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

THE MOLECULAR WEIGHT OF EGG ALBUMIN
I. IN ELECTROLYTE-FREE CONDITION

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For large molecules of complicated structure, such as the proteins, the classical methods of molecular-weight determination (except that of the osmotic pressure) are not sufficiently sensitive. The diffusion measurements of Herzog[2] have been used to calculate the molecular weight but, depending on the formula employed, the value for egg albumin was found to be 73,000 or 17,000. In addition to this uncertainty in the value found, there is the possibility of alteration occurring in the protein during the length of time required for diffusion in his experiments.

Until the present the best method of estimating the molecular weights of the proteins has been through the measurement of the osmotic pressure. Such a method is rendered quite uncertain, however, by the effects arising from the Donnan membrane equilibria and other causes. Lillie's measurements[3] were made on egg white simply diluted with water to precipitate the globulins. As he was therefore dealing with a mixture of egg albumin, conalbumin and ovomucoid his results cannot be used in the estimation of the molecular weight of egg albumin. Sørensen's very careful measurements,[4] which were corrected for the membrane equilibrium, gave 34,000 as the most probable value for the molecular weight of electrolyte-free egg albumin. Adair,[5] on the basis of his own refined osmotic pressure measurements of hemoglobin, questions Sørensen's interpretation and considers that the Sørensen value should be doubled, giving 66,000 as the true molecular weight.

A direct method free from membrane complications has recently been developed by one of us.[6] It depends upon the establishment of an equilibrium between the centrifuging and the diffusion of the material as the result of prolonged centrifuging at constant temperature.

Theoretical Part

Since in the previous paper the kinetic derivation was given of the expression for the molecular weight, the thermodynamic derivation[7] will be given here.

[1] American-Scandinavian Fellow.

[2] Herzog, *Z. Chem. Ind. Kolloide*, **2**, 2 (1907); *Z. Elektrochem.*, **16**, 1003 (1910).

[3] Lillie, *Am. J. Physiol.*, **20**, 127 (1907).

[4] Sørensen, *Medd. Carlsberg Lab.*, **12**, 348 (1917).

[5] Adair, *Proc. Cambridge Phil. Soc., Biological Sciences*, **1**, 75 (1924).

[6] Svedberg and Fåhraeus, THIS JOURNAL, **48**, 430 (1926).

[7] Svedberg, *Z. physik. Chem.*, **121**, 65 (1926). Lewis and Randall, "Thermodynamics," McGraw-Hill Book Co., New York, **1923**, p. 244.

In a solution subjected to a centrifugal field of force, the free energy F per mole of solute is a function of x the distance from the axis of rotation, p the hydrostatic pressure, and c the concentration of the dissolved substance. The change in free energy from point to point in the solution is then $dF = (\partial F/\partial x)dx + (\partial F/\partial p)dp + (\partial F/\partial c)dc$. If the centrifuging is continued long enough to enable the solute to reach equilibrium between centrifugal sedimentation and diffusion, then $dF = 0$. For a dilute solution, $\partial F/\partial x = -M\omega^2 x$; $\partial F/\partial p = MV$; $\partial F/\partial c = RT/c$; $dp = \rho\omega^2 x dx$, where M is the molecular weight, ω the angular velocity, V the partial specific volume of the solute, R the gas constant, T the absolute temperature and ρ the density of the solution.

Substituting, $0 = -M\omega^2 x dx + MV\rho\omega^2 x dx + (RT/c)dc$, or $dc/c = [M(1 - V\rho)\omega^2 x dx]/RT$. After integrating we obtain

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

Apparatus and Method

The ultracentrifuge used and the method employed for the determination of the molecular weight of a colored protein, hemoglobin, have already been described.[8] A small amount, about 0.04 cc., of the solution to be studied is introduced into a quartz cell possessing plane-parallel windows and is rotated at constant temperature in the special centrifuge until equilibrium between centrifuging and diffusion is reached. Then the variation of concentration of the solution with distance from the axis of rotation is determined photographically.

