THE SEDIMENTATION CONSTANTS OF THE RESPIRATORY PROTEINS

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The ultracentrifugal technique developed in this laboratory enables us to define the mass and shape properties of a high molecular substance by means of two data, the molecular weight and the sedimentation constant (Svedberg, 1934). The latter one, which is a function of the mass and shape of the molecule, is the easiest to measure with a fairly high degree of precision. The error in the mean value from a few—say ten—absolute determinations is about 1 per cent. Relative measurements can be arranged so as to give an accuracy of around 2 per cent in a single experiment. The amount of substance required for a determination is of the order of 0.001 gram. If the molecules to be studied possess characteristic light absorption in the visible or ultraviolet part of the spectrum, it is possible to make the measurement regardless of the presence of other substances in the solution. In many cases the method further allows us to measure simultaneously the sedimentation constants of the different molecular species in a mixed solution. The determination of the sedimentation constant therefore constitutes an efficient tool for the study of high molecular substances dissolved in native fluids. On the following pages a report will be given of an attempt to characterize the respiratory proteins throughout the animal kingdom by their sedimentation constants (see Svedberg and Hedenius, 1933; Svedberg, 1933).

Method

A small quantity of the solution to be studied (0.1 to 1.5 cc.), enclosed in a sector-shaped cell between plane-parallel windows of crystalline quartz, is exposed to the influence of a very strong field of force in a special centrifugal instrument, the ultracentrifuge. In order to avoid convection currents due to temperature disturbances, the rotation takes place in hydrogen of 20 mm. pressure and the surface of the solution is covered with a layer of oil. From time to time photographs of the rotating column of solution are taken so as to record the movement of the boundary between solvent and sedimenting solute. A concentration scale is photographed on the same plate. The pictures are then reg-

istered by means of a microphotometer and the curves obtained used for the calculation. If the field of force is strong enough, the rate of settling can be measured and from this the sedimentation constant, defined as the velocity of sedimentation in a field of unit strength reduced to water of 20° as solvent, can be calculated.

If x is the distance from the centre of rotation, t the time, ω the angular velocity, η the viscosity and ρ the density of the solvent, η_0 the viscosity and ρ_0 the density of water of 20°, and V the partial specific volume of the solute, the sedimentation constant is given by the expression:

$$s_{20}^{\circ} = dx/dt \,.\, 1/\omega^2 x \,.\, \eta/\eta_0 \cdot \frac{1 - V\rho_0}{1 - V\rho}.$$

From previous protein investigations of this laboratory it is known that V varies very little from one protein to the other. The fact that V only figures in a correction term makes it unnecessary to determine it for every protein studied. This circumstance is of considerable importance because of the difficulty of procuring enough material for density determinations in the case of many of the invertebrates. In the following the mean value $V = 0.745$ was used.

The active group of the respiratory pigments confers upon these proteins characteristic absorption bands in the visible and near ultraviolet spectrum. If a suitable wave-length is chosen for the illumination, it is therefore always possible to measure the sedimentation of the molecules of a respiratory pigment undisturbed by the presence of other blood proteins. A sample of blood can be studied in the ultracentrifuge directly after drawing it and eventually diluting it with a salt solution (or laking the blood corpuscles) without subjecting it to any chemical treatment at all. This simplified procedure eliminates serious causes of error in the case of the more unstable pigments.

Four types of respiratory blood proteins are known, red pigments (erythrocruorin, hemoglobin), green pigments (chlorocruorin), blue pigments (hemocyanin) and pigments of reddish-brown color (hemerythrin) (Redfield, 1933). As a rule we have used the mercury lines 577–79 mμ and 546 mμ in combination with a Wratten K3 filter for the study of the red and green proteins, the mercury line 366 mμ with a nickel oxide glass filter for the hemocyanins as well as for mixtures of red or green proteins with hemocyanins and line 546 mμ with a Wratten n:r 77 filter for the hemerythrins. The pictures in the visible spectrum have been taken on Ilford rapid process panchromatic plates while for the ultraviolet we have used Imperial process plates.

