23.78, 30.04 cc. of 0.01 *N* HCl. Calcd. for $C_{10}H_{18}O_3NAs_4$: N, 2.82. Found: 2.75, 2.78.

Summary

1. β-Hydroxyethylarsonic acid has been obtained in a crystalline form.

2. A series of aryl arseno-ethanols has been prepared by the simultaneous reduction of β-hydroxyethylarsonic acid and various aromatic arsonic acids.

3. One member of the previously unknown series of unsymmetrical aliphatic-aromatic tri-arseno compounds has been made.

SAINT PAUL, MINNESOTA

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

THE MOLECULAR WEIGHT OF HEMOCYANIN

BY THE SVEDBERG AND EUGEN CHIRNOAGA[1]

RECEIVED NOVEMBER 26, 1927 PUBLISHED MAY 5, 1928

Hemocyanin is the respiratory blue pigment of the blood of certain lower animals: molusca, crustacea and arachnoides.[2,3,4] Both in physiological function and in composition it shows a certain analogy to hemoglobin, the respiratory pigment of the blood of higher animals. The hemocyanin molecule has a metallic constituent, copper, while hemoglobin contains iron. Hemocyanins from different species seem to differ in their copper content. Griffiths[5] found for *Cancer*, *Homarus* and *Sepia* about 0.33%; Heinze[6] for *Octopus vulgaris* 0.38%; Burdel[7] and Begemann[8] for *Helix pomatia* about 0.27%; Alsberg and Clark[9] for *Limulus polyphemus* 0.28%; while Redfield, Coolidge and Shotts[10] in a very careful investigation found for the same species 0.173%. The differences in the copper content as well as in other properties, for example, the oxygen combining curve, seem to indicate that there exist different kinds of hemocyanin.

Because of the physiological importance of hemocyanin it was thought to be of interest to study its actual molecular weight in solution by means of the new method of ultracentrifuging, already applied to the study of hemoglobin, egg albumin, phycoerythrin and phycocyan.

[1] Fellow of the International Education Board.

[2] Frédéricque, *Arch. Zoöl.*, **7**, 535 (1878).

[3] Ch. Dhéré, *Arch. de Physiol. et Pathol. gen.*, **16**, 985 (1916); **18**, 221 (1919).

[4] J. Botazzi, *ibid.*, **18**, 1 (1919).

[5] Griffiths, *Compt. rend.*, **114**, 496 (1892).

[6] Heinze, *Z. physiol. Chem.*, **33**, 370 (1901); **43**, 290 (1904).

[7] Burdel, *Thèses*, Fribourg, **1922**.

[8] Begemann, *Proefschrift*, Utrecht, **1924**.

[9] Alsberg and Clark, *J. Biol. Chem.*, **8**, 1 (1910).

[10] Redfield, Coolidge and Shotts, *ibid.*, **76**, 185 (1928).

This paper deals with hemocyanin extracted from the blood of the vineyard snail, *Helix pomatia*.

Experimental

Preparation of Material.—Nine hundred cc. of blood was obtained from 800 hibernating snails. The shells were cut into opposite the heart, recognized by its blue color. The heart was pierced by a needle and the blood allowed to drop into a crucible.[11] The freezing point depression of fresh blood was 0.45°.

For the isolation of hemocyanin the blood was transferred to collodion bags and put to dialyze in a box kept at 0°, according to the procedure followed by Dhéré.[12] The distilled water flowing into the vessel containing the collodion bags was kept saturated with toluene in order to prevent the growth of bacteria. After two weeks a voluminous precipitate was deposited in each bag, leaving a slightly blue supernatant liquid. After centrifuging, the precipitate dissolved readily in dilute solutions of sodium chloride, sodium phosphate, acetic acid and other electrolytes. A second dialysis was continued until the conductivity of the liquid had fallen at room temperature to 6.1×10^{-6} mhos.

Under the microscope the deposit showed numerous crystals, most of them forming crosses, five and six branched stars and several clearly revealed the octahedral shape. Fig. 1 shows dendrite crystals from a solution dialyzed against distilled water. Fig. 2 reproduces individual crystals precipitated by electrodialysis from a dilute solution which would no longer give any deposit even after prolonged ordinary dialysis. Good photographs were obtained with difficulty because hemocyanin is extremely sensitive to alkali, dissolving in contact with the glass of the microscopic slide. Well shaped crystals maintain their form only for a short period of time, shrinking on drying. This suggests that they are of a semi-liquid nature. Unlike Dhéré we did not use alcohol or fuchsine in photographing our crystals, thereby avoiding the possibility of producing denaturation of the protein.