Fig. 1 shows a vertical section of the rotor and the supporting shaft without the cell holder in place. The lower diagram represents the rotor from beneath. The main portion of the rotor was turned on a lathe from a blank of an especially tough alloy used for certain separator parts. The surface of the rotor that rests on the shaft must have a certain curvature to allow the rotor to assume slightly different positions on the shaft in the self-balancing process, while the centrifuge is being brought up to the desired speed. The protecting disk, containing four sectorial apertures, and screwed on to the lower part of the rotor, serves as a deflector for the stream of microscopic oil droplets proceeding upwards from the bearing. This stream is thrown out towards the periphery and passes through the openings in the protecting disk; but during the short time required for the droplets to reach the cell windows, the rotor has turned sufficiently to cause the droplets to impinge on the brass screen and deposit there instead of on the windows.

The cell holder consists of three brass rings screwed together as indicated in Fig. 2. The cells are made of two quartz windows 20 × 15 × 5 mm.

[8] Svedberg and Rinde, THIS JOURNAL, **46**, 2677 (1924). See also Ref. 6.

and an intermediate glass plate 1.84 mm. thick from which is cut a 5° sector-shaped opening. These three plates are cemented together with de Khotinsky cement, yielding a plane-parallel, sectorial cell of 2.00 mm. thickness as the container for the solution to be studied. Two pairs of cells having inside lengths of 9 mm. and 15 mm., respectively, are arranged in the cell holder as in Fig. 2. The cells are separated from the metal by thin ebonite plates and are provided with brass covers lined with a thin layer of rubber. By means of suitably located apertures in the thin brass screen that fits immediately under the cell holder in the rotor, two solutions may be studied at the same time in the cells whose outer ends are at different distances from

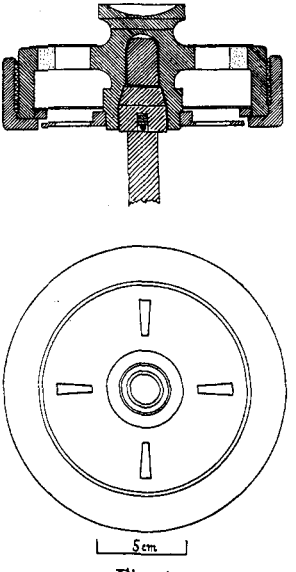

Fig. 1.

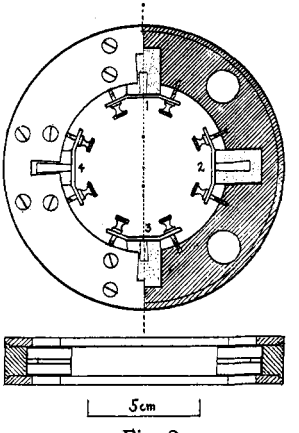

Fig. 2.

the axis of rotation. Thus, one diaphragm used permitted light to pass only through the region 47.5 mm. to 41.5 mm. from the axis of rotation in Cell 4, in Cell 3 from 41.5 to 36.0 mm., and in Cell 2 from 36.0 to 32.5 mm. Cell 2 was used for a standard ($Ca(NO_3)_2$ for the ultraviolet region) in order to adjust the incident light intensity of the lamp to the same value for a series of photographic exposures. To prevent the rise of disturbing convection currents due to evaporation from the solution in the cell, and to protect the protein from the action of the oxygen and carbon dioxide of the air, the solution was covered with a layer of vacuum oil. Both the protein and the cell cement are entirely inactive toward this oil.

Since egg albumin, in common with most proteins, has an absorption band only in the short ultraviolet region of the spectrum, a quartz mercury lamp was used as the source of illumination. The photographic lens used was a biconvex quartz lens of 46 cm. focal length for light of wave length 253 $m\mu$.

