that of HCN and that of $CHCl_3$, 2.93 − 1.05 = 1.88, or the difference between CH_3CN and CH_3CCl_3,[18] 3.45 − 1.5 = 1.95. The value 2.0×10^{-18} observed by Snoek differs from the calculated values by less than the experimental error.

In the nitromethanes, the moment of the nitro group should act in the C—N line[17] and the moment relations should resemble those in the methyl cyanides. The moment 3.42×10^{-18} found for nitromethane is considerably lower than the approximate value 3.78×10^{-18} calculated by Höjendahl[19] from the measurements of Pohrt on the vapor and higher than the values 3.04,[20] 3.05[19] and 3.13[21] found in solution. The moment of CCl_3NO_2 should be approximately equal to the difference between that of CH_3NO_2 and that of CH_3CCl_3, 3.42 − 1.5 = 1.92, which is in excellent agreement with the value 1.88 in Table II.

(18) Van Arkel and Snoek, *Z. physik. Chem.*, **B18**, 159 (1932).
(19) Höjendahl, "Thesis," Copenhagen, 1928; *Physik. Z.*, **30**, 391 (1929).
(20) Hunter and Partington, *J. Chem. Soc.*, 312 (1932).
(21) Weissberger and Sängewald, *Ber.*, **65**, 701 (1932).

In spite of the fact that the dipoles of the carbonyl, nitro and cyanide groups are larger than those which have previously been examined in such close proximity to one another, there is no evidence of any exceptionally large induced shift of charge or alteration of valence angle in the molecules. In these aliphatic molecules, any shifts of charge other than those due to simple inductive effect are too small to be detected.

Summary

The dielectric constants of the vapors of phosgene, hydrogen cyanide, nitromethane and chloropicrin have been measured and used to calculate the dipole moments of the molecules of these substances. The values thus obtained, together with the moments of other similar molecules, are found to conform closely to the moments calculated on the assumption of a central tetrahedral carbon atom.

PRINCETON, NEW JERSEY RECEIVED JUNE 14, 1934

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

The Molecular Weight of Erythrocruorin. II.

BY THE SVEDBERG AND INGA-BRITTA ERIKSSON-QUENSEL

In a previous communication[1] a report has been given of the determination by centrifugal methods of the molecular weight, the sedimentation constant and the *P*H stability range of the red proteins dissolved in the blood of the lugworm, *Arenicola marina* and the earthworm, *Lumbricus terrestris*. Both with regard to molecular mass and electrochemical properties such marked differences were found between the respiratory pigments in question on the one hand and the hemoglobin of the vertebrates on the other that it was proposed to revive the name erythrocruorin formerly used by Ray Lankester for the red blood proteins of the invertebrates. The sedimentation constant of *Arenicola* erythrocruorin is 57.4×10^{-13}, that of *Lumbricus* erythrocruorin 60.9×10^{-13}. By means of a mixture test it could be proved that the difference between the two constants expressed by the above values is real. The molecular weight was found to be around 2,800,000 in both cases. The experimental error in the sedimentation constant is probably less than 1%. The molecular weight, however, being determined by means of the sedimentation equilibrium method, which is difficult to handle in case of very heavy molecules, may be in error to an extent of about 10%.[1a]

(1) Svedberg and Eriksson, THIS JOURNAL, **55**, 2834 (1933).

In the course of a comparative study of the respiratory proteins throughout the animal kingdom, Mrs. Astrid Hedenius discovered several other varieties of erythrocruorin characterized by different sedimentation constants.[2] It seemed to us desirable to determine the *P*H stability range and the molecular weight for at least one representative of each of these new types of erythrocruorin.

At the present time ten different sedimentation constants are known from the red blood pigments, *viz.*, 60.9, 57.4, 33.7, (22.4), 16.3, (11.6), (7.1), 4.4, 3.5, 1.9×10^{-13}. Erythrocruorin characterized by the two first constants was

(1a) Recent determinations by means of a more accurate method of observation have given a slightly higher value, *viz.*, 3,200,000.
(2) Svedberg and Hedenius, *Nature*, **131**, 325 (1933); *J. Biol. Chem.*, **103**, 311 (1933); Biological Bulletin, **66**, 191 (1934).

studied in the previous paper, 4.4 is the constant of hemoglobin and the values in parentheses only occur as dissociation or association products. There remain therefore only four independent erythrocruorin varieties to be studied in detail, *viz.*, those with the constants 33.7, 16.3, 3.5 and 1.9.