It may be added that electrodialysis produces total deposition of hemocyanin from its solutions in less than twenty-four hours, while the conductivity of the liquid drops to that of distilled water. If the solution has been very dilute the precipitate will dissolve again through the action of the alkali of the glass on breaking the current and shaking.

The ultramicroscope reveals no discrete particles in a solution of hemocyanin, but the cone of light can be seen distinctly even at a dilution of 0.0001%.

[11] We wish to express our thanks to Dr. S. Bock for his kind assistance in procuring the snails and for his kind advice as to the best way of drawing their blood.

[12] Ch. Dhéré, *Thèses*, Fribourg, 1909.

The precipitate obtained after dialyzing the blood three times down to a specific conductivity of 6×10^{-6} mhos was used for making up the solutions of different P_H for the following measurements.

The isoelectric point was assumed to be at P_H 4.7 as stated by Quagliariello[13] for hemocyanin from *Octopus*. After the completion of the present investigation, the isoelectric point of our hemocyanin material was determined in this Laboratory by A. Tiselius. He found by means of cataphoresis measurements P_H 5.2.[14]

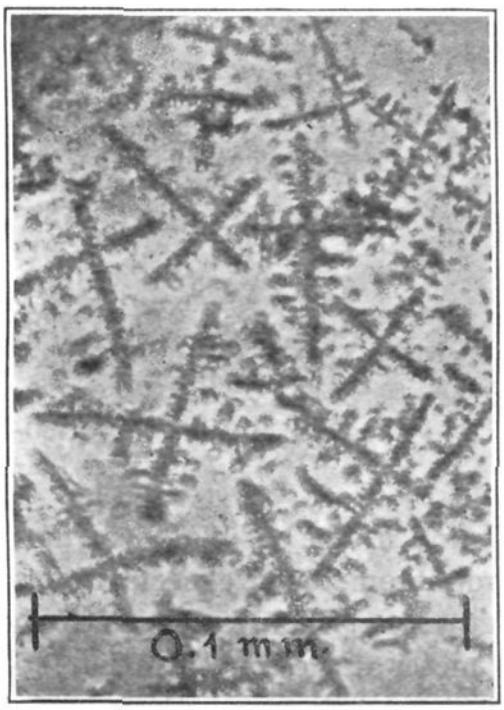

Fig. 1.

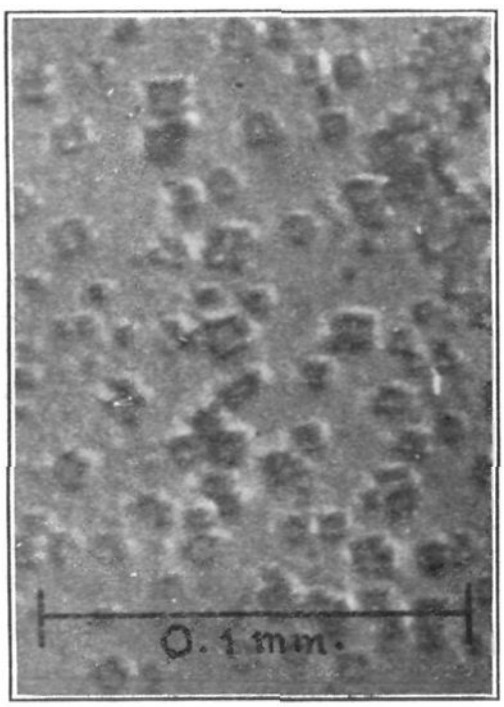

Fig. 2.

Specific Volume.—The partial specific volume of hemocyanin was determined pycnometrically at 19.5° and calculated according to the formula $V = [w - (l - h)]/\rho h$, where V is the partial specific volume, w the weight of solvent in the pycnometer, l the weight of solution, h the weight of the protein and ρ the density of solvent.

The concentration of the solutions was determined by transferring one cc. of solution to a porcelain crucible, evaporating down to coagulation on a water-bath and then drying to constant weight at 105°. From the weight so found, the content of salt in one cc. of solution was subtracted. Another method employed was to coagulate at 82–83° one cc. of solution in a small flask, wash with boiled water and dry to constant weight as

[13] Quagliariello, *Atti acad. med. chir. Napoli*, **74** (1920); *Die Naturwissensch.*, **11**, 261 (1923).