In order to obtain maximum sensitivity in the determination of concentration by the photographic method, light of a wave length corresponding to a strong absorption band of the substance should be used. To remove unsuitable wave lengths, light filters must be inserted. Of course, the isolation of a certain wave length in the visible offers no great difficulty, but none of the ordinary filters is satisfactory for the short ultraviolet. In order to ascertain the exact requirements for the present investigation, the variation of absorption with wave length for a layer 2 cm. thick of a 0.09% solution of crystallized, salt-free egg albumin was determined by means of a Judd-Lewis sector photometer. In Fig. 3 the resulting curve is plotted, using as ordinates the logarithms of the ratio of the incident light to the transmitted light, $\log I_0/I$. The curve is equivalent to that of a 0.9% solution in the centrifuge, a strength very frequently employed. The required filter should evidently transmit only light of wave length shorter than 290 mμ. A combination of gaseous chlorine and bromine[9] seems to be practically the only suitable filter for the isolation of this region.

The filters were made of 8 cm. lengths of glass tubing of 44mm. inside diameter and provided with side arms for filling, and the ends were closed by quartz plates. Vaseline was used as a seal for the chlorine cell, but for the bromine cell phosphorus pentoxide paste was more satisfactory. The bromine reacted too rapidly with the vaseline, consequently producing on the windows a film opaque to the short ultraviolet. The inside wall of the chlorine filter was coated with a lampblack varnish to diminish reflection, but since the light passing through the bromine filter close to the lens is nearly parallel no protection against reflection was needed for this filter. The respective filters were filled with well-dried chlorine at atmospheric pressure and bromine at its partial pressure for ordinary room temperatures of about 150 mm. at 17°. The chlorine filter required refilling at only two-month intervals, but the bromine filter deteriorated with sufficient rapidity to necessitate weekly cleaning and refilling.

In Fig. 3 approximate values of $\log I_0/I$ are plotted against wave length for the two filters to show their suitability for use in the present investigation. Although the yellow line of the mercury spectrum at 579 mμ is not absorbed, the photographic plates used were not sensitive to this region, so actually the only effective lines are those at 265 mμ, the strong resonance band at 254 mμ, and the line at 248 mμ. Since the protein has so strong an absorption below 248 mμ, the shorter, weak lines of the mercury spectrum are probably completely absorbed even by very low concentrations of the protein and therefore produce a negligible effect on the photographic plate.

The quartz optical system is indicated diagrammatically in Fig. 4.

[9] Peskov, *J. Phys. Chem.*, **21**, 382 (1917).

The mercury lamp is represented by 1, 2 is a 5cm. water filter to remove the infra-red rays, 3 an electromagnetic shutter for making the exposures,

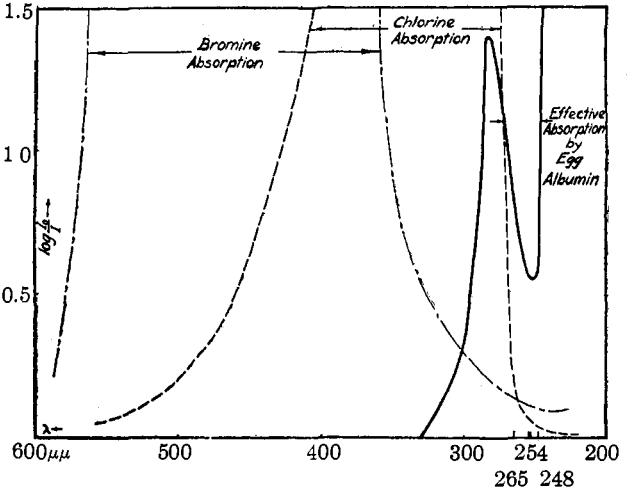

Fig. 3.—Relative light absorptions of salt-free egg albumin and gaseous chlorine and bromine. Full line: 0.09% egg albumin in 2 cm. thickness of layer; dotted lines: gaseous chlorine and bromine in 8 cm. thickness of layer.