The survey of the respiratory proteins carried out by A. Hedenius has shown that hemoglobin characterized by the sedimentation constant 4.4×10^{-13} and the molecular weight 69,000 is to be found only in the five highest classes of the vertebrates, *viz.*, Mammalia, Aves, Reptilia, Amphibia, Pisces. The blood of the animals belonging to the lowest class of the vertebrates, the Cyclostomata, possesses a red respiratory protein which with regard to sedimentation constant, molecular weight and isoelectric point resembles closely the pigment found in the blood corpuscles of certain invertebrates. We have therefore termed the pigment of the Cyclostomata erythrocruorin. In view of the fact that a whole series of red respiratory proteins of different sedimentation constants and molecular weights are now known it might be advisable to decide upon using the name erythrocruorin—which is in fact the older term—for the whole red series and reserve the name hemoglobin as a special term for the variety which under normal conditions possesses the molecular weight 69,000 and the sedimentation constant 4.4×10^{-13} and which is characteristic of the higher vertebrates.

The Sedimentation Constant and Molecular Weight of Erythrocruorin from the Blood of Planorbis Corneus.—The snails were taken from a pond near Upsala, and kept living in a well-aired aquarium. The blood was drawn by sucking with a capillary pipet after injuring the foot of the animal. A full-sized individual gave 0.5 cc. of blood with an erythrocruorin content of about 1.6%. For the ultracentrifugal runs the blood can be diluted 10–20 times. The specific volume was determined pycnometrically using 10 cc. of blood which had been dialyzed against distilled water for three days in the cold. The value 0.745 was obtained, which is almost identical with the value found for erythrocruorin of *Lumbricus*, *viz.*, 0.740. The isoelectric point was measured by Dr. K. O. Pedersen of this Laboratory. He found the value P_H 4.77,[3] which is slightly higher than that of the *Arenicola* erythrocruorin, 4.56. The ultracentrifugal technique of measurement was the same as the one described in the previous communication. As a rule the blood was drawn immediately before a determination. We found, however, that *Planorbis* blood like other kinds of sensitive blood samples may be kept for many months without the slightest change in the sedimentation constant if preserved in frozen condition in a refrigerator at −7°.

The sedimentation constant was determined in the P_H range 0.7 to 12.6. In Table I and Fig. 1 the measurements are summarized.[4]

Table I and Fig. 1 show that erythrocruorin from *Planorbis* is stable from P_H 3 to 7.8 with a mean value for the sedimentation constant of

TABLE I

ERYTHROCRUORIN OF *Planorbis*, SEDIMENTATION VELOCITY MEASUREMENTS

Dilution of blood 15 times, corresponding to a concentration of erythrocruorin of about 0.16%; centrifugal force about 150,000 times gravity; thickness of column of solution, 1.20 cm.; optical and photographic conditions as in previous communication.

Solvent	Total molar	P_H of soln.	$s_{20} \times 10^{13}$		
HCl, KCl	0.4	0.7	..	..	11.3
HCl, KCl	.2	1.0	21.9	17.5	12.4
HCl, KCl	.2	1.0	..	15.6	12.3
HCl, KCl	.16	1.3	..	..	11.4
Na citrate, HCl	.23	1.5	23.3	17.4	11.8
HCl, KCl	.13	1.5	..	..	10.5
HCl, KCl	.12	1.7	..	..	11.1
HCl, KCl	.11	2.0	21.5	16.3	11.3
HCl, KCl	.11	2.0	22.8	..	12.4
HCl, KCl	.106	2.2		28.6	
HCl, KCl	.106	2.2		33.4	
HCl, KCl	.103	2.5		32.5	
HCl, KCl	.101	3.0		34.3	
HAc, NaAc, KCl	.12	4.0		31.5	
HAc, NaAc, KCl	.12	4.0		33.1	
HAc, NaAc, KCl	.12	4.6		34.3	
HAc, NaAc, KCl	.12	5.0		35.0	
KH_2PO_4, Na_2HPO_4, KCl	.12	5.5		34.7	
KH_2PO_4, Na_2HPO_4, KCl	.12	6.0		33.3	
KH_2PO_4, Na_2HPO_4, KCl	.12	6.5		33.2	
KH_2PO_4, Na_2HPO_4, KCl	.12	6.8		34.6[a]	
KH_2PO_4, Na_2HPO_4, KCl	.12	7.1		34.1	
KH_2PO_4, Na_2HPO_4, KCl	.12	7.5		33.1	
KH_2PO_4, Na_2HPO_4, KCl	.12	7.8		33.7	
KH_2PO_4, $Na_2B_4O_7$, NaCl	.20	8.2		32.2	
KH_2PO_4, $Na_2B_4O_7$, NaCl	.20	8.5		30.3	
Na_2HPO_4, KCl	.105	8.9		29.0	
KH_2PO_4, $Na_2B_4O_7$, NaCl	.20	9.0		30.2	
Na_2CO_3, $Na_2B_4O_7$, NaCl	.20	9.5		28.5	
Na_2HPO_4, NaOH, KCl	.1505	9.5		30.3	
Na_2HPO_4, NaOH, KCl	.151	9.8		29.4	
Na_2CO_3, $Na_2B_4O_7$, NaCl	.20	10.0		26.8	
Na_2CO_3, $Na_2B_4O_7$, NaCl	.20	10.0		26.1	
Na_2HPO_4, NaOH	.052	10.2		27.5	
Na_2HPO_4, NaOH	.055	10.7		26.2	
Na_2CO_3, $Na_2B_4O_7$, NaCl	.20	10.8		22.3	
Na_2CO_3, $Na_2B_4O_7$, NaCl	.20	10.8		25.7	
Na_2HPO_4, NaOH	.06	11.0		22.1	
Na_2HPO_4, NaOH	.09	11.4		22.0	
Na_2HPO_4, NaOH, NaCl	.27	11.5		25.4	
Na_2HPO_4, NaOH, NaCl	.27	11.8		22.2	
Na_2HPO_4, NaOH	.1	12.1		16.7	
Na_2HPO_4, NaOH, NaCl	.27	12.5		18.8	
Na_2HPO_4, NaOH	.15	12.6		16.9[b]	