[14] The Referee has kindly drawn our attention to a recent paper by E. and E. Stedman, *Biochem. J.*, **21**, 533 (1927), where the isoelectric point of hemocyanin from *Helix pomatia* is given as P_H 5.3.

before. The results obtained by the two methods agree very well. The figures for the partial specific volume of the protein in solutions at various P_H and concentrations are given in Table I. Except in alkaline solutions, P_H 8, the partial specific volume is practically independent of P_H.

TABLE I

PARTIAL SPECIFIC VOLUME FOR HEMOCYANIN FROM *Helix pomatia*

Solvent	P_H	Concentration, %	V
Phosphate buffer	8.0	6.10	0.755
Phosphate buffer	8.0	3.05	.753
Phosphate buffer	8.0	1.53	.754
Phosphate buffer	7.0	9.40	.735
Phosphate buffer	7.0	4.70	.735
Phosphate buffer	7.0	2.35	.736
Phosphate buffer	7.0	1.18	.737
Acetate buffer	5.6	3.38	.732
Acetate buffer	5.6	1.69	.731
Acetate buffer	4.7	5.29	.735
Acetate buffer	4.7	2.64	.738
Acetate buffer	4.7	1.32	.738
Acetate buffer	3.8	3.47	.733
Acetate buffer	3.8	1.74	.730
Acetate buffer	3.8	0.87	.733

Light Absorption.—The light absorption in the visible spectrum was measured with a König-Martens spectrophotometer at concentrations between 1.2 and 1.7% and in the ultraviolet with a Judd-Lewis spectrophotometer at the concentration 0.05%. The positions of the absorption maxima agree with those found by Dhéré and Burdel.[12,15] In the visible spectrum we found an absorption band between 610 and 530$\mu\mu$; in the ultraviolet there is a first absorption band between 360 and 310$\mu\mu$ and a second one much more pronounced between 290 and 260$\mu\mu$, with a maximum at 278$\mu\mu$. Table II gives the values of the extinction coefficients per unit concentration, $\epsilon/c = 1/dc \cdot \log I/I_0$, for solutions of P_H 3.8, 4.7, 5.6, 7.0 and 8.0 in the visible spectrum.

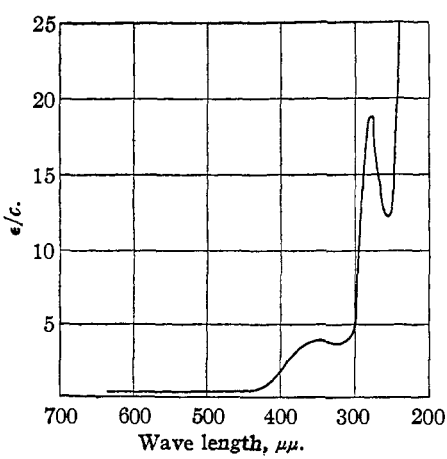

Fig. 3.

The extinction coefficient cannot be measured with great accuracy in the Judd-Lewis spectrophotometer and for this reason the figures for

[15] Ch. Dhéré and A. Burdel, *Journ. de Physiol. et Pathol. gen.*, **18**, 685 (1919–20).

the ultraviolet are not tabulated. The whole absorption curve in the visible and the ultraviolet spectrum for a solution of *P*H 4.7 and one weight per cent. concentration is given in Fig. 3. In order to bring out more clearly the maximum in the visible spectrum, part of the curve, from 650 to 400$\mu\mu$, is reproduced in larger scale in Fig. 4.

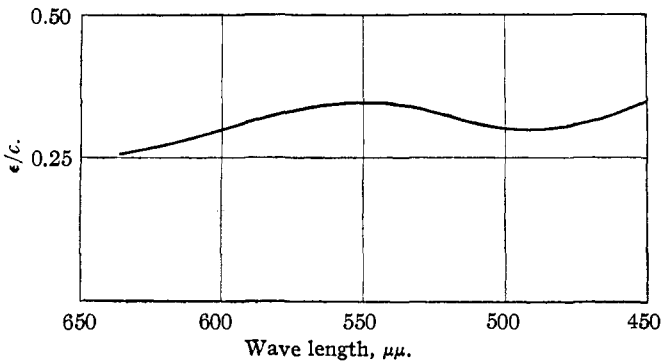

Fig. 4.

