be diphenyl ketimine hydrochloride. Calcd. for $C_{13}H_{12}NCl$: N, 6.4; Cl, 16.3. Found: N, 6.6; Cl, 18.2.

In conclusion, the writer wishes to express his appreciation to Dr. Franklin, under whose kind guidance the work presented here has been carried out.

Summary

1. It is shown in this paper that the amidines are to be regarded as the ammonia analogs of the carboxylic acids. The term carbazylic acid has been used to denote these acids.

2. The alkyl and aryl cyanides have been shown to be the anammonides of the carbazylic acids.

3. The preparation of a number of salts of the different carbazylic acids has been described.

4. The ammonation of the acid anammonides in the presence of an acid has been demonstrated.

5. The decomposition of the salts of the carbazylic acids, when heated with a metallic amide, proceeds with the formation of a hydrocarbon and an alkali salt of cyanamide.

6. The difference in behavior of hydrocyanic acid and cyanogen from that of other nitriles toward potassium amide in liquid ammonia solution has been noted and an explanation offered.

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

THE MOLECULAR WEIGHTS OF SERUM ALBUMIN AND OF SERUM GLOBULIN

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The two chief protein constituents of blood serum are serum albumin and serum globulin. Crystallized serum albumin was obtained as early as 1894,[1] while serum globulin crystals have not as yet been observed. The fact that serum albumin is easily crystallized has been taken as an indication of its homogeneity. Recently, however, the validity of this conclusion has been questioned by Sörensen,[2] who found that by fractional crystallization a series of protein samples with different solubilities could be obtained from serum albumin. The osmotic pressures of the various fractions were, however, the same. The question as to whether serum globulin is a simple protein or a mixture has been the object of much experi-

[1] A. Gürber, *Würzburger Physiol.-med. Ges.*, **1894**, 113; **1895**, 26.

[2] S. P. L. Sörensen, "Proteins," The Fleischmann Company, New York, **1925**, p. 40.

mental work and discussion. By the fractional precipitation of serum globulin it is possible to obtain protein samples differing in solubility and in chemical composition, the so-called euglobulins and pseudoglobulins; this fact has been taken as a proof of the complex nature of serum globulin.[3] Sörensen found somewhat higher osmotic pressures for the pseudoglobulin fractions than for the euglobulin fractions[4] and so did Adair.[5]

The molecular weights of serum albumin and of the serum globulin fractions have been calculated by Sörensen and by Adair from their osmotic pressure measurements. Sörensen gives 45,000 and Adair 62,000 for serum albumin. Sörensen's globulin fractions gave values from 140,000 (euglobulins) to 80,000 (pseudoglobulins), while Adair found for euglobulin 174,000 and for pseudoglobulin fractions 150,000–130,000.

Cohn has made an attempt to estimate the molecular weights of these proteins from analytical data.[6] For serum albumin he arrives at the value 45,000, and for serum globulin he finds 27,000 as the minimal molecular weight, which value he multiplies by three in order to obtain a molecular weight of 81,000 in agreement with Sörensen's osmotic pressure determinations.

In view of the uncertainty involved in many of the analytical data used by Cohn in his calculations and the numerous and uncontrollable sources of error in osmotic measurements, it was thought advisable to undertake an investigation of the molecular weights of these important proteins by means of the centrifugal methods already used in this Laboratory for the determination of the molecular weights of hemoglobin,[7] egg albumin,[8] phycocyan,[9] phycoerythrin[9] and hemocyanin.[10]

Experimental

The protein material used in this investigation was prepared from horse blood. All determinations were carried out in buffer solutions at the isoelectric point, namely, at P_H 4.8 for serum albumin and at 5.5 for serum globulin, where the stability of the proteins is greatest and all effects due to the presence of protein ions are at a minimum. A study of the P_H limits within which these proteins are stable will be carried out later.

[3] (a) E. Marcus, *Z. physiol. Chem.*, **28**, 559 (1899); (b) E. Fuld and K. Spiro, *ibid.*, **31**, 132 (1900); (c) E. P. Pick, *Hofmeisters Beitr.*, **1**, 351, 393 (1901); (d) O. Porges and K. Spiro, *ibid.*, **3**, 277 (1902).

[4] Ref. 2, p. 52.

[5] G. S. Adair, *Scandinavian Arch. Physiol.*, **49**, 76 (1926).

[6] (a) E. J. Cohn, J. L. Hendry and A. M. Prentiss, *J. Biol. Chem.*, **63**, 721 (1925); (b) E. J. Cohn, *Physiol. Reviews*, **5**, 349 (1925).

[7] (a) T. Svedberg and R. Fåhraeus, THIS JOURNAL, **48**, 430 (1926), (b) T. Svedberg and J. B. Nichols, *ibid.*, **49**, 2920 (1927).

[8] T. Svedberg and J. B. Nichols, *ibid.*, **48**, 3081 (1926).

[9] T. Svedberg and N. B. Lewis, *ibid.*, **50**, 525 (1928).

[10] T. Svedberg and E. Chirnoaga, *ibid.*, **50**, 1399 (1928).