4 the chlorine filter, 5 the totally reflecting quartz prism, 6 and 8 are the windows of the centrifuge, 7 the quartz cell which is rotated, 9 the bromine filter immediately below the quartz lens 10, and 11 is the photographic plate on which may be made a maximum of twenty exposures.

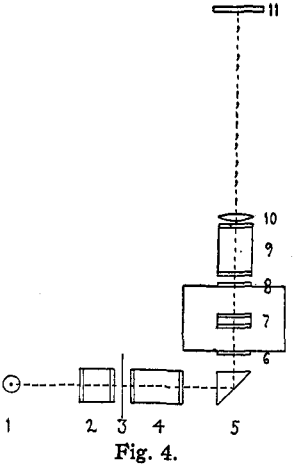

Fig. 4.

As mentioned above, one of the centrifuge cells contained a solution of pure calcium nitrate to act as a standard in adjusting the different exposures to the same incident light intensity. The concentration used should be such that the absorption is approximately the same as that of the protein solution to be studied. The automatic microphotometer used for registering the plates gives a nearly linear relation between galvanometer deflection of the photometer thermopile and the concentration when the underexposure portion of the photographic curve is used. Therefore, the variation in intensity of the mercury-arc lamp for different exposures may be corrected for by multiplying by the ratio of the galvanometer deflections produced by the calcium nitrate portions of the exposures. For very low exposures, however,

the slight plate fog disturbs this correction so that accurate results are not obtained. If the range in concentration when equilibrium is attained is too great to fall within the linear region of the photometer curve, then two or more exposures at different lengths of time must be taken. Generally, four different times of exposure, giving an eight-fold variation in time, were made to eliminate any exposure effects.

The ordinary length of the column of solution was 0.5 cm., the magnification of the lens 2.017 and that of the microphotometer 10 so that 0.0496 cm., the difference $x_2 - x_1$ of Equation 1, corresponded to 1.00 cm. on the microphotometric curve.

Experimental Part

Crystallized egg albumin was prepared according to Sørensen's method[10] and dialyzed for 18 days in collodion bags immersed in flowing, distilled water at 0°, saturated with toluene. The conductivity at 17° for a 6.6% solution was 1×10^{-4} mhos. Centrifuging experiments carried out on solutions made from stock solutions purified only by crystallization and dialysis indicated that some impurity was still present. Therefore, immediately before use the samples were further purified by means of the Pauli electrodialyzer[11] for a period of one and one-half to two days at a current density of 0.6 milliampere per sq. cm. The P_H value of the salt-free protein was then 4.8 to 4.9, slightly on the alkaline side of the iso-electric point.

The partial specific volume of the material was determined pycnometrically at 19.2°. As with hemoglobin the value varies little with concentration and was taken as 0.749 for dilute solutions. Concentrations of the protein were determined by drying a sample to constant weight in an electric oven at 105°.

Effect of concentration on the molecular weight was studied only through the range of 0.45 to 1.5%. However, this is actually equivalent to a range of 0.3 to 2.2% as may be seen from Tables II, III and IV. Solutions of higher concentration than 1.5% gave inconclusive results because of the range of exposures required to include the concentration range. For example, in an experiment with a 2.4% solution a range of exposures from 40 seconds to 18 minutes was necessary. The longer exposures gave very erratic results, due in part to increased fog on the plate and also to some phenomenon similar to halation (possibly fluorescence of the gelatin of the emulsion), causing a spreading of the image of the equilibrium exposure where there was a steep change in blackening of the plate.

One experiment was made using a 56cm. chlorine filter alone to remove the long-waved ultraviolet and the visible portions of the spectrum. Even though much visible light was transmitted, causing the image to be partially out of focus and the accuracy of concentration measurement to be low, comparable results were obtained.

[10] Sørensen, Ref. 4, p. 12.

[11] Pauli, *Biochem. Z.*, **152**, 355 (1924).