TABLE II

LIGHT ABSORPTION FOR HEMOCYANIN FROM *Helix pomatia*

Wave length, μμ	*P*H 3.8, 1.75%	*P*H 4.7, 1.32%	*P*H 5.6, 1.69%	*P*H 7.0, 1.17%	*P*H 8.0, 1.52%
			ϵ/c		
635	0.199	0.253	0.297	0.262	0.267
605	.229	.294	.339	.308	.304
579	.248	.325	.377	.335	.329
556	.249	.345	.395	.346	.343
538	.238	.341	.401	.344	.352
522	.216	.341	.396	.346	.336
506	.206	...	.361	.347	.341
492	.200	.299	.360	.377	.375
479	...	...	.349	...	.317
468	.197	...	.346	...	.322

The light absorption varies slightly with the age of the solution, though the general shape of the curve remains exactly the same. Since our solutions were of different ages, from one day to several weeks, no weight should be put on the small variation of ϵ/c with *P*H as shown in Table II within the region *P*H 4.7 to 8.0. At the acid side of the isoelectric point, however, the absorption in the visible spectrum is decidedly lower and the maximum seems to be slightly shifted toward the red. As demonstrated by the centrifuging experiments, the huge hemocyanin molecule which exists in solution at and above the isoelectric point is broken up into smaller units at *P*H 3.8 and the decrease in light absorption is probably a consequence of this disintegration.

Determination of the Molecular Weight

A. Sedimentation Velocity Method.[16,17]—The molecular weight is given by

$$M = \frac{RT\,s}{D(1 - V\rho)}$$

where R is the gas constant, T the absolute temperature, s the specific sedimentation velocity defined as $[dx/dt \cdot 1/\omega^2 x]$, D the diffusion constant of the solute, V its partial specific volume, ρ the density of the solution, x the distance from the center of rotation, t the time and ω the angular velocity of the centrifuge.

The sedimentation velocity is calculated from the movement of the boundary between pure solvent and solution towards the periphery and the diffusion constant from the amount of blurring of the same boundary as described in previous communications.

In order to make the values from different runs comparable, the specific sedimentation velocities were all reduced to 20° by means of the relation $s_1/s_2 = \eta_2/\eta_1$, and the diffusion constants by means of the relation $D_1/D_2 = T_1\eta_2/T_2\eta_1$, where η is the viscosity of the solution. Variations in the intensity of the lamp were corrected for by using a standard solution of potassium chromate of suitable strength. In the earlier runs Hauff Ultra Rapid plates were used; later it was found that Imperial Process plates gave better results.

The changes in concentration taking place in the solution enclosed in the centrifuge cell could not be followed photographically in the visible light absorption band 570$\mu\mu$ on account of the small absorption and therefore in the first series of experiments long wave ultraviolet light (366$\mu\mu$) was used corresponding to the absorption maximum at 360$\mu\mu$. This light was isolated from the other radiations of the mercury lamp by means of a nickel glass filter.

Our first run was made with a solution of P_H 8.0 and concentration 3.05% at a speed of 7000 r.p.m. (centrifugal force 2565 times that of gravity). It was found that the sedimentation equilibrium expected in analogy to the behavior of the other proteins previously investigated was not attained. Instead the molecules were centrifuged down, leaving a sharp boundary between solution and solvent. Three successive experiments were carried out with the above solution at speeds of 4000, 8000 and 11,000 r.p.m., all other conditions being equal. The obtained values for the diffusion constant indicated a variation approximately inversely proportional to the centrifugal force applied. At the same time it was noticed that during centrifuging some change took place in the solution, starting from the bottom of the cell, causing less light

[16] Svedberg, *Z. physik. Chem.*, **127**, 51 (1927).

[17] Svedberg and Nichols, THIS JOURNAL, **49**, 2920 (1927).

absorption and consequently undue darkening of the photographs in the affected region. This darkening extended rapidly upwards, altering the blurring of the boundary and therefore preventing accurate calculation of the diffusion constant. The effect was more marked with old than with fresh solutions. It was assumed to be due to reduction of the hemocyanin produced by the activity of bacteria present in the solutions since the medium was alkaline.[18,19]

A few experiments carried out at P_H 3.8 indicated a breaking up of the molecule into smaller units of varying size. Because of the instability of the protein both in alkaline and extremely acid solutions, we decided to limit the present investigation of the molecular weight to solutions near the isoelectric point, which offered better conditions of stability.[20]

Long wave ultraviolet light was used down to a concentration of 0.68%. Several experiments made at this point could not be calculated on account of insufficient contrast in the photographs between solution and solvent and therefore we passed over to short ultraviolet light isolated from the radiation of the mercury arc by means of chlorine and bromine filters. Even in this case at a dilution of 0.1% the cell of 2 mm. thickness used in previous investigations had to be replaced by one 8 mm. thick in order to have sufficiently strong absorption to permit the taking of suitable photographs.