[a] The sample had been kept frozen at −7° for nine months.

[b] Traces of low-molecular component noticeable.

(3) Pedersen, *Kolloid-Z.*, **63**, 274 (1933).

(4) In cases where more than one determination was carried out at the same or nearly the same P_H, the mean value has been used for the diagram.

33.7×10^{-13}. From eighteen sedimentation measurements on *Planorbis* blood diluted with 1% sodium chloride Mrs. A. Hedenius found the mean value 33.8×10^{-13}.

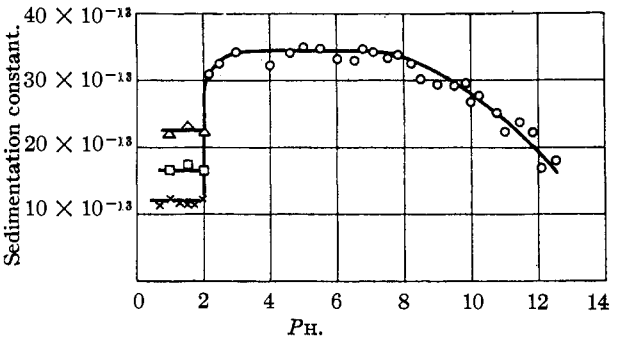

Fig. 1.

In solutions more alkaline than P_H 7.8 the sedimentation constant decreases, indicating gradual decomposition of the molecule. On the acid border of the stability region the molecule of sedimentation constant 33.7 disappears and three new components with the constants 22.4, 16.7 and 11.6 are formed. These molecules are stable in the range P_H 0.7 to 2.0. The values are not far from the sedimentation constants 23.5, 16.9, 12.0 which are known from the study of other proteins and which represent molecules of weight about one-half, one-quarter or one-sixth of the molecule of *Planorbis* erythrocruorin. It therefore seems reasonable to assume that this molecule actually dissociates into such fragments at the acid border of the stability region.

The molecular weight was determined by means of sedimentation equilibrium measurements. Table II summarizes the results and Table III gives the complete data of run No. 2.

TABLE II

ERYTHROCRUORIN OF *Planorbis*, SUMMARY OF SEDIMENTATION EQUILIBRIUM RUNS

Solvent, acetate or phosphate buffer 0.02 M + 0.1 M in KCl; concn. of protein 0.1–0.2% at start; centrifugal force about 360 times gravity (speed 2040–2910 r. p. m.); time of centrifuging about 75 hours.