Preparation of Material.—Serum albumin was prepared in the following manner.[11] Horse serum free from hemoglobin and containing toluene as a preservative was mixed with an equal volume of a saturated ammonium sulfate solution. After standing for one day at 5°, the globulin precipitate was centrifuged off. To the solution was added slowly 0.1 *N* sulfuric acid half saturated with ammonium sulfate until a permanent cloudiness appeared. Toluene was added and the mixture was left to crystallize at room temperature. After a period of three days, the crystals were filtered off and washed with a weak solution of ammonium sulfate. The serum albumin thus obtained was recrystallized three times and for eight days dialyzed in collodion bags against flowing distilled water saturated with toluene at 0°. In order to remove the last traces of globulin it was finally electrodialyzed in a Pauli apparatus[12] for thirty-six hours at a current density of 0.6 mil. amp. per sq. cm. The concentration of the stock solution made up from this material (called in the following serum albumin I) was 0.76%.

Measurements with the ultracentrifuge showed that the protein of the above solution had been partly split into some non-centrifugible substance. We therefore prepared a second batch of serum albumin, working as quickly as possible. Only one process of crystallization was performed. The dialysis was continued for four days only and the electrodialysis for three days. A stock solution containing 2.80% of organic substance in acetate buffer at P_H 4.8 (0.02 *N* with regard to acetate ion) was made up (serum albumin II). The centrifuging experiments showed that this solution was much less decomposed.

During the work done on proteins in this Laboratory we have often had the opportunity to observe that an elaborate process of purification furnishes a material less homogeneous with regard to molecular weight than does a cruder but quicker method. The preparation of serum albumin is a typical example of this phenomenon. We have the impression that the living organisms always contain proteins of well defined molecular weights, but that many of these proteins are so unstable that they are often decomposed during the process of separation and purification.

Serum globulin was prepared in the following way.[13] The precipitate obtained after half saturating the serum with ammonium sulfate was washed with half saturated ammonium sulfate solution and dissolved in 10% sodium chloride solution, using toluene as a preservative. The protein was again precipitated by half saturating with ammonium sulfate, washed and dissolved as before. This process was repeated a third time, the washing being continued until the biuret reaction was negative. The precipitate was dissolved again in 10% sodium chloride solution and dialyzed against a phosphate buffer of P_H 5.5, 0.19 *M* in KH_2PO_4 and 0.009 *M* in Na_2HPO_4. The strength of this solution (serum globulin I) was 2.60%.

A second batch was made in the following way. The material was precipitated three times with ammonium sulfate, washed and dissolved in phosphate buffer and dialyzed against the same phosphate buffer until the outer liquid did not show the presence of sulfate and chloride. The stock solution had a strength of 3.25% (serum globulin II).

Part of the serum globulin material was fractionated according to Sörensen[14] by means of ammonium sulfate. The centrifugal study of the different fractions

[11] (a) Compare H. T. Krieger, "Ueber Darstellung krist. tierisch. Eiweissstoffe," Dissertation, Strassburg, **1899**; (b) S. P. L. Sörensen, *Medd. Carlsberg Lab.*, **12**, 12 (1915).

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

[13] Compare G. Kauder, *Arch. exptl. Pathol.*, **22**, 411 (1886).

[14] Ref. 2, p. 44.

showed that a rapid decomposition of the protein took place during the process of fractionation, more and more non-centrifugible material being formed.

Specific Volume.—The partial specific volume was determined pycnometrically at 19.5°. The amount of protein in solution was found by means of the following methods: (a) heating the solution until coagulation occurred, filtering and washing in a Gooch crucible and drying to constant weight at 105°; (b) drying at 105° to constant weight and subtracting the amount of salt in the sample as known from the composition of the buffer. The two methods gave consistent results.

No change of specific volume with concentration was observed for the range 0.75–3.0%. As mean value we obtained for serum albumin 0.748 and for serum globulin 0.745. These figures are equal within the limits of experimental error. It is worth noticing that according to previous determinations in this Laboratory[7,8,9] the values for egg albumin, hemoglobin, phycocyan and phycoerythrin are almost identical with the value found for serum albumin and serum globulin. These six proteins are, therefore, probably built up according to a plan common to all of them. On the other hand hemocyanin from *Helix*[10] and from *Limulus*[15] have a specific volume of 0.735 near their isoelectric points.

Light Absorption.—Serum albumin as well as serum globulin absorb only in the short-waved ultraviolet part of the spectrum. The measurements were carried out by means of a Judd-Lewis spectrophotometer in 20 mm. cells for solutions of concentration 0.20, 0.10 and 0.05%. The toluene dissolved in the solutions and serving as a preservative was removed by bubbling moist nitrogen through them before measuring the light absorption. The specific extinction coefficient $\epsilon/c = 1/d \log I_0/I$, where c is the concentration in %, I_0 the intensity of the light beam after passing a layer of solvent d cm. thick and I the intensity after passing the same thickness of solution, is plotted against wave length in Fig. 1. The absorption curve of serum albumin (A) has a maximum at 278 $\mu\mu$ and a minimum at 250 $\mu\mu$; the corresponding values for serum globulin, curve (B), are 285 $\mu\mu$ and 255 $\mu\mu$. The positions of the maxima and minima are identical within the limits of error. The values of the extinction coefficients are, however, much higher for serum globulin than for serum albumin. This is probably due to the high tryptophane content of serum globulin compared with that of serum albumin.