For those species the blood of which contains a respiratory protein

of high molecular weight at least two determinations have been made, one for the blood in question alone, and one in mixture with the blood of a standard species. This last kind of measurement enables us to compare all the sedimentation determinations with a few well-established constants. The absence of a double boundary in the case of a mixture of two proteins of high molecular weight, and accordingly low diffusion, has been taken as a criterion of the identity of the sedimentation constants (limit of error about ± 2 per cent). On the other hand, the existence of two different sedimentation constants lying close together has always been checked by applying this mixture test.

The blood samples have mostly been diluted down to a pigment concentration of 0.1–0.3 per cent in order to avoid viscosity errors. A few check runs on undiluted blood have been done, however. As a rule we have used 1 per cent NaCl as diluting agent. The natural pH of the blood is often very close to the alkaline stability limit of the respiratory protein contained in it. In such cases, where more than one kind of molecular mass exists at this border, the samples have been diluted with a suitable buffer solution to keep the pH down. The sedimentation constant of the state of aggregation which possesses the widest pH stability range has always been chosen to characterize the protein in question. The sedimentation constants of dissociation or aggregation states observed have been given in parenthesis in the tables.

Most of the invertebrates have their blood pigment dissolved in the plasma. The echinoderms, the lamellibranchians, some of the worms and, of course, all the vertebrates, however, have red blood corpuscles. To bring the pigment into solution the erythrocytes were laked with distilled water and filtered off. Sodium chloride was then added to bring the salt content up to 1 per cent. In cases where we had at our disposal a quantity of blood sufficient for separation of the pigment from colorless proteins eventually present the corpuscles were washed on the centrifuge with sea water or with 1 per cent NaCl solution before laking.

It was found during this investigation that the blood samples obtained from invertebrate animals were often apt to deteriorate rapidly even when kept at 0° C. This was especially pronounced in the case of very small species where it was impossible to avoid contamination of the blood by coelomic fluid or gland excretions. At least one run for each species was therefore carried out immediately after drawing the blood. Tests with samples of frozen blood, however, showed that even the most unstable types could be stored in this state for months without deteriorating. During the latter part of this investigation constant use was made of this circumstance.

TABLE I

Chætopoda: Polychæta, Hirudinea

Pigment dissolved in plasma. Standard species for mixture test, *Nereis virens* (Sars). Solvent, 1 per cent NaCl; thickness of column of solution 1.2 cm.; centrifugal force about 65,000 times gravity.

	Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
					per cent
Type of pigment: erythrocruorin	Eunicidæ: Lumbrinereis fragilis (Müll.) Kristineberg, Sweden	10		58.0	–
		20	Nereis virens	59.8†	±3
	Nereidæ: Nereis virens (Sars) Kristineberg, Sweden	10		58.6*	–
		20		61.5*	–
		15		56.6	–
		30		57.6‖	–
		20	Lumbrinereis fragilis	61.5†	–
		20	Arenicola marina	57.0*	–
		15	Eumenia crassa	59.0	–
		20	Pectinaria belgica	54.1	–
		13	Polymnia nebulosa	59.1	–
		20	Sabella pavonia	53.8	–
		20	Hæmopis sanguisuga	57.6	–
		20	Hirudo medicinalis	58.5	–
		20	Lumbricus terrestris	58.4*	–
		10	Planorbis corneus	56.2	–
		20	Helix pomatia	59.6§	–
		20	Sepia officinalis	58.9	–
	Arenicolidæ: Arenicola marina (L.) Kristineberg, Sweden	20	Nereis virens	57.8*	±1
	Scalibregmidæ: Eumenia crassa Oersted Kristineberg, Sweden	10		61.4	–
		10	Nereis virens	57.3	±3