Imperial Eclipse plates were used in all of the experiments and were developed for three minutes in Ilford Metol developer for soft effects.

Results

Four series of measurements are given below: one on crystallized, dialyzed egg albumin to show the effect of a small amount of impurity on the values of the molecular weight, and three on egg albumin that was further purified by electrodialysis.

It should be noted that the mean concentrations given in the tables do not represent the average of the individual concentrations for the different exposures, but are back-calculated (using the end value of the concentration in one of the exposures as the starting point) from the averaged values of the quantities log c_2/c_1 determined from the concentrations in the individual exposures. Otherwise, errors would have been introduced in combining the data obtained from different lengths of exposure. This is due to the fact that the calcium nitrate standard gives no information as to the variation in light intensity between two different lengths of exposure, but only between exposures of the same length. Since after equilibrium was reached the values for the molecular weight remained constant for many hours, in general each determination consisted of exposures taken at several different times and various lengths of exposures, giving from three to eight values for each $x_2 - x_1$ interval.

Table I gives the results obtained for an unelectrodialyzed sample. Six exposures were used for the calculations corresponding to 15- and 30-second exposures at 28.5 and 35 hours after starting the centrifuge, duplicate exposures being taken at the latter time. The method of calculation

TABLE I

EXPT. 8. UNELECTRODIALYZED EGG ALBUMIN

Concentration, 0.78 g. per 100 cc.; conductivity, 7×10^{-5} mhos. in a 1.56% solution at 17°; $V = 0.749$; $T = 288°$; ρ, the density of the solution, 1.0004; b, the distance of outer end of solution from axis of rotation, 4.73 cm.; length of column of solution, 0.54 cm.; thickness of column, 0.200 cm.; $x_2 - x_1$ interval, 0.0496 cm.; speed, 10,550 r.p.m. ($\omega = 351.7\ \pi$); aperture of the quartz lens, f40; the number of exposures was 3 in every case except the first where it was 6.

Distances, cm.		Mean concns., g. per 100 cc.			Calcd. values for a mixture 94% of 34,000 + 6% of 170,000
x_2	x_1	c_2	c_1	M (obs.)	
4.680	4.630	1.0455	0.9149	45,300	47,600
4.630	4.581	0.9149	.8077	42,750	43,700
4.581	4.531	.8077	.7128	43,300	40,400
4.531	4.482	.7128	.6386	38,500	38,000
4.482	4.432	.6386	.5754	36,950	37,800
4.432	4.382	.5754	.5196	36,550	36,300
4.382	4.333	.5196	.4708	35,700	35,450
4.333	4.283	.4708	.4277	35,200	35,100
4.283	4.233	.4277	.3890	35,150	35,000

and significance of the last column will be explained under the heading, "Discussion of Results."

Tables II, III and IV give the results obtained for electrodialyzed, salt-free egg albumin at different concentrations. In the case of the 0.45% albumin the values were calculated from 5-, 7- and 10-second exposures taken 35.5, 41 and 47 hours after the start of the centrifuging. For the 1.1% solution the exposures used were 10, 15, 30 and 45 seconds in length at times of 37, 49 and 59.5 hours after the start. This is probably the most reliable of the series because the material used was a sample electrodialyzed for the experiment immediately after the completion of the preparation of

TABLE II

EXPT. 22. 0.45% ELECTRODIALYZED EGG ALBUMIN

Concentration, 0.45 g. per 100 cc.; conductivity, 9.9×10^{-6} mhos. at 17° in a 0.9% solution after being electrodialyzed for 30 hours at a current density of 0.6 milliamps. per sq. cm.; $V = 0.749$; $\rho = 0.9997$; $T = 290°$; $b = 4.73$ cm.; length of column of solution, 0.522 cm.; speed, 10,740 r.p.m. ($\omega = 358.0\ \pi$); same thickness of cell and aperture of lens as before.