Our data obtained by ultracentrifuging of hemocyanin solutions at P_H 4.7 soon brought to light the strange phenomenon of a strong variation of the diffusion "constant" with concentration of the protein solution.

TABLE III

SEDIMENTATION VELOCITY AND DIFFUSION FOR HEMOCYANIN FROM *Helix pomatia*

Concentration, 3.05%; P_H, 8.0 (phosphate buffer); V = 0.753; T = 291.5; length of column of solution, 0.99 cm.; thickness of column, 0.20 cm.; speed, 11,000 r.p.m. (ω = 366.7π); time of exposure, 20 secs.

Time interval, min.	Δx cm. per 30 min.	x, mean cm.	$s_{20°}$, cm./sec.	$D_{20°}$, cm.²/day
30– 60	0.054	3.796	5.79×10^{-12}	1.48×10^{-3}
60– 90	.063	3.854	6.80	0.99
90–120	.058	3.914	6.11	1.31
120–150	.062	3.974	6.44	0.95
150–181	.065	4.039	6.69	1.05
181–219	.060	4.111	6.08	1.41
219–248	.066	4.182	6.47	1.24
		Mean	6.34×10^{-12}	1.20×10^{-3}

[18] Alsberg, *J. Biol. Chem.*, **23**, 495 (1915).

[19] Phisalix, *Compt. rend. soc. biol.*, **52**, 729 (1900).

[20] For the isoelectric point we had accepted Quagliariello's value, P_H 4.7. As stated above the isoelectric point of the material used was actually P_H 5.2. An investigation on the stability region of the same hemocyanin material carried out in this Laboratory by F. F. Heyroth has shown that there is no change in molecular weight between P_H 4.7 and 5.2.

TABLE IV

SEDIMENTATION VELOCITY AND DIFFUSION FOR HEMOCYANIN FROM *Helix pomatia*

Concentration, 0.17%; P_H 4.7 (acetate buffer, 0.02 N with regard to Na); V = 0.738; T = 291.8; length of column of solution, 0.95 cm.; thickness of column, 0.80 cm.; speed, 11,000 r.p.m. (ω = 366.7 π); time of exposure, 40 secs.

Time interval, min.	Δx, cm. per 30 min.	x, mean cm.	$s_{20°}$ cm./sec.	$D_{20°}$, cm.²/day
60– 90	0.089	3.897	9.56×10^{-12}	11.0×10^{-3}
90–120	.095	3.989	9.91	16.1
120–150	.098	4.085	9.77	17.3
150–180	.104	4.186	10.30	14.8
180–210	.095	4.286	9.16	15.2
210–240	.105	4.386	10.20	15.5
		Mean	9.8×10^{-12}	15.0×10^{-3}

M = 4.93×10^6

This fact had not been met with in the study of the other proteins investigated by this method. However, the change in the values of D with the centrifugal force observed at P_H 8 was no longer encountered with isoelectric solutions.

Details of two typical runs under widely different conditions are given in Tables III and IV.

In Table V all the determinations of diffusion constant and specific

TABLE V

SEDIMENTATION VELOCITY AND DIFFUSION FOR HEMOCYANIN FROM *Helix pomatia*

Concn., %	P_H	R.p.m.	$D_{20°} \times 10^5$, cm.²/sec.	$s_{20°} \times 10^{13}$, cm./sec.
3.05	8.0	4000	3.82	5.63
3.05	8.0	8000	2.34	6.57
3.05	8.0	11000	1.47	6.60
3.05	8.0	11000	1.38	6.37
2.60	4.7	4000	4.50	...
2.60	4.7	11000	4.70	8.15
1.36	4.7	3000	7.67	...
1.36	4.7	4000	9.55	8.80
0.68	4.7	4000	9.50	8.60
0.68	4.7	6000	8.73	8.96
0.34	4.7	6000	14.5	9.30
0.34	4.7	4000	12.0	9.30
0.34	4.7	4000	10.2	9.25
0.34	4.7	8000	13.0	8.94
0.17	4.7	4000	11.0	9.05
0.17	4.7	8000	14.6	9.70
0.17	4.7	8000	12.3	9.60
0.17	4.7	11000	15.3	9.80
0.074	4.7	4000	14.0	8.55
.074	4.7	6000	15.1	9.90
.074	4.7	8000	15.6	10.3
.051	4.7	6000	16.5	...
.049	4.7	10000	16.0	...