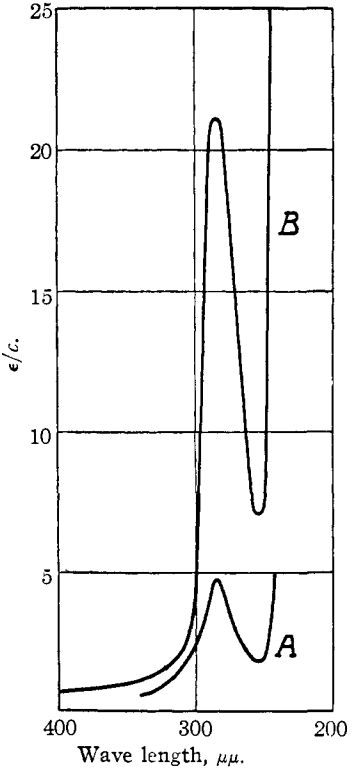

Fig. 1.

The centrifuging experiments have shown that in dilute solutions serum albumin is partly transformed into a non-centrifugible substance. In order to check the influence of this decomposition upon the light absorption some measurements were also made in a 2.80% solution using a 1-mm. absorption cell. Only a slight decrease in light absorption was observed, indicating that the specific extinction coefficient is almost the same for serum albumin and the decomposition products in question.

[15] Unpublished determinations by Dr. F. F. Heyroth.

Determination of the Molecular Weight

A. Sedimentation Velocity Method.[16]—Because of the more detailed information with regard to uniformity of molecular species and variation of diffusion and molecular weight with concentration which the sedimentation velocity method furnishes, this procedure was first applied.

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.

The specific sedimentation velocity and the diffusion constant were calculated from the photographic records as described in previous papers. The values were all reduced to 20° by means of the relations $s_1/s_2 = \eta_2/\eta_1$ and $D_1/D_2 = T_1/T_2 \cdot \eta_2/\eta_1$, where η is the viscosity of the solution.

A few changes in the apparatus were made in order to improve the experimental conditions. Thus a new oil cooler was inserted in the oil circulation system so that the temperature of the oil entering the turbines could be lowered from 25 to 15°. This caused a drop of temperature in the cell from about 30 to about 25°. When working with such unstable proteins as serum albumin and serum globulin, it is of great importance to keep the temperature of the cell as low as possible. Cells of three different thicknesses were used, namely, 2, 6 and 12 mm., in order to obtain suitable light extinction for solutions of various concentrations. The middle or sector plate of the cells previously made of glass was replaced by an ebonite plate. The quartz plates were cemented to the ebonite plate and the steel collar surrounding the plates by means of a cement composed of 2 parts of shellac and 1 part of pine tar heated together at 125° for five hours. These new cells endured the enormous strain in the centrifuge much better than the old ones.

The height of column of solution in the cells was 12 to 14 mm. The speed was chosen as high as possible, namely, around 42,000 r.p.m. corresponding to a centrifugal force about 100,000 times the force of gravity. The protein solutions used in the centrifuging experiments were made up from the stock solution by dilution with buffer immediately before each run and the dissolved toluene was removed by bubbling moist nitrogen through it.

As source of light a vertical quartz mercury lamp with a ground quartz plate as a diffuser was used. Chlorine and bromine quartz filters isolated a suitable range of wave length below 290 $\mu\mu$, and a quartz water filter re-

[16] (a) T. Svedberg, *Z. physik. Chem.*, **127**, 51 (1927); (b) T. Svedberg and J. B. Nichols, THIS JOURNAL, **49**, 2920 (1927).

moved the infra-red radiations. The photographs were taken on Imperial Process plates by means of a quartz lens of 100.5 cm. focal length. Metol-hydroquinone developer was used; the time of development was one minute.

TABLE I

SERUM ALBUMIN I, SEDIMENTATION VELOCITY RUN

Concentration, 0.12%; electrolyte-free solution; $V = 0.748$; $\rho = 1.000$; length of column of solution, 0.90 cm.; thickness of column, 1.20 cm.; speed, 42,100 r.p.m. ($\omega = 1403.3\pi$); source of light, mercury lamp, light filters, chlorine and bromine, aperture of objective, F: 36; plates, Imperial Process; time of exposure, 30 sec.; metol-hydroquinone developer; time of development, 1 min.

Sedimentation

Time interval, hours	T	Δx per half hour, cm.	Mean x, cm.	Centrif. force, $\omega^2 x$	$s_{20}°$, cm./sec. per cm./sec.^{2}
1.5–2.0	298.8	0.090	5.29	10.38×10^7	4.25×10^{-13}
2.0–2.5	298.9	.090	5.38	10.45×10^7	4.18×10^{-13}
2.5–3.0	299.0	.100	5.46	10.61×10^7	4.56×10^{-13}

Diffusion

Time of diffusion, min.	T	Mean[a] z, cm.	$D_{20}°$, cm.^{2}/sec.
65	298.6	0.060	8.75×10^{-7}
95	298.8	.080	10.58×10^{-7}
125	298.9	.105	13.82×10^{-7}
155	299.0	.120	14.19×10^{-7}

Mixture of different molecules; 43% non-centrifugible substance.