Distances, cm. x_2	Distances, cm. x_1	Exposures used	Mean concns., g. per 100 cc. c_2	Mean concns., g. per 100 cc. c_1	M
4.680	4.630	6	0.7394	0.6677	33,600
4.630	4.581	6	.6677	.6028	34,000
4.581	4.531	7	.6028	.5411	36,300
4.531	4.481	8	.5411	.4868	35,900
4.482	4.432	8	.4868	.4396	35,050
4.432	4.382	7	.4396	.3980	34,600
4.382	4.333	6	.3980	.3602	35,050
4.333	4.283	5	.3602	.3269	34,500
4.283	4.233	5	.3269	.2970	34,500
				Av.	34,830

TABLE III

EXPT. 17. 1.1% ELECTRODIALYZED EGG ALBUMIN

Concentration, 1.1 g. per 100 cc.; conductivity, 3.42×10^{-5} mhos. at 18° for a 6.6% solution after electrodialyzing for 30 hours at a current density of 0.6 milliamp. per sq. cm.; $V = 0.749$; $\rho = 1.0012$; $T = 288$; $b = 4.73$ cm.; length of column of solution, 0.556 cm.; speed, 10,700 r.p.m. ($\omega = 356.7\ \pi$).

Distances, cm. x_2	Distances, cm. x_1	Exposures used	Mean concns., g. per 100 cc. c_2	Mean concns., g. per 100 cc. c_1	M
4.680	4.630	6	1.7408	1.5713	33,800
4.630	4.581	6	1.5713	1.4260	32,400
4.581	4.531	5	1.4260	1.2980	31,800
4.531	4.482	5	1.2980	1.1713	35,100
4.482	4.432	6	1.1713	1.0580	35,150
4.432	4.382	6	1.0580	0.9586	34,400
4.382	4.333	6	0.9586	.8667	35,600
4.333	4.283	6	.8667	.7832	36,200
4.283	4.233	3	.7832	.7100	35,400
				Av.	34,430

TABLE IV

EXPT. 28. 1.5% ELECTRODIALYZED EGG ALBUMIN

Concentration, 1.5 g. per 100 cc.; conductivity, 1.2×10^{-5} mhos. at 17° for a 6.0% solution electrodialyzed 48 hours at 0.6 milliamp. per sq. cm.; $V = 0.749$; $\rho = 1.0022$; $T = 291°$; $b = 4.73$ cm.; length of column of solution, 0.510 cm.; speed, 10,950 r.p.m. ($\omega = 365\ \pi$).

Distances, cm. x_2	x_1	Exposures used	Mean concns., g. per 100 cc. c_2	c_1	M
4.680	4.630	3	2.1914	1.9775	32,850
4.630	4.581	4	1.9775	1.7830	33,500
4.581	4.531	5	1.7830	1.5923	36,950
4.531	4.482	6	1.5923	1.4250	36,650
4.482	4.432	5	1.4250	1.2862	34,200
4.432	4.382	6	1.2862	1.1564	35,900
4.382	4.333	6	1.1564	1.0426	35,650
4.333	4.283	4	1.0426	0.9398	35,550
4.283	4.233	4	0.9398	.8500	35,050
				Av.	35,150

a fresh stock solution of egg albumin. Table IV gives the results for a 1.5% solution based on exposures 10, 20, 40 and 80 seconds in length taken at times of 33.5, 39 and 43 hours after the start. The original photometric records for the 20- and 80-second exposures at 43 hours of centrifuging are reproduced in Fig. 5. The ordinates represent galvano-