No.	P_H of soln.	Mol. wt.
1	5.0 acetate	1,240,000
2	5.3 phosphate	1,291,000
3	6.8 phosphate	1,620,000
4	7.3 phosphate	1,214,000
	Mean	1,341,000

There is no systematic drift with distance from center of rotation, showing that the *Planorbis* erythrocruorin is monodisperse. The molecular weight is very nearly one-half that of the erythrocruorin from *Arenicola* and *Lumbricus*. From the sedimentation constant 33.7×10^{-13} and the molecular weight 1,300,000 we get for the molar frictional constant $f = M(1 - V\rho)/s$ the value 9.9×10^{16}. A spherical molecule of the same specific volume would have the frictional constant $f_0 = 8.3 \times 10^{16}$. The dissymmetry number f/f_0 is therefore 1.2.[5]

The Sedimentation Constant and Molecular Weight of Erythrocruorin from the Blood of Daphnia Pulex.— The erythrocruorin molecule of one-half the weight of the *Planorbis* erythrocruorin has not been found as a normal constituent of any blood protein. The next step, or one-fourth of the *Planorbis* pigment, is represented in the blood of certain crustaceans. The only species which we could procure in sufficient quantity was *Daphnia pulex*. The use of such a small species (length about 2 mm.) has, however, the serious drawback that the blood cannot be drawn in pure state. Our material was treated in the following way. The animals were washed on a suitable net, dried quickly on filter paper and then put in a small bag of filtering cloth and pressed gently. A red solution containing blood and body fluid dripped off and was filtered through paper and then frozen. Immediately before each run a small piece of the red ice was thawed, mixed with a buffer solution and centrifuged. The use of the mercury lines 577–579 and 546 mμ for taking the sedimentation pictures made it possible to study the erythrocruorin of the sample without any disturbing action from other proteins present. Because of the impossibility of isolating this erythrocruorin in pure state the specific volume could not be determined. When correcting the

TABLE III

ERYTHROCRUORIN OF *Planorbis*, SEDIMENTATION EQUILIBRIUM RUN NO. 2

Spec. volume, 0.745; density of solvent, 1.0034; abs. temp., 293°; length of column of soln., 0.475 cm.; thickness of column of soln., 1.20 cm.; dist. of outer end of soln. from center of rotation, 5.95 cm.; speed 2280 r. p. m.; source of light, mercury lamp; light filter, Wratten K3; aperture of objective, F:36; plates, Ilford rap. proc. panchromatic; time of exp., 10, 20, 30 sec.; expt. made after 48, 53, 77 hours of centrifuging.

Distances, cm. x_2	x_1	Concn. ratio c_2/c_1	Number of exposures	Mol. wt.
5.85	5.80	1.289	9	1,475,000
5.80	5.75	1.290	11	1,498,000
5.75	5.70	1.279	11	1,457,000
5.70	5.65	1.195	11	1,066,000
5.65	5.60	1.183	11	1,015,000
5.60	5.55	1.237	10	1,291,000
5.55	5.50	1.223	9	1,235,000
			Mean	1,291,000

(5) Recent determinations by means of a more accurate method of observation have given a slightly higher value for the molecular weight, *viz.*, 1,600,000, corresponding to a dissymmetry number of 1.38.

sedimentation constants for density effects by the salts added the value found for *Planorbis* erythrocruorin, or 0.745, was used.

The sedimentation constant was determined in the P_H range 4.0–12.1. Table IV and Fig. 2 summarize the measurements. In cases where more than one determination was carried out at the same P_H the mean value has been used for the diagram.

Table IV and Fig. 2 show that erythrocruorin from *Daphnia* contains two components. The higher and predominant one is stable from P_H 4.5 to 10.5 with a mean value for the sedimentation constant of 16.3×10^{-13} within the stability range. Mrs. A. Hedenius[2] found from three measurements on *Daphnia* blood diluted with 1% sodium chloride the value 16.9. The percentage of the lower component increases with P_H, reaching 100% at the P_H where the higher component disappears.

TABLE IV

ERYTHROCRUORIN OF *Daphnia*, SEDIMENTATION VELOCITY MEASUREMENTS

Dilution of blood, 5 times; centrifugal force about 200,000 times gravity; thickness of column of soln., 1.20 cm., optical and photographic conditions as in Table I.