sedimentation velocity are given. Fig. 5 demonstrates the variation of diffusion constant and sedimentation velocity with concentration at a P_H of 4.7. Both the table and the curve show that at a concentration of 0.1% and below, the values of D remain independent of concentration, indicating that we have reached a region of normal diffusion conditions. The values of s also show a slight variation with concentration. This change, however, is not at all comparable to that of the diffusion constant.

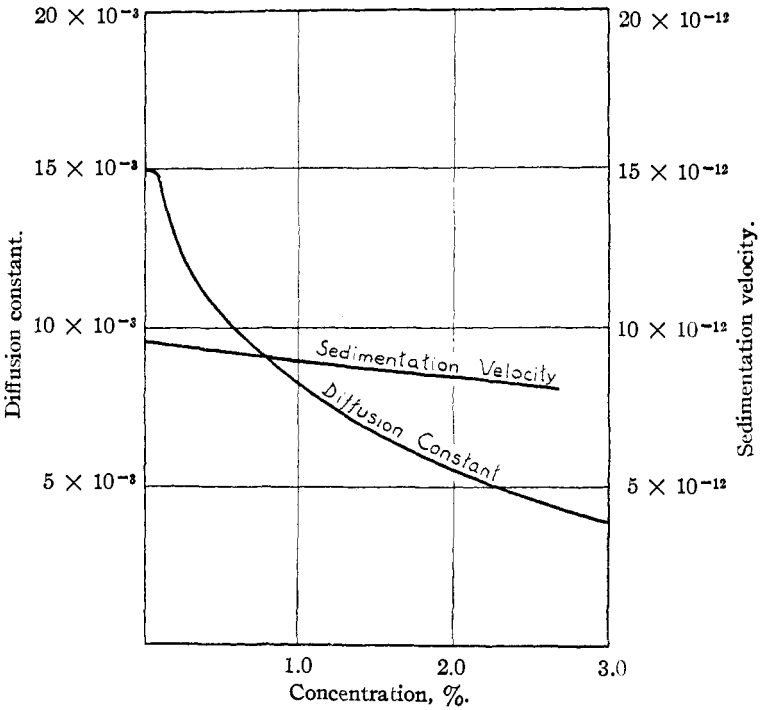

Fig. 5.

Taking for D the mean value 15.4×10^{-3} from the five determinations below 0.1% and for s the mean value 9.42×10^{-12} from the 11 determinations below 0.4%, we get for the molecular weight 4.93×10^6.

B. Sedimentation Equilibrium Method.[21,22,23]—The molecular weight is given by

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

where R, T, V, ρ and ω have their usual meaning and c_1 and c_2 are the concentrations at the distances x_1 and x_2 from the center of rotation.

[21] Svedberg and Fåhraeus, THIS JOURNAL, 48, 430 (1926).
[22] Svedberg, *Z. physik. Chem.*, **121**, 65 (1926).
[23] Svedberg and Nichols, THIS JOURNAL, 48, 3081 (1926).

This method is based on the equilibrium reached in the cell during centrifuging between diffusion and centrifugal force and may only be applied when the conditions insure normal diffusion. It was found by the sedimentation velocity method that in the case of hemocyanin it is essential to study only very dilute solutions, less than 0.1% in weight. Because of the enormous molecular weight only a very low centrifugal force was permissible. Even at a speed of only 3000 r.p.m. no equilibrium could be obtained but the solute molecules and the solvent separated clearly. Therefore speeds of about 1000 r.p.m., giving a centrifugal force only 50–60 times greater than gravity, were used in the following experiments. With columns of solution not longer than 3–4 mm., equilibrium was attained after two to three days of centrifuging.

Standard solution and plates were the same as for the sedimentation velocity runs.

Details of a typical run are given in Table VI. In Table VII a summary of results from eight experiments is given, all carried out with solutions of P_H 4.7 and at concentrations below 0.09%; three exposures were used for the calculation of each molecular weight value.