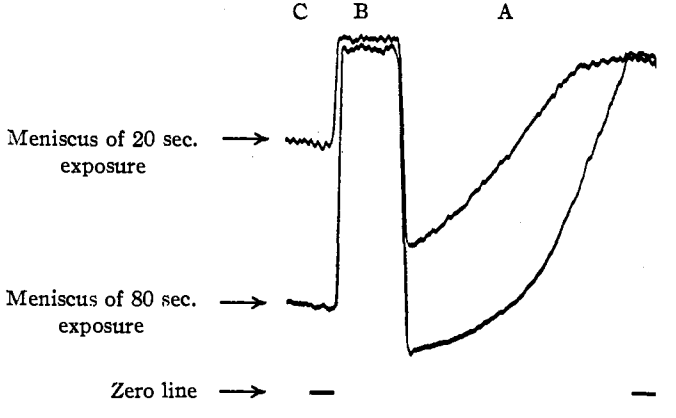

Fig. 5.

meter deflections and the abscissas distances. The 20-second exposure is the upper curve. Region A is the equilibrium curve of the albumin, B is the oil layer covering the meniscus and C is the calcium nitrate standard.

Discussion of Results

It is evident from the drift in the values obtained for the molecular weight of egg albumin of only moderate purity (Table I) that there is

more than one size of molecule present. By means of the equation[12]

$$C_x = \sum_{1}^{r} c_r \cdot nA \cdot \frac{e^{-n\,B(b^2 - x^2)}}{1 - e^{-nA}} \qquad (2)$$

where $B = M(1 - V\rho)\omega^2/2RT$ and $A = B(b^2 - a^2)$, the distribution of sizes may be calculated from the system of simultaneous equations obtained. The significance of most of these quantities has already been given in the derivation of Equation 1, but of those introduced here, C_x refers to the total concentration of material at the distance x from the axis of rotation after the establishment of the equilibrium, c_r is the original concentration of the size of molecule r times the smallest molecule m assumed to be present, b is the distance of the outer end of the cell, and a the distance of the meniscus of the solution from the axis of rotation.

Making a preliminary assumption of a mixture of molecules 1, 2, 3 and 4 times 17,000, an unsuccessful attempt was made to calculate the distribution. Then, in order to obtain an idea of the probable range of sizes to be expected, an analysis was made of the theoretical sedimentation equilibrium curves developed by Mason and Weaver.[13] An egg albumin solution containing only molecules of size 34,000, subjected to the experimental conditions indicated under Table I, would be represented approximately by a curve developed by $\alpha = 1$. From this series of curves it was noted that molecules having a sedimentation equilibrium curve developed by $\alpha = 0.2$ would be almost completely centrifuged from the meniscus region and would increase very rapidly in concentration near the outer end of the cell. Therefore, a mixture composed mostly of molecules of size 34,000 and a small percentage of relatively large molecules, about five times the size of the first, should give a relation between molecular weight and distance from the axis of rotation quite similar to that experimentally found.

Calculations showed that a mixture of 94% of molecules 34,000 and 6% of molecules $5 \times 34{,}000$, or 170,000, satisfied the observed drift in molecular weight better than any other mixture, although 92% of molecules 34,000 and 8% of molecules $4 \times 34{,}000$, or 136,000, gave a fair agreement. The curve calculated for the former mixture is given as the full line in Fig. 6, and the observed values are indicated as circles. The agreement is all that could be expected. Several curves are also given in dotted line representing hypothetical mixtures of molecules not far removed from 34,000 to show the great difference in character of the drift of such mixtures from the first mixtures mentioned above.

There are two possible explanations for the presence of a small amount

[12] Developed by Rinde of this Laboratory, in the course of his as yet unpublished investigations on the sedimentation equilibria established in gold sols after prolonged centrifuging.

[13] Mason and Weaver, *Phys. Rev.*, **23**, 412 (1924).

of material of molecular weight approximately 170,000. Either it is the remainder of the globulins that are not removed until the electrolyte content is extremely low or else this represents the average size of a small quantity of egg albumin aggregated by the salts still present. Although it is more probable that unprecipitated globulins were the main cause, since a test for globulins was actually obtained, there may have been a slight aggregation as well. Egg albumin is quite sensitive to salts—as will be shown in a later paper.