Solvent	Total molar	P_H of soln.	$s_{20} \times 10^{13}$	Low-molecular component, %
HAc, NaAc, NaCl	0.17	4.0	10.2	...
HAc, NaAc, NaCl	.17	4.6	15.9	...
HAc, NaAc, NaCl	.17	5.0	16.8	15
KH_2PO_4, Na_2HPO_4, NaCl	.17	5.5	15.3	...
KH_2PO_4, Na_2HPO_4, NaCl	.17	5.5	16.4	...
KH_2PO_4, Na_2HPO_4, NaCl	.17	6.0	15.9	17
KH_2PO_4, Na_2HPO_4, NaCl	.17	6.4	16.8	31
KH_2PO_4, Na_2HPO_4, NaCl	.17	6.8	16.5 and 2.8	38
KH_2PO_4, Na_2HPO_4, NaCl	.17	6.8	16.8	..
KH_2PO_4, Na_2HPO_4, NaCl	.17	7.4	16.1	...
KH_2PO_4, Na_2HPO_4, NaCl	.17	7.4	16.0	31
KH_2PO_4, Na_2HPO_4, NaCl	.17	8.0	16.3	48
KH_2PO_4, $Na_2B_4O_7$, NaCl	.17	8.6	16.5	...
Na_2HPO_4, KCl	.15	8.9	16.7	45
$Na_2B_4O_7$, Na_2CO_3	.17	9.5	15.9 and 3.8	43
Na_2HPO_4, NaOH	.07	10.2	16.8	...
$Na_2B_4O_7$, Na_2CO_3, NaCl	.17	10.8	14.0 and 3.7	73
Na_2HPO_4, NaOH	.06	11.0	14.3	64
Na_2HPO_4, NaOH, NaCl	.27	11.5	.. 2.5	100[a]
Na_2HPO_4, NaOH, NaCl	.27	12.1	.. 2.6	100[a]

[a] Centrifugal force 260,000 times gravity.

Owing to the great instability of the *Daphnia* erythrocruorin, probably caused by the presence of enzymes from the body fluid, we found it impossible to carry out equilibrium measurements on the material at our disposal. The molecular weight cannot, therefore, be determined directly. Among the crustaceans with hemocyanin in their blood there is, however, a group possessing a pigment of sedimentation constant 16.9×10^{-13} as the normal constituent and another group with a hemocyanin of constant 23.5 which at the alkaline end of the stability range dissociates into molecules of constant 16.9. Some preliminary sedimentation equilibrium measurements on these hemocyanins has shown that the one of constant 23.5 probably possesses one-half the weight of the *Planorbis* erythrocruorin and the one of constant 16.9 probably one-quarter the weight of this erythrocruorin. We therefore believe that we are justified in drawing the conclusion that the higher component of the *Daphnia* erythrocruorin is one-quarter of the *Planorbis* erythrocruorin. The lower component has a sedimentation constant close to that of ovalbumin and therefore probably a molecular weight of 34,500. This would mean that it is one-twelfth of the higher component.

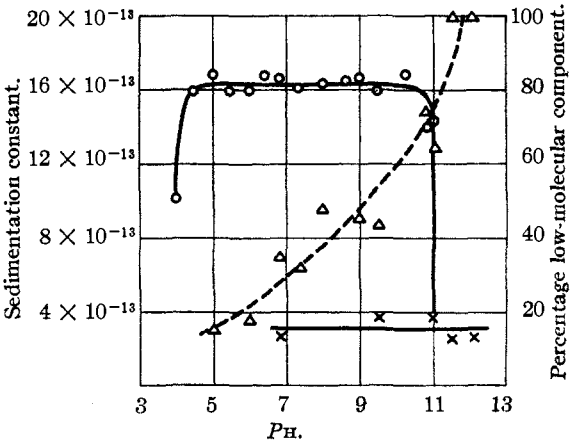

Fig. 2.

The Sedimentation Constants and Molecular Weights of the Erythrocruorins from the Blood of Arca Pexata, Notomastus Latericius, Chironomus Plumosus, Thyone Briareus, Myxine Glutinosa and Petromyzon Fluviatilis.—Regular blood pigments of the weights one-sixth, one-twelfth and one-sixteenth the weight of the *Planorbis* erythrocruorin have not been observed. The next step corresponds to one twenty-fourth of the *Planorbis* erythrocruorin and is realized in the hemoglobin of the vertebrate animals. We then arrive at a protein of one forty-eighth the weight of *Planorbis* erythrocruorin. This pigment we have found in the corpuscles of the blood clam *Arca pexata*. The corpuscles of the marine worm *Natomastus latericius* and the blood plasma of the *Chironomus* larvae contain an erythrocruorin variety of about the same weight but highly dissymmetrical. The next, and last,

step downward, or one ninety-sixth of the *Planorbis* erythrocruorin, we have found in the corpuscles of the Cyclostomat *Petromyzon fluviatilis*. Intermediate states probably representing mixtures of one forty-eighth and one ninety-sixth of the *Planorbis* erythrocruorin are present in the corpuscles of the sea cucumber *Thyone briareus* and the Cyclostomat *Myxine glutinosa*. Owing to the difficulty of procuring sufficient material of some of these animals the P_H stability range and the specific volume have only been determined for the *Petromyzon* erythrocruorin. On the other varieties we have made equilibrium measurements, the sedimentation constant having already been determined in 1% sodium chloride solution by Mrs. A. Hedenius.[2] The position of the isoelectric points has been ascertained by Dr. Pedersen.