[a] "Mean z" is the mean of the distances from $c = 25$ to $c = 50$ and from $c = 50$ to $c = 75$, the concentration in the unchanged part of the solution being taken as 100 (compare THIS JOURNAL, **49**, 2922 (1927)).

The data of a run with the material "serum albumin I" are given in Table I. The distinct drift in the values of the diffusion constant with time indicates the presence of molecules or particles of various sizes. Pure buffer was not formed in the top part of the cell during centrifuging as usual. The light absorption indicated that about 43% of the organic matter was not centrifugible under the present experimental conditions. The material "serum albumin I," therefore, does not represent a pure protein but rather a mixture of molecules of different weight, some of them being so small that they were not influenced by the enormous centrifugal field applied.

In Tables II and III are given the results of two typical runs with the material "serum albumin II." In this case there is no drift in the diffusion constant with time. Pure buffer was formed in the top part of the cell when solutions of concentrations 1.25 to 0.75% were centrifuged. In more dilute solutions (0.50 and 0.25%), however, pure buffer was not formed. The concentration of the non-centrifugible substance increased with the dilution. The fact that there is no drift in the diffusion constant

shows that the material "serum albumin II" contains protein molecules of the same weight and shape. The serum albumin is therefore to be regarded as homogeneous with regard to molecular weight. The determinations also show that in dilute solution the serum albumin molecule breaks up into small units. The molecular weight of the centrifugible

TABLE II

SERUM ALBUMIN II, SEDIMENTATION VELOCITY RUN

Concn., 0.50%; acetate buffer, P_H 4.8 (0.02 M in total acetate); $V = 0.748$; $\rho = 1.000$; length of col. of soln., 1.10 cm.; thickness of col., 0.60 cm.; speed, 40,000 r.p.m. ($\omega = 1333\pi$); source of light, mercury lamp; light filters, chlorine and bromine; aperture of lens F: 36; plates, Imperial Process; exposure, 60 sec.; metol-hydroquinone developer, 1 min. Not centrifuged to pure buffer.

Sedimentation

Time interval, hours	T	Δx per half hour, cm.	Mean x, cm.	Centrif. force, $\omega^2 x$	$s_{20}°$, cm./sec. per cm./sec.^{2}
1.0–1.5	299.6	0.080	5.01	8.79×10^7	4.35×10^{-13}
1.5–2.0	299.7	.085	5.09	8.93×10^7	4.54×10^{-13}
2.0–2.5	299.8	.080	5.18	9.08×10^7	4.19×10^{-13}
2.5–3.0	299.9	.080	5.26	9.22×10^7	4.12×10^{-13}
3.0–3.5	299.9	.080	5.34	9.36×10^7	4.06×10^{-13}
3.5–4.0	299.9	.080	5.42	9.50×10^7	4.00×10^{-13}
				Mean	4.21×10^{-13}

Diffusion

Time of diff., min.	T	Mean z, cm.	$D_{20}°$, cm.^{2}/sec.
67.5	299.5	0.050	5.71×10^{-7}
97.5	299.6	.063	6.26×10^{-7}
127.5	299.8	.073	6.40×10^{-7}
157.5	299.9	.080	6.21×10^{-7}
187.5	299.9	.088	6.31×10^{-7}
217.5	299.9	.095	6.34×10^{-7}
			Mean 6.20×10^{-7}

Molecular weight = 65,000; 14% non-centrifugible substance.

TABLE III

SERUM ALBUMIN II, SEDIMENTATION VELOCITY RUN

Concn., 1.25%; buffer, V and ρ as in Table II; length of col., 1.43 cm.; thickness of col., 0.20 cm.; speed, 40,100 r.p.m. ($\omega = 1367\pi$); exposure, 40 sec.; optical and photographic conditions as in Table II.—Centrifuged to pure buffer.

Sedimentation

Time interval, hours	T	Δx per half hour, cm.	Mean x, cm.	Centrif. force, $\omega^2 x$	$s_{20}°$, cm./sec. per cm./sec.^{2}
1.0–1.5	299.6	0.060	..	.....	.....
1.5–2.0	299.8	.070	4.72	8.32×10^7	4.01×10^{-13}
2.0–2.5	299.9	.065	4.80	8.46×10^7	3.64×10^{-13}
2.5–3.0	300.0	.075	4.88	8.61×10^7	4.12×10^{-13}
3.0–3.5	300.0	.080	4.96	8.74×10^7	4.33×10^{-13}
3.5–4.0	300.0	.090	5.05	8.89×10^7	4.79×10^{-13}
				Mean	4.18×10^{-13}

TABLE III (*Concluded*)

Diffusion

Time of diffusion, min.	T	Mean z, cm.	$D_{20°}$, cm.²/sec.
73.5	299.4	0.053	5.92×10^{-7}
103.5	299.7	.065	6.27×10^{-7}
133.5	299.9	.070	5.61×10^{-7}
163.5	300.0	.080	5.96×10^{-7}
193.5	300.0	.090	6.38×10^{-7}
223.5	300.0	.093	5.90×10^{-7}
			Mean 6.01×10^{-7}

