the starch and be present in the liberated fatty acids was shown. As the fatty acids occurring in the germ are present as glycerides, the aqueous filtrate from the hydrolysis was examined for glycerol; a negative result was obtained. This is added evidence that the fatty acids identified were not present extraneously in the starch.

Experimental Part

Mixed Fatty Acids from Rice Starch.—The rice starch[4] which was used as the starting material had a negligible amount of extractable extraneous material, using petroleum ether as the solvent. In the course of the work a total of twenty pounds was used. The hydrolysis[1b] was carried out using hydrochloric acid and a much more concentrated suspension of the rice starch. Five pounds of the starch yielded a suspension of 3.5 liters; the acid solution was made up by diluting 525 cc. of concentrated hydrochloric acid to 1.5 liters. The filtrate from the hydrolysis, which had a dark brown color, was reserved for the examination for glycerol. The fat by hydrolysis, obtained by

[1] (a) Sostegni, *Gazz. chim. ital.*, **15**, 376 (1885); (b) Taylor and Nelson, THIS JOURNAL, **42**, 1726 (1920); (c) Aoi, *J. Chem. Soc., Japan*, **44**, 755 (1923).

[2] Taylor and Iddles, *Ind. Eng. Chem.*, **18**, 713 (1926).

[3] Taylor and Lehrman, THIS JOURNAL, **48**, 1739 (1926).

[4] The author wishes to thank Stein, Hall and Co., Inc., New York City, for their kindness in supplying this material.