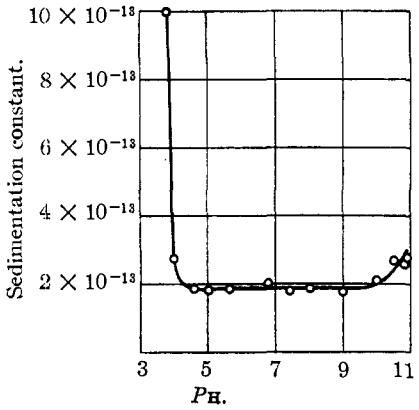

Fig. 3.

The corpuscles from the blood of *Arca* and *Thyone* were freed from plasma by centrifuging and then washed with sea water on the centrifuge. The samples were brought from Woods Hole to Upsala in frozen condition packed in "dry ice" (solid carbon dioxide). After being hemolyzed the stromata were filtered off and the solution stored at −7°. The corpuscles from *Notomastus*, *Myxine* and *Petromyzon* were treated similarly. In these cases the animals were sent living to Upsala, *Notomastus* and *Myxine* from Kristineberg on the west coast of Sweden and *Petromyzon* from Elvkarleby. The *Chironomus* larvae were collected near Upsala. In the latter case the blood was drawn simply by cutting the skin and sucking with a capillary pipet. All the samples were stored at −7°.

In Table V our molecular weight determinations as well as the measurements of the sedimentation constants by Mrs. Hedenius and those of the isoelectric points by Dr. Pedersen are summarized. Table VI and Fig. 3 give our measurements of the P_H stability range for *Petromyzon* erythrocruorin.

TABLE V

MOLECULAR WEIGHTS, SEDIMENTATION CONSTANTS, MOLECULAR SYMMETRY AND ISOELECTRIC POINTS OF ERYTHROCRUORINS FROM THE BLOOD OF *Arca*, *Notomastus*, *Chironomus*, *Thyone*, *Myxine* AND *Petromyzon*

Species	Mol. wt.	$s_{20} \times 10^{13}$	Molecular symmetry	Isoelectric point
Arca pexata	33,600[a]	3.5	Spherical	Between 5 and 6
Notomastus latericius	36,400[a]	2.1	Non-spherical	..
Chironomus plumosus	31,400[c]	2.0	Non-spherical	5.40
Thyone briareus	23,600[b]	2.6	Non-spherical	About 5.8
Myxine glutinosa	23,100[b]	2.3	Non-spherical	About 6
Petromyzon fluviatilis	19,100[b]	1.9	Nearly spherical	5.6

[a] Mean from two sedimentation equilibrium determinations. [b] Mean from three determinations. [c] Mean from four determinations.

TABLE VI

ERYTHROCRUORIN OF *Petromyzon*, SEDIMENTATION VELOCITY MEASUREMENTS

Concentration of protein about 0.2%; centrifugal force 260,000–360,000 times gravity; thickness of column of soln., 0.6 cm.; optical conditions as in Table I.

Solvent	Total molar	P_H of soln.	$s_{20} \times 10^{13}$
HAc, NaAc, NaCl	0.19	3.8	10.0[a]
HAc, NaAc, NaCl	.19	4.0	2.74
HAc, NaAc, NaCl	.19	4.6	1.82
HAc, NaAc, NaCl	.19	5.0	1.84
KH_2PO_4, Na_2HPO_4, NaCl	.19	5.8	1.87
KH_2PO_4, Na_2HPO_4, KCl	.12	6.8	2.06
KH_2PO_4, Na_2HPO_4, NaCl	.19	7.4	1.79
KH_2PO_4, Na_2HPO_4, KCl	.12	8.0	1.92
KH_2PO_4, $Na_2B_4O_7$, NaCl	.20	9.0	1.79
Na_2CO_3, $Na_2B_4O_7$, NaCl	.20	10.0	2.12
Na_2CO_3, $Na_2B_4O_7$, NaCl	.20	10.5	2.79
Na_2CO_3, $Na_2B_4O_7$, NaCl	.20	10.8	2.72
Na_2CO_3, $Na_2B_4O_7$, NaCl	.20	10.8	2.80

[a] Polydisperse aggregation product.