Molecular weight = 67,200; centrifuged to pure buffer.

part was found to be the same in concentrated solutions where no decomposition had taken place as in the very dilute (0.25%) solutions where

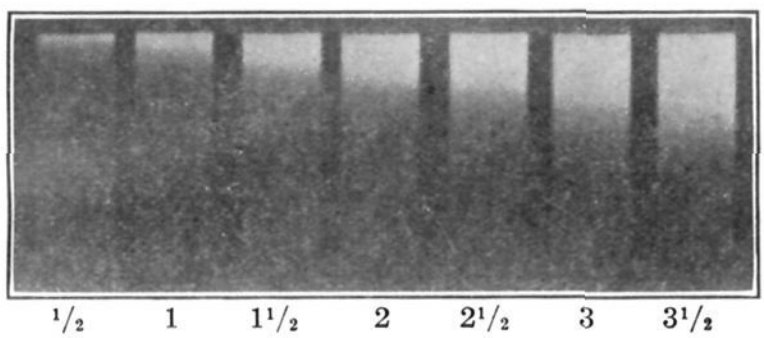

Fig. 2.

about 29% of the protein was decomposed. This shows that the decomposition leads immediately to very small molecules without giving rise to any appreciable amount of intermediate products. The behavior of the material "serum albumin I" indicates that the elaborate purification used in that case has caused a rather complicated decomposition and aggregation process to take place, the result being a less pure material than when a cruder and quicker purification method was used (Material II).

The photographs from which the data of Table II have been derived are reproduced in Fig. 2. The corre-

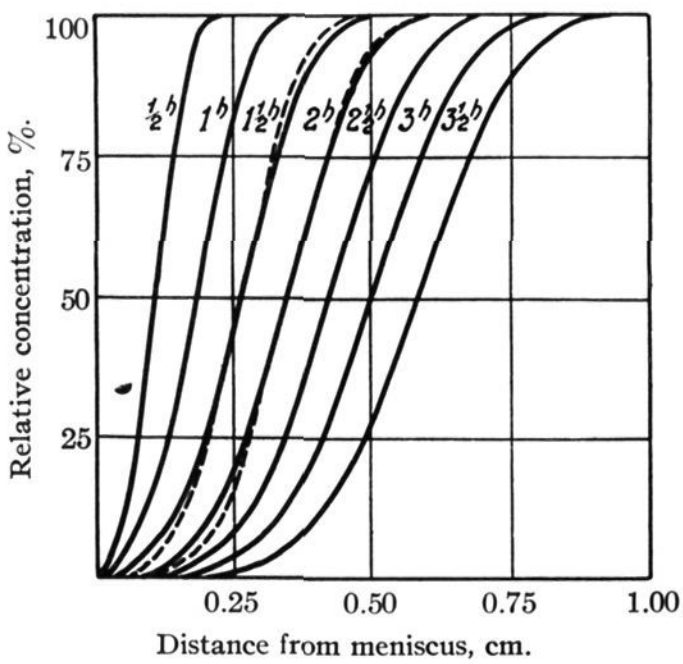

Fig. 3.

sponding concentration curves, corrected for the sector shape of the cell and the variation of centrifugal force with distance from the center of rotation,[16] are given in Fig. 3. The dotted curves for one and one-half and tow hours represent the theoretical diffusion curves of a substance of only one molecular species if subjected to the same experimental conditions. The deviation from the experimentally determined variation of concentration is within the limits of experimental error.

In order to ascertain whether the formation of the non-centrifugible substance in the dilute serum albumin solutions is a reversible or an irreversible process, the following experiment was performed. A 0.25% solution was precipitated by saturating with ammonium sulfate. A measurement of the light absorption of the liquid from which the precipitate had been filtered off showed that all the albumin and its decomposition products had really been completely salted out. The biuret reaction was also negative. The precipitate was dissolved in water, the solution was dialyzed and electrodialyzed and the albumin studied in the ultracentrifuge at a concentration of 1.1% in an acetate buffer of P_H 4.8, as usual. The protein this time sedimented with normal molecular weight, leaving pure buffer in the top part of the cell, showing that the breaking up of the serum albumin in dilute solution is a reversible process.

TABLE IV

SERUM ALBUMIN II, SUMMARY OF SEDIMENTATION VELOCITY MEASUREMENTS

Concn., g. per 100 cc.	Mean speed, r.p.m.	$s_{20}°$, cm./sec. per cm./sec.^{2}	$D_{20}°$, cm.^{2}/sec.	Mol. wt.
1.25	40,100	4.18×10^{-13}	6.01×10^{-7}	67,200
1.00	42,100	4.48×10^{-13}	6.35×10^{-7}	68,200
0.75	41,600	4.00×10^{-13}	5.97×10^{-7}	65,000
0.50	40,000	4.21×10^{-13}	6.20×10^{-7}	65,600
0.25	39,900	4.18×10^{-13}	5.97×10^{-7}	67,700
		Mean 4.21×10^{-13}	Mean 6.10×10^{-7}	Mean 66,740

The results of all the sedimentation velocity runs with serum albumin are summarized in Table IV. The mean value of the molecular weight is 66,740 ± 2000.