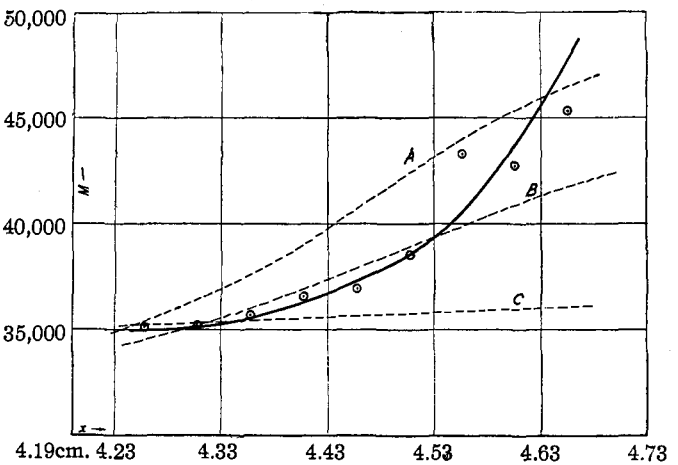

Fig. 6.—Relation between molecular weight and distance. Full line: calculated curve for a mixture of molecules, 94% of 34,000 and 6% of 170,000. Circles: experimental values from Table I. Dotted lines: hypothetical mixtures of molecules. *A*. 40% of 17,000, 10% of 34,000, 10% of 51,000 and 40% of 68,000. *B*. 30% of 17,000, 30% of 34,000, 20% of 51,000 and 20% of 68,000. *C*. 95% of 34,000 and 5% of 68,000.

When the electrolyte content was made negligible through further purification by electrodialysis, the last portions of the globulins apparently were precipitated, or the aggregating influence was removed. Immediately, the drift in the values of the molecular weight disappeared and consistent results were obtained, whereas before they had been somewhat erratic. The conductivity of these electrodialyzed solutions was generally not more than one-fourth that of the solutions only dialyzed, so they were much purer.

Certain mixtures would be very difficult to detect by means of the sedimentation equilibrium. For instance, a mixture composed of 5% of molecules 17,000, 90% of 34,000 and 5% of 68,000 would give a drift from only 35,500 to 34,000, which would appear uniform within the limits of the present experimental error. Again, a mixture composed of 95% of 34,000 and 5% of molecules 68,000, indicated in Fig. 6, would seem to satisfy the

values obtained under Table IV, except for the two values nearest the outer end, which, however, are sufficiently low to render such a mixture improbable. Therefore, it cannot *definitely* be decided that small amounts of molecules not greatly different in size from 34,000 are absent until determinations are made at sufficiently high speeds (about 40,000 r.p.m.), so that the distribution may be calculated from the initial period of sedimentation before the equilibrium is established. Only under such conditions would there be sufficient separation for accurate distribution results.

As the result of the present investigation it may be concluded with confidence that egg albumin in salt-free conditions is composed for the most part, perhaps almost exclusively, of molecules of weight 34,500 ± 1000 for dilute solutions. This estimate is based more on the general trend of the whole investigation than merely on the few experiments reported above. Thus Sørensen did actually succeed in avoiding membrane uncertainties in his estimate of approximately 34,000, and Cohn's[14] minimal molecular weight of 33,800, based on all of the available analytical evidence, also represents the true molecular weight.

In salt-containing solutions and in solutions at P_H values differing much from the iso-electric point the conditions are not nearly so simple; therefore this material will form the subject of a subsequent paper.

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

Summary

1. The centrifugal sedimentation equilibrium method for the determination of the molecular weight of the proteins has been further amplified to include the colorless proteins.

2. It has been shown that in egg albumin of only moderate purity there was a small amount present of some material of molecular weight approximately 170,000.

3. Further purification removed this foreign material, and the results obtained indicated strongly that in electrolyte-free solutions egg albumin is a uniform substance of molecular weight 34,500 ± 1000.

UPSALA, SWEDEN

[14] Cohn, Hendry and Prentiss, *J. Biol. Chem.*, **63**, 721 (1925).