For *Petromyzon* erythrocruorin the specific volume 0.751 was obtained as a mean of two determinations on the dialyzed pigment. This value is almost identical with the specific volume of the other varieties of erythrocruorin so far studied. When calculating the equilibrium runs on the pigments from *Arca*, *Notomastus*, *Chironomus*, *Thyone* and *Myxine* the same value was used.

The erythrocruorin from *Arca* is within the limits of error identical with ovalbumin both with regard to sedimentation constant and molecular weight. It represents the normal

molecular unit of the proteins. Its molecular weight is one-half that of hemoglobin and the molecule therefore possesses two hemin groups, provided the iron content is the same as for hemoglobin. The erythrocruorins from *Notomastus* and *Chironomus* have also about the same molecular weight as ovalbumin but the sedimentation constant is low, indicating a highly dissymmetrical molecule. An equilibrium run at high speed indicated the presence of a small amount of molecules of lower weight. The pigments of *Thyone*, *Myxine* and *Petromyzon* have lower molecular weights ranging from 23,600 to 19,100 and sedimentation constants from 2.6 to 1.9. The simplest explanation of the behavior of these erythrocruorins seems to be that they represent mixtures of the molecules 34,500 and 17,250, that is, mixtures of molecules with two and one hemin group per molecule, respectively. In the *Petromyzon* erythrocruorin the latter component is predominant. The *P*H stability curve (Fig. 3) shows that this protein is stable from *P*H 4.5 to 10.0 with a mean value for the sedimentation constant of 1.87×10^{-13}. Both at the acid and the alkaline border aggregation occurs. In solutions more alkaline than *P*H 11 the pigment turns brown and irregular values are found. The isoelectric points of these blood proteins are all situated between *P*H 5 and 6.

In Table VII we have made an attempt to survey the different types of the red respiratory proteins from the point of view of sedimentation constant and molecular weight.[6] The iron content for all the types has been assumed to be the same as for hemoglobin (sedimentation constant 4.4).

TABLE VII

SEDIMENTATION CONSTANTS AND MOLECULAR WEIGHTS OF THE RED BLOOD PROTEINS

Number of hemin groups per molecule	Sedimentation constant	Molecular weight: From sedimentation equilibrium measurements	Molecular weight: From number of hemin groups per molecule
1	1.9	19,100	17,250
2	3.5	34,500	34,500
4	4.4	68,000	69,000
(8)	(7.1)	(150,000)	138,000
(16)	(11.6)	(280,000)	280,000
24	16.3	(410,000)	420,000
(48)	(22.4)	(830,000)	840,000
96	33.7	1,600,000	1,680,000
192	57.4	3,200,000	3,360,000
	60.9		

(6) The observed molecular weights of the proteins of sedimentation constants 7.1 (serum globulin), 11.6 (phycoerythrin), 16.3 (Pandalus-hemocyanin), 22.4 (Homarus-hemocyanin), are given according to recent equilibrium measurements by one of us (I.-B. E.-Q.).

The sedimentation constants in parentheses have only been observed as dissociation or association products. Molecular weights in parentheses have not been determined for red pigments but are known from measurements on other proteins. The molecules of constants 60.9 and 57.4 have probably the same weight but slightly different shape. The molecules assumed to contain 16, 24 and 48 hemin groups are found as dissociation products of the erythrocruorin molecule containing 96 hemin groups (*Planorbis*). The molecule of 8 hemin groups has been observed as association product of hemoglobin with 4 hemin groups from the blood of certain reptiles and amphibians.[2] Some of the relationships which we have tried to emphasize in Table VII may not seem very strongly proved. Similar regularities have, however, been observed among other groups of proteins. Thus the behavior of certain hemocyanins makes it very probable that the molecule of sedimentation constant 16.3 is one-half of the molecule of sedimentation constant 22.4 (or 23.5) and this one in its turn one-quarter of the molecule of constant 57.4.

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Summary

1. The sedimentation constant and *P*H stability range of the erythrocruorins from *Planorbis corneus*, *Daphnia pulex* and *Petromyzon fluviatilis* as well as the molecular weights of the erythrocruorins from *Planorbis corneus*, *Arca pexata*, *Notomastus latericius*, *Chironomus plumosus*, *Thyone briareus*, *Myxine glutinosa* and *Petromyzon fluviatilis* have been determined by means of the ultracentrifugal methods.