In Tables V and VI are given the results of two typical runs with serum globulin (Material I and II, respectively). The photographs and the concentration curves corresponding to the data of Table V are reproduced in Figs. 4 and 5. No drift in the diffusion constant occurs and pure buffer is formed in the top part of the cell at all concentrations studied (1.00 to 0.12%). It is therefore obvious that serum globulin is a homogeneous substance with regard to molecular weight. The results of all sedimentation velocity runs with serum globulin are given in Table VII. The mean value of the molecular weight is 104,400 ± 3000.

As already mentioned under "Preparation of Material" an attempt was

TABLE V

SERUM GLOBULIN I, SEDIMENTATION VELOCITY RUN

Concn., 0.72%; phosphate buffer, P_H 5.5 (0.19 M in KH_2PO_4 and 0.009 M in Na_2HPO_4); $V = 0.745$; $\rho = 1.018$; length of col., 1.33 cm.; thickness of col., 0.20 cm.; speed, 41,400 r.p.m. ($\omega = 1380\pi$); exposure, 40 sec.; optical and photographic conditions as in Table I.

Sedimentation

Time interval, min.	T	Δx per 20 min., cm.	Mean x, cm.	Centrif. force, $\omega^2 x$	$s_{20}°$, cm./sec. per cm./sec.^{2}
20–40	298.4	0.060	4.72	8.83×10^7	5.01×10^{-13}
40–60	298.5	.075	4.79	9.00×10^7	6.11×10^{-13}
60–80	298.7	.070	4.86	9.13×10^7	5.61×10^{-13}
80–100	298.9	.065	4.93	9.26×10^7	5.12×10^{-13}
100–120	299.0	.065	4.99	9.38×10^7	5.02×10^{-13}
120–140	299.1	.075	5.06	9.50×10^7	5.71×10^{-13}
140–160	299.1	.075	5.13	9.69×10^7	5.60×10^{-13}
160–180	299.1	.075	5.21	9.83×10^7	5.52×10^{-13}
				Mean	5.46×10^{-13}

Diffusion

Time of diff. min.	T	Mean z, cm.	$D_{20}°$, cm.^{2}/sec.
32	298.3	0.040	7.95×10^{-7}
52	298.5	.040	4.87×10^{-7}
72	298.6	.045	4.41×10^{-7}
92	298.8	.055	5.17×10^{-7}
112	299.1	.060	5.02×10^{-7}
132	299.1	.067	5.31×10^{-7}
152	299.1	.068	4.75×10^{-7}
172	299.1	.072	4.71×10^{-7}
		Mean	5.27×10^{-7}

Molecular weight = 104,500.

TABLE VI

SERUM GLOBULIN II, SEDIMENTATION VELOCITY RUN

Concn., 0.75%; buffer, V and ρ as in Table V; length of col., 1.45 cm.; thickness of col., 0.20 cm.; speed, 41,700 r.p.m. ($\omega = 1390\pi$); exposure, 30 sec.; optical and photographic conditions as in Table I.

Sedimentation

Time interval, min.	T	Δx per 20 min. cm.	Mean x, cm.	Centrif. force, $\omega^2 x$	$s_{20}°$, cm./sec. per cm./sec.^{2}
20–40	296.0	...	..	.....	.....
40–60	296.3	0.060	4.64	8.85×10^7	5.23×10^{-13}
60–80	296.5	.070	4.71	8.97×10^7	5.99×10^{-13}
80–100	296 6	.065	4.77	9.10×10^7	5.48×10^{-13}
100–120	296.7	.070	4.84	9.23×10^7	5.80×10^{-12}
120–140	296.8	.070	4.91	9.36×10^7	5.70×10^{-13}
140–160	296 9	.078	4.98	9.50×10^7	6.24×10^{-13}
160–180	296.9	.077	5.06	9.65×10^7	6.07×10^{-13}
				Mean	5.79×10^{-13}

TABLE VI (*Concluded*)

Diffusion

Time of diff., min.	T	Mean z, cm.	$D_{20}°$ cm.²/sec.
25	295.7	0.030	6.12×10^{-7}
45	296.3	.040	5.97×10^{-7}
65	296.5	.048	5.90×10^{-7}
85	296.5	.055	5.93×10^{-7}
105	296.7	.058	5.31×10^{-7}
125	296.8	.060	4.76×10^{-7}
145	296.9	.065	4.81×10^{-7}
165	296.9	.070	4.90×10^{-7}
			Mean 5.46×10^{-7}

Molecular weight = 106,800.