TABLE VI

SEDIMENTATION EQUILIBRIUM OF HEMOCYANIN FROM *Helix pomatia*

Concentration, 0.051%; P_H = 4.7; length of column of solution, 0.315 cm.; thickness of column, 0.80 cm.; distance of the bottom of the cell from axis of rotation, 4.73 cm.; T = 288.3; speed, 1180 r.p.m. ($\omega = 39.3\,\pi$).

Distances, cm.		Mean concn., %		
x_2	x_1	c_2	c_1	M
4.720	4.685	0.0683	0.0548	5.64×10^6
4.685	4.635	0.0548	0.0448	5.20
4.635	4.585	0.0448	0.0377	4.51
4.585	4.535	0.0377	0.0312	4.99
4.535	4.485	0.0312	0.0260	4.87
4.485	4.435	0.0260	0.0214	5.25
			Mean	5.08×10^6

TABLE VII

MOLECULAR WEIGHT OF HEMOCYANIN FROM *Helix pomatia*

Concn., %	Length of column of solution, cm.	M
0.082	0.37	5.05×10^6
.082	.39	5.22
.074	.39	4.84
.074	.46	4.79
.051	.32	5.07
.051	.38	5.06
.049	.30	5.24
.049	.33	5.15
	Mean	5.08×10^6

Discussion of Results

All the experimental evidence reviewed in this paper indicates five million as the most probable value for the actual molecular weight of hemocyanin from *Helix pomatia* in dilute solution near the isoelectric point. Assuming the molecules to be spherical, we obtain 12.0×10^{-7} cm. for its radius by means of Stokes' law, $r = \sqrt{9\eta s/2\Delta\rho}$, where η is the viscosity of the solution, s the specific sedimentation velocity and $\Delta\rho$ the difference in density between the protein molecule and the solvent. The density of the protein molecule was taken as the reciprocal of the partial specific volume experimentally determined. The radius may also be calculated from the Einstein formula, $r = RT/6\pi\eta ND$, where D is the diffusion constant and N the Avogadro constant. In this way we get $r = 12.2 \times 10^{-7}$ cm. The fact that these two values agree so closely indicates that the particle cannot deviate much from the spherical shape. This particle size is about at the limit of visibility in the ultramicroscope for metallic particles when illuminated with light from the carbon arc. However, particles of an organic substance can only be seen when much larger and therefore it is obvious that we cannot expect to make the molecule of hemocyanin visible in the ultramicroscope.

In order to ascertain whether the particles are of uniform size, we carried out an experiment at a speed of 25,000 r.p.m., corresponding to a centrifugal force 37,000 times greater than that of gravity, with a solution at P_H 4.7 and of concentration 0.051% at a temperature of 23°, taking photographs every three minutes, the whole experiment lasting only half an hour. During such a short interval of time no appreciable diffusion takes place, and if the solution contains molecules of the same size, the photographs must display a sharp boundary between solvent and solution. Fig. 6 shows that same photograph (upper picture) in comparison with a similar one (lower picture) taken during the centrifuging of a gold sol at five minute intervals and at a speed of 22,000 r.p.m. and a temperature of 28.5°.[24] The difference in their appearance is obvious. While the boundary formed by the centrifuged hemocyanin molecules is quite sharp, that in the gold sol is blurred and becomes more so as the particles travel further toward the bottom of the cell. The gold colloid used was prepared by reducing gold chloride by phosphorus and is known to be one of the most uniform synthetic colloids. The radius (determined by H. Rinde) ranges from 1.5–3.3$\mu\mu$, the maximum in the distribution curve being at 2.5$\mu\mu$.

The sharp boundary shown in the photograph, the constancy of the molecular weight determined at various distances from the center of rotation in the sedimentation equilibrium experiment (Table VI) and the constancy with time of the value of the diffusion constant obtained in

[24] Rinde, *Dissertation*, Upsala, 1928.

the sedimentation velocity method (Table IV) all justify the conclusion that, notwithstanding their size and enormous weight, the hemocyanin particles are entitled to be considered as real molecules acting as single units.