TABLE I—*Continued*

	Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
					per cent
Type of pigment: erythrocruorin	Amphictenidæ: Pectinaria belgica (Pall.) Kristineberg, Sweden	5		54.6(11.7)	–
		20	Nereis virens	54.3(11.7)	±0
	Terebellidæ: Polymnia nebulosa (Mont.) Kristineberg, Sweden	2		56.4	–
		2	Nereis virens	58.3	±1
Type of pigment: chlorocruorin	Chlorhæmidæ: Brada villosa Rathke Kristineberg, Sweden	—		56.0‡	
		2		56.3‡	
	Serpulidæ: Serpula vermicularis L. Kristineberg, Sweden	4		58.8	
	Sabella pavonia Sav. Kristineberg, Sweden	8		52.7	
		6	Nereis virens	54.4	±1
Type of pigment: erythrocruorin	Hirudinidæ: Hirudo medicinalis L. South Europe	25		58.5	–
		25	Nereis virens	57.5	±1
	Hæmopis sanguisuga L. Upsala, Sweden	35		57.7	–
		35	Nereis virens	56.5	±2
			Mean	57.5	±1.5

* Thickness of column of solution 0.8 cm.
† Thickness of column of solution 1.0 cm.
‡ Thickness of column of solution 0.2 cm.; mercury lines 405–436 mμ; Wratten nr 50 filter, Imperial Process plates.
§ Centrifugal force 39,000 times gravity.
|| Centrifugal force 21,000 times gravity; blood frozen for six months, determined by Mrs. I.-B. Eriksson-Quensel.

The following buffer solutions were used:

pH 5.0. 0.0059 M acetic acid, 0.0141 M Na-acetate, 0.8 per cent NaCl.
pH 6.8. 0.01 M Na_2HPO_4, 0.01 M KH_2PO_4, 0.1 M KCl.
pH 8.0. 0.019 M Na_2HPO_4, 0.001 M KH_2PO_4, 0.1 M KCl.

Table II

Chætopoda: Polychæta

Pigment enclosed in corpuscles. Type of pigment, erythrocruorin; solvent, 1 per cent NaCl; thickness of column of solution, 1.2 cm.; centrifugal force about 180,000 times gravity.

Origin of blood	$s_{20°} \times 10^{13}$
Glyceridæ:	
Glycera Rouxii Aud. and M. E.	
Kristineberg, Sweden	3.56
	3.44
Mean	3.50
Capitellidæ:	
Notomastus latericeus Sars	
Kristineberg, Sweden	2.18
	1.98
Mean	2.08

Table III

Chætopoda: Oligochæta

Pigment dissolved in plasma. Type of pigment, erythrocruorin. Standard species for mixture test, *Lumbricus terrestris* L. Solvent, 1 per cent NaCl; thickness of column of solution 1.2 cm. Centrifugal force about 65,000 times gravity.

Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
				per cent
Lumbricidæ:				
Lumbricus terrestris L.				
Upsala, Sweden	20		60.6	—
	20		60.0*	
	20		63.1†	—
	20	Nereis virens	63.0‡	—
	20	Tonicella marmorea	62.1	—
	40	Sepia officinalis	59.9	—
	30	Eisenia fœtida	65.6	—
	20	Arion ater	57.7	—
Eisenia fœtida Sav.				
Upsala, Sweden	20		63.5	
	30	Lumbricus terrestris	63.4	±4
		Mean	61.9	

* Blood frozen for 16 days.
† Blood frozen for 49 days.
‡ Thickness of column of solution, 0.8 cm.

In the tables they will be denoted by their pH. The additions of neutral salt have been made in order to repress the disturbances of the sedimentation caused by the electric charge of the protein molecules (Donnan effect).

Sedimentation Constants

Scolecida

Among the lower worms one representative of the nemerteans was examined, but it was found impossible to draw enough blood from it for a determination of the sedimentation constant.