TABLE VII

SERUM GLOBULIN, SUMMARY OF SEDIMENTATION VELOCITY MEASUREMENTS

Subs.	Concn., g. per 100 cc.	Mean speed, r.p.m.	$s_{20}°$, cm./sec. per cm./sec.²	$D_{20}°$ cm.²/sec.	Mol. wt.
Ser. glob. II	1.00	42,000	5.50×10^{-13}	5.37×10^{-7}	103,400
Ser. glob. II	0.75	41,700	5.79×10^{-13}	5.46×10^{-7}	106,800
Ser. glob. I	.72	41,400	5.46×10^{-13}	5.27×10^{-7}	104,500
Ser. glob. I	.53	41,900	5.54×10^{-13}	5.49×10^{-7}	101,800
Ser. glob. I	.12	41,400	6.01×10^{-13}	5.45×10^{-7}	105,700
			Mean 5.66×10^{-13}	Mean 5.41×10^{-7}	Mean 104,400

made to study the "euglobulins" and "pseudoglobulins" obtained by fractionating serum globulin with ammonium sulfate. It was found,

1/3 2/3 I 1 1/3 1 2/3 2 2 1/3 2 2/3 3

Hours.

Fig. 4.

however, that the process of fractionation causes decomposition of the globulin, more and more non-centrifugible substance appearing during the course of the operations. From our experiences, therefore, it seems possible that the so-called pseudoglobulins are just artificial products representing mixtures of undecomposed serum globulin and decomposition products generated by the fractionating process. This important question will be dealt with fully in a subsequent communication.

B. Sedimentation Equilibrium Method.[7a,8,17]—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.

Because of the decomposition of serum albumin observed in the sedimentation velocity experiments, this protein was only studied in relatively high concentration using a 2 mm. cell. On the other hand, serum globulin, which was found to be stable also in dilute solution, was investigated at lower concentrations by means of a cell 8 mm. in thickness.

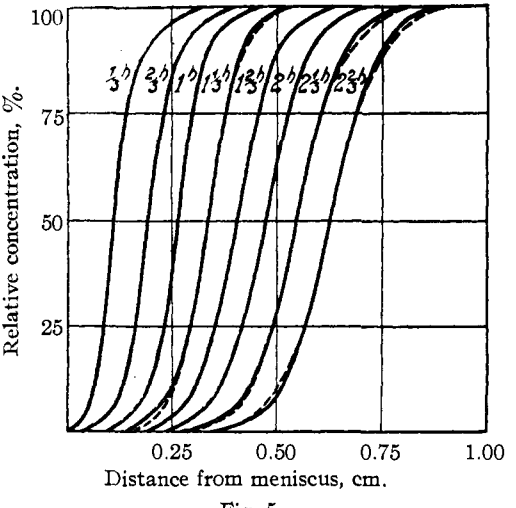

Fig. 5.

The source of light, the light filters and the lens were similar to those used in the sedimentation velocity runs. The same plates and the same developer were used. Pictures were taken with three different times of exposure in order to obtain suitable photographic densities for the various concentrations. Changes in the intensity of the lamp were corrected for by having another cell of the centrifuge filled with a solution of potassium chromate of suitable strength and photographing this cell at the same time as the cell filled with the protein solution.

TABLE VIII

SERUM ALBUMIN II, SEDIMENTATION EQUILIBRIUM RUN

Concn., 1.00%; acetate buffer, PH 4.8 (0.02 M in total acetate); V = 0.748; ρ = 1.000; T = 293.2; length of col. of soln., 0.50 cm.; thickness of col., 0.20 cm.; dist. of outer end of sol. from axis of rotation, 4.73 cm.; speed, 8200 r.p.m. ($\omega = 273.3\pi$); optical and photographic conditions similar to those in Table I; aperture of lens, F: 30; time of exposure 1, 2 and 4 min.; exposures made after 36, 40 and 44 hours of centrifuging.

Distances, cm. x_2	x_1	Mean concn., % c_2	c_1	No. of exposures	Mol. wt.
4.63	4.58	1.300	1.154	11	68,000
4.58	4.53	1.154	1.022	11	69,600
4.53	4.48	1.022	0.909	10	68,600
4.48	4.43	0.909	.810	10	67,700
4.43	4.38	.810	.724	10	67,200
4.38	4.33	.724	.645	10	69,100
				Mean	68,400

[17] Svedberg, *Z. physik. Chem.*, **121**, 65 (1926).

TABLE IX

SERUM GLOBULIN II, SEDIMENTATION EQUILIBRIUM RUN

Concn., 0.15%; phosphate buffer, P_H 5.5 (0.19 M in KH_2PO_4 and 0.009 M in Na_2HPO_4); V = 0.745; ρ = 1.018; T = 291; length of col., 0.50 cm.; thickness of col., 0.80 cm.; speed, 6920 r.p.m. (ω = 230.7π); times of exposure, 20, 40 and 60 sec.; exposures made after 38, 44 and 48 hours of centrifuging; other data as in Table VIII.

Distances, cm.		Mean concn., %		No. of	
x_2	x_1	c_2	c_1	exposures	Mol. wt.
4.68	4.63	0.215	0.189	2	105,600
4.63	4.58	.189	.167	10	102,500
4.58	4.53	.167	.147	10	106,800
4.53	4.48	.147	.130	10	104,100
4.48	4.43	.130	.116	10	105,000
4.43	4.38	.116	.104	7	101,800
4.38	4.33	.104	.093	2	97,900
				Mean	103,400

Details of a run with serum albumin (Material II) are given in Table VIII. A typical serum globulin run is shown in Table IX (Material II). The results of all the sedimentation equilibrium runs are summarized in Table X.