The variation of diffusion "constant" with concentration noted in the sedimentation velocity measurements can be explained on the assumption that in concentrated solutions, probably on account of their unusual size, and therefore proximity, there are forces at work acting between the molecules, preventing a completely free movement, which results in a greater frictional force opposed to the diffusion. As the dilution increases and with it the distance between molecules, these intermolecular forces become weaker, until at a certain limit concentration they disappear and the molecules are completely free to move about. This is the state which we have previously termed normal diffusion conditions. It must be clearly understood that in our conception no compact groups of molecules are formed which would modify the total molecular surface. On this assumption it can be theoretically proved that the diffusion constant will change with the concentration of the solution, while the sedimentation velocity should not appreciably do so. A simple calculation shows that in a 3% solution of hemocyanin (the concentration present in the snail blood) the average distance between the surfaces of adjacent molecules is only about one and a half times the molecular diameter.

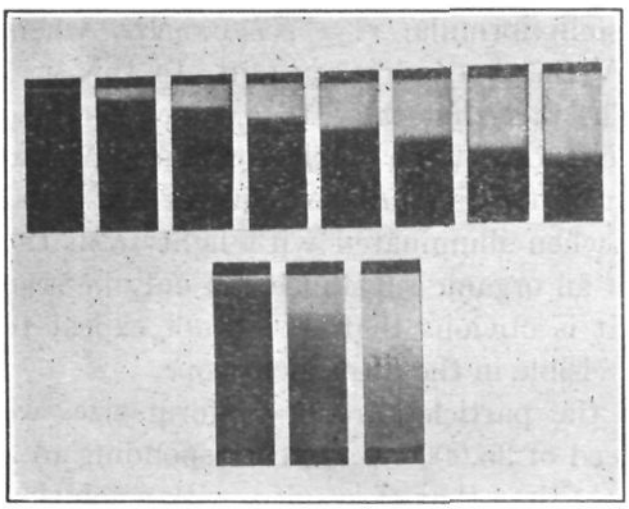

Fig. 6.

Summary

1. The centrifugal methods have been applied to the study of the molecular weight of hemocyanin from *Helix pomatia*.

2. The unexpected phenomenon of a strong variation of its diffusion constant with concentration down to a certain low limit of concentration has been encountered and an assumption has been put forward to explain it.

3. Both the sedimentation velocity and sedimentation equilibrium methods for determining the molecular weight have resulted in good agreement in the figure of 5,000,000 ± 5% as the most probable value in dilute solution at P_H 4.7.

4. Within the limits of experimental error all the molecules of a dilute solution of hemocyanin from *Helix pomatia* at P_H 4.7 are found to be of equal weight and size and this protein is therefore probably to be regarded as a chemical individual.

5. As to shape, the calculations based on our experimental data indicate that at P_H 4.7 the molecules are practically spherical with a radius of 12.1×10^{-7} cm.

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES. XIII. PROPERTIES OF GAMMA-DELTA-DIHYDROXY-CARBONYL DERIVATIVES AND THEIR BEARING ON THE POLYMERIZATION OF POLYSACCHARIDES

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RECEIVED NOVEMBER 30, 1927 PUBLISHED MAY 5, 1928

In previous communications[2] the important role played by adjacent hydroxyl groups in the γ,δ-positions to the carbonyl group has been emphasized and evidence submitted of the tendency of such compounds to readily lose a molecule of water with subsequent formation of a highly polymerized product. The bearing of such phenomena on the problem of the constitution of cellulose and related compounds has been repeatedly indicated.[2]

Further evidence is now available in that a second dihydroxy carbonyl derivative, namely, methyl γ,δ-dihydroxy amyl ketone has been synthesized and found to show a similar behavior to that of the methyl γ,δ-dihydroxy butyl ketone previously investigated.[2c]

Thus in presence of a trace of concentrated sulfuric acid at about 90°, it readily loses a mole of water per mole of the ketone, yielding a viscous, resinous substance which is found to be a polymerized product having a molecular weight in benzene solution of 553–590.

The molecular weight of the corresponding product from methyl γ,δ-dihydroxy butyl ketone is shown to vary between 395 and 445 in phenol solution and to increase to 3706 when camphor is used as the solvent.

Assuming the methyl γ,δ-dihydroxy amyl ketone to possess the butylene oxide structure, the change may be represented as follows.

[1] Constructed from the dissertation presented by C. Pauline Burt to the Faculty of the Graduate School of Yale University, June, 1925, in candidacy for the degree of Doctor of Philosophy.

[2] (a) Hibbert, *Chem. Met. Eng.*, **22**, 838 (1920); (b) *J. Ind. Eng. Chem.*, **13**, 256, 334 (1920); (c) Hibbert and Timm, THIS JOURNAL, **45**, 2433 (1923).