Annelida

A considerable number of species belonging to this group have been studied. In most cases the respiratory pigment is in solution and sufficient quantities of blood may easily be drawn. A large worm like *Arenicola* may yield up to 0.3 cc., while it requires about a dozen individuals of a small species like *Serpula* to make up 0.1 cc. *Nephthys ciliata, Panthalis oerstedi, Thelepus cincinnatus* and *Terebellides stroemi* were tried without success. Worms with red blood corpuscles are scarce and it is not always easy to extract the pigment without injuring it. With *Echiurus pallasi* and *Priapulus caudatus* we were unsuccessful. Erythrocruorin, chlorocruorin, and hemerythrin but not hemocyanin have been found within this group of animals.

Tables I–III contain the determinations of sedimentation constants.

By adopting the mixture test we were able to prove that the dissolved erythrocruorin and chlorocruorin of all the Polychæta and Hirudinea species have the same sedimentation constant with a mean value of 57.5×10^{-13}. The Oligochæta have a slightly higher constant, viz., 61.9×10^{-13}. Two different and very low constants, 3.50 and 2.08 $\times 10^{-13}$, were found for the erythrocruorin contained in the corpuscles of the glyceride and capitellide worms. The hemerythrin from the corpuscles of the sipunculoideans has also a low constant, but owing to the scarcity of the material at our disposal (*Phascolosoma vulgare*), accurate values for the sedimentation constant could not be obtained. We have therefore refrained from including these data in the tables. The erythrocruorin of *Pectinaria* is very unstable and decomposes easily, giving rise to a product of sedimentation constant 11.7×10^{-13}. A dissociation product of the same constant has been observed at the alkaline border of the stability range in the case of *Arenicola* erythrocruorin (Svedberg and Eriksson-Quensel, 1933). Sedimentation equilibrium measurements indicate that the appearance of this product represents the breaking up of the molecule into 16 equal parts of weight 207,000.

TABLE IV

Crustacea: Phyllopoda, Malacostraca

Pigment dissolved in plasma. Standard species for mixture test, *Pandalus borealis* Krøyer. Solvent, 1 per cent NaCl or buffer pH 5. Thickness of column of solution 1.2 cm.; centrifugal force, 90,000–165,000 times gravity.

	Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
					per cent
Type of pigment: erythrocruorin	Daphnidæ: Daphnia pulex de Geer Upsala, Sweden			16.5* † 17.2* 17.1*	
			Palinurus vulgaris	16.8*	
	Bopyridæ: Athelges sp. Kristineberg, Sweden	7	Planorbis corneus	19.3	
Type of pigment: hemocyanin	Caridídæ: Pandalus borealis Krøyer Kristineberg, Sweden	5		16.6	
		5	Palinurus vulgaris	16.8	
		5	Pagurus striatus	18.1	
		5	Homarus vulgaris	17.2	
	Palæmon fabrici Rathke	15	Homarus vulgaris	15.6	

TABLE IV—*Continued*

Origin of blood		Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
					per cent
Type of pigment: hemocyanin	Palinuridæ: Palinurus vulgaris Latr.				
	Naples, Italy	8		17.2	
		9		15.3	
		8	Pandalus borealis	15.8	±6
		10	Daphnia pulex	18.1	
	Paguridæ:				
	Pagurus striatus Latr.				
	Naples, Italy	7		16.0‡	
		10	Pandalus borealis	16.7‡	±8
	Eupagurus bernhardus (L.)				
	Kristineberg, Sweden	5	Homarus vulgaris	17.1‡	
		5		16.2‡(21.6)	
		5		17.4‡(22.5)	
			Mean	16.9 (22.1)	

* Thickness of column of solution 0.8 cm.
† Centrifugal force 45,000 times gravity.
‡ Solvent buffer pH 5.