TABLE X

SUMMARY OF SEDIMENTATION EQUILIBRIUM MEASUREMENTS

Subs.	Concn. at start, %	Mean speed, r.p.m.	Mol. wt.
Serum albumin II	0.92	8540	67,900
Serum albumin II	1.00	8200	68,400
Serum globulin I	0.10	7000	102,900
Serum globulin I	.15	6900	103,100
Serum globulin II	.15	6920	103,400
	Mean for serum albumin		68,150
	Mean for serum globulin		103,100

The values of the molecular weight calculated from measurements at different distances from the center of rotation were identical within the limits of error as demonstrated by Tables VIII and IX. The study of serum albumin and serum globulin by means of the sedimentation equilibrium method, therefore, confirms the result arrived at by means of the sedimentation velocity method, namely, that these proteins are to be regarded as homogeneous with regard to molecular weight. The mean value of the molecular weight found by the equilibrium method is 68,150 ± 2000 for serum albumin and 103,100 ± 3000 for serum globulin. These figures are identical within the limits of error with those furnished by the velocity method.

Discussion of Results

The measurements described in this paper have shown that serum albumin and serum globulin isolated from horse blood by means of a rapid

process of purification must be regarded as two proteins homogeneous with regard to molecular weight. The value for serum albumin is 67,500 ± 2000 and that for serum globulin 103,800 ± 3000. This is rather surprising when compared with the results of earlier investigations of these proteins. As already mentioned, Sörensen found that serum albumin could be divided into a series of fractions possessing widely different solubilities.[2] His figure for the molecular weight calculated from osmotic measurements was 45,000, which is about 33% lower than our value. The fact brought to light by our experiments that serum albumin is a very unstable protein easily decomposed during the process of purification and always partly decomposed in dilute solutions seems to indicate that the discrepancy might be explained by assuming that Sörensen's carefully purified albumin had undergone partial decomposition. An abnormally high osmotic pressure would probably have been the result of such a change. Adair's serum albumin, which showed an osmotic pressure corresponding to a molecular weight of 62,000, seems to have been less decomposed. The different solubilities found by Sörensen in his different serum albumin fractions could be interpreted by assuming that serum albumin, although homogeneous with regard to molecular weight, is a mixture of two or more proteins of different chemical properties. The simplest explanation, however, seems to be that the differences in solubility are caused by the presence in the fractions of varying amounts of decomposition or aggregation products.

The fact that serum globulin was found to be homogeneous with regard to molecular weight is perhaps still more surprising than the behavior of serum albumin. Most of the previous work on this protein seemed to indicate that serum globulin was to be regarded as a rather complicated mixture of euglobulins and pseudoglobulins. Our fractionation experiments controlled by measurements in the ultracentrifuge, although only of a preliminary nature, have shown, however, that this protein also is liable to break up during the usual process of fractionation. It seems, therefore, probable that the so-called euglobulins and pseudoglobulins do not exist in the blood but are to be regarded as artificial products.

In a previous communication attention was drawn to the fact that the molecular weights of hemoglobin, phycocyan and phycoerythrin as determined by the centrifugal methods were all very nearly simple multiples of the molecular weight of egg albumin.[9] It is of considerable interest to note that the molecular weight of serum albumin is now found to be almost exactly two times the molecular weight of egg albumin, while the molecular weight of serum globulin is three times the weight of egg albumin. Thus we now have six proteins of widely different origin obeying this rule of simple multiples. Perhaps of equal importance is the information that there exist proteins possessing widely different chemical and physical

properties but having approximately the same molecular weight. We now know two such pairs, hemoglobin and serum albumin on the one hand and phycocyan and serum globulin on the other. The detailed discussion of these regularities will be reserved for a subsequent communication.

Summary

1. The centrifugal sedimentation velocity and sedimentation equilibrium methods have been applied to the study of the molecular weights of serum albumin and serum globulin in buffer solutions at their isoelectric points, using various protein concentrations.

2. The serum albumin was found to have a molecular weight of 67,500 ± 2000 and the serum globulin 103,800 ± 3000, both being independent of concentration.

3. It was found that within the limits of experimental error both these proteins are homogeneous with regard to molecular weight and that they therefore may represent pure chemical individuals.

4. It was found that both these proteins, but especially the serum albumin, are rather unstable substances easily decomposed during the process of purification. Serum albumin is always partly, although reversibly, decomposed in dilute solution. These facts seem to afford an explanation for the discrepancies between the results of various investigators working on the proteins of the serum.

5. It was pointed out that the molecular weights of serum albumin and serum globulin are, like the molecular weights of hemoglobin, phycocyan and phycoerythrin, very nearly simple multiples of that of egg albumin.

6. It was pointed out that these new molecular weight determinations show that there exist proteins of different chemical composition and widely different properties but possessing very nearly the same molecular weight. Hemoglobin and serum albumin with molecular weights near 68,000 and phycocyan and serum globulin with molecular weights near 105,000 are two such pairs.

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