2. These red respiratory proteins form together with hemoglobin, which is strictly limited to the five higher classes of the vertebrates, a system of molecules built up of units of weight 34,500 (and $^1/_2 \times$ 34,500) in simple proportions.

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

The Ultracentrifugal Study of Pomelin

BY LAURA KREJCI[1] AND THE SVEDBERG

In two recent communications Saunders[2] has reported the results of an investigation concerning the amount of nitrogen extracted from orange seed meal by solutions of different salts. He has found that with one exception, and that a logical one, the amounts extracted are identical regardless of the salt used as extracting agent. This circumstance is of especial interest in view of Gortner's findings[3] that wheat flour yields widely different amounts of nitrogen on extraction with solutions of various salts; the potassium halides, in particular, show a marked lyotropic series. Saunders has suggested that this difference in behavior may be a consequence of the fact that the orange seed protein, pomelin, is a well-defined crystalline globulin, while wheat flour contains a complex mixture of proteins and might therefore be expected to yield varying amounts of nitrogen on treatment with different salts. Since, however, solutions of different salts in varying concentration have been found to extract different amounts of protein even from seed material which is known to contain crystalline globulin,[4] and since it is also possible that different salts may extract the same amounts of nitrogen from a meal and yet not extract a chemical entity, it seemed to be of interest to make an ultracentrifugal study of the orange seed globulin in order to determine more precisely than would otherwise be possible the character of the material extracted by the salts from the orange seed meal. At the suggestion of Dr. Saunders such a study was undertaken in this Laboratory.

Material Used

Dr. Saunders was kind enough to provide the material for the investigation, both orange seed meal and a sample of dried pomelin. The orange seed meal had been prepared in the same manner as for the extraction studies. The seeds were ground in a mill, extracted repeatedly with benzene to remove the oil, air-dried and sifted. The dried pomelin had been prepared by the ammonium sulfate-dialysis method described by Saunders,[5] and dried with alcohol and ether.

The dried pomelin was rejected in favor of the orange seed meal as the source of protein for use in the ultracentrifuge because of the possibility that the alcohol and ether used during the preparation might have caused denaturation. However, near the completion of the study two runs were made with solutions of the dried pomelin in order to determine the effect if any of the preparation process on the protein.

Comparison of Chloride, Bromide and Iodide Extracts. Sedimentation Velocity Measurements.—In order to make a comparison of the protein matter extracted by the different salts, portions of the orange seed meal were extracted with half normal solutions of potassium chloride, potassium bromide and potassium iodide. After the treatment described below, the extracts were studied in the ultracentrifuge by the sedimentation velocity method. The results are listed in Table I.[6]

TABLE I

COMPARISON OF EXTRACTS OF ORANGE SEED MEAL. SEDIMENTATION VELOCITY MEASUREMENTS

Centrifugal force about 100,000 times gravity; thickness of column of solution, 1.2 cm.; source of light, mercury lamp; light filter, chlorine and bromine; plates, Imperial Process; exposure time, one minute; metol hydroquinone developer, one minute. Solvent in all cases: 0.5 *N* KCl.

Description of treatment	$s_{20} \times 10^{13}$ KCl extract			KBr extract			KI extract		
Pptd. once with $(NH_4)_2SO_4$	..	..	..	..	11.6	..	..	..	..
Pptd. 3 times with $(NH_4)_2SO_4$	..	11.3	..	..	..	..	2.8	11.4	..
Pptd. 4 times with $(NH_4)_2SO_4$	1.5	11.2	..	..	..	..	..	..	..
Fraction pptd. by dilution	..	11.8	16.9	..	12.0	16.7	..	11.3	17.0
Fraction not pptd. by diln.	0.8	11.7	..	1.5	..	..	1.7	..	..
Second extract of meal, pptd. once with $(NH_4)_2SO_4$	..	11.0	..	..	..	..	..	..	..

(1) Fellow of the American Scandinavian Foundation.

(2) Saunders, THIS JOURNAL, **53**, 696 (1931); Rotha and Saunders, *ibid.*, **54**, 342 (1932).

(3) Gortner, Hoffman and Sinclair, "Colloid Symposium Monograph," The Chemical Catalog Co., Inc., New York, 1928, Vol. V, p. 187.

(4) Osborne and Harris, *Am. J. Physiol.*, **14**, 151 (1905).

(5) Saunders, Ref. 2, p. 697.

(6) In all cases the sedimentation constant has been corrected for the density and viscosity of the salt present to a basis of sedimentation in pure water. See Svedberg and Stamm, THIS JOURNAL, **51**, 2177 (1929).