TABLE V

Crustacea: Malacostraca

Pigment dissolved in plasma. Type of pigment, hemocyanin. Standard species for mixture test, *Homarus vulgaris* M. E. Solvent, 1 per cent NaCl or buffer pH 5. Thickness of column of solution 1.2 cm. Centrifugal force 67,000–150,000 times gravity.

Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
				per cent
Squillidæ:				
Squilla mantis Latr.				
Naples, Italy	10		23.7†	
	10	Homarus vulgaris	23.4†	±3
Nephropsidæ:				
Nephrops norvegicus (L.)				
Kristineberg, Sweden	8		24.4	
	8	Homarus vulgaris	24.7	±1
Homarus vulgaris M. E.				
Havstenssund, Sweden	5		24.6*	
	10		24.3†	
	10	Pandalus borealis	24.2	
	10	Palæmon fabrici	21.7	
	10	Eupagurus bernhardus	22.1†	
	6	Cancer pagurus	22.4	
	10	Carcinus mænas	22.3†	
	6	Astacus fluviatilis	23.7	
	10	Hyas araneus	22.3†	
	8	Nephrops norvegicus	24.4	
	10	Squilla mantis	22.7†	
	10	Calocaris macandreæ	22.6	
	7	Planorbis corneus	23.9	
	10	Planorbis corneus	25.3	
	10	Planorbis corneus	24.8	
	10	Sepia officinalis	23.0	
	12	Octopus vulgaris	22.0	
	5	Chiridothea entomon	22.5	
Astacus fluviatilis L.				
Dådran, Sweden	5		23.8	
	10		25.0	
	10		24.1‡	
	6	Homarus vulgaris	23.4	±1

TABLE V—*Continued*

Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
				per cent
Majidæ:				
Hyas araneus (L.)				
Kristineberg, Sweden	6		24.2†	
	5	Homarus vulgaris	22.2†	
Maja squinado Latr.				
Naples, Italy	1		26.7	
Cancridæ:				
Cancer pagurus L.				
Havstenssund, Sweden	5		23.0(15.7)	—
	5	Homarus vulgaris	22.5†	±0
Carcinus maenas (L.)				
Kristineberg, Sweden	6		24.1†(16.4)	
	5	Homarus vulgaris	22.2†	±0
Idotheidæ:				
Chiridothea entomon L.				
Härnösand, Sweden	10		23.0(16.1)	
	4	Homarus vulgaris	22.7†(16.0)	±1
		Mean	23.5(16.1)	±1

* Thickness of column of solution 1.0 cm.
† Solvent buffer pH 5.
‡ Thickness of column of solution 0.8 cm.

Arthropoda

Among the numerous species of this group possessing respiratory blood proteins there are several large forms, such as the lobster and the horseshoe crab, which yield large quantities of blood. In the case of *Balanus* and *Branchipus* we were unable to collect enough respiratory pigment for a determination of the sedimentation constant. It is doubtful whether the former has any respiratory pigment at all. Species with blood corpuscles are not to be found among the Arthropoda, all species belonging to this group having their pigment dissolved in the blood plasma. Hemocyanin is the commonest pigment; erythrocruorin is also represented; but chlorocruorin and hemerythrin do not occur here. Tables IV–VII contain the determinations of sedimentation constants.

As shown by the mixture test, five different sedimentation constants

were found within this group, viz., 17, 24, 34×10^{-13} for the crustaceans; 17, 34, 57×10^{-13} for the xiphosurans; 34×10^{-13} for the scorpionideans, and the very low constant 2.0×10^{-13} for the insects (*Chironomus* larvæ).

The crustacean hemocyanin of constant 23.5×10^{-13} gives a dissociation product of constant 16.1×10^{-13} at the alkaline border of the stability range. The natural pH of the blood is such that this com-

TABLE VI

Crustacea: Malacostraca;
Arachnomorpha: Xiphosura, Scorpionidea

Pigment dissolved in plasma. Type of pigment, hemocyanin. Solvent, 1 per cent NaCl; centrifugal force about 115,000 times gravity. Thickness of column of solution 1.2 cm.

Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
				per cent
Thalassinidæ:				
Calocaris macandreæ Bell				
Kristineberg, Sweden	4		34.0	
	4	Homarus vulgaris	33.9	
	4	Planorbis corneus	33.7	±4
Limulidæ:				
Limulus polyphemus (L.)				
Woods Hole, Mass.	5		34.1(61.2)(16.7)	
	4		33.6*(56.9)(16.3)	
Chactidæ:				
Euscorpius carpaticus (L.)				
Naples, Italy	11		36.6	
	11	Planorbis corneus	31.9	±5
		Mean	34.0	

* Solvent buffer pH 5.

ponent is almost always observed in the fresh blood. With time the pH decreases spontaneously and the dissociation goes back reversibly. The appearance of this product probably means the breaking up of the molecule into two equal parts. As shown by Table IV, the molecule of constant 16.9 is the normal one in a number of crustacean species both in the case of hemocyanin and erythrocruorin.

TABLE VII

Eutracheata: Diptera

Pigment dissolved in plasma. Type of pigment, erythrocruorin. Solvent, 1 per cent NaCl. Centrifugal force about 216,000 times gravity. Thickness of column of solution 0.8 cm.

Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
Chironomidæ:				
Chironomus plumosus (larvæ) L.				
Storvik, Sweden	20		1.8	
	20		2.1	
Chironomus sp.				
Upsala, Sweden	12		2.0	
	12		1.9	
	8		2.1	
	8		1.8*	
		Mean	2.0	

* Solvent buffer pH 8.

Limulus presents the unique example of a species the blood of which contains hemocyanin of three different sedimentation constants not only at the alkaline border but also near the isoelectric point. A detailed investigation of the *Limulus* blood by Mrs. I.-B. Eriksson-Quensel has shown that these components exist throughout the whole stability

TABLE VIII

Amphineura: Placophora

Pigment dissolved in plasma. Type of pigment, hemocyanin. Solvent, 1 per cent NaCl. Thickness of column of solution 1.2 cm. Centrifugal force about 65,000 times gravity.

Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
				per cent
Chitonidæ:				
Tonicella marmorea Fabr.				
Kristineberg, Sweden	2.0		60.4	
	2.5		62.8*	
	2.5	Lumbricus terrestris	59.4	±5
		Mean	60.9	

* Centrifugal force 32,000 times gravity.

range. At the acid border the two highest components disappear, giving rise to a new component of constant 23×10^{-13}. According to determinations by K. O. Pedersen, all the components have the same isoelectric point.

Mollusca

From some of the big forms of this group it is easy to draw large amounts of blood in a very pure state. Thus a single individual of the tropical snail *Achatina fulva* yields 50 cc. of blood and a medium-sized

TABLE IX

Conchifera: Gastropoda

Pigment dissolved in plasma. Type of pigment, erythrocruorin. Solvent, 1 per cent NaCl; thickness of column of solution 1.2 cm. Standard species for mixture test, *Planorbis corneus* (L.); centrifugal force 65,000–115,000 times gravity.

Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
Limnæidæ:				
Planorbis corneus (L.)				
Upsala, Sweden	10		35.1* †	
	10		31.4*	
	15		33.8	
	10	Nereis virens	33.6	
	10	Athelges sp.	34.0	
	12	Homarus vulgaris	32.2	
	15	Homarus vulgaris	34.2	
	15	Homarus vulgaris	32.4	
	15	Calocaris macandreæ	32.2	
	15	Euscorpius carpathicus	30.5	
	12	Helix pomatia	33.7†	
	15	Helix pomatia	35.7†	
	15	Octopus vulgaris	33.9	
	20	Planorbis umbilicatus	32.2	
Planorbis umbilicatus (Müll.)				
Upsala, Sweden	10		36.7*	
	10		36.9*	
	10		37.2*	
	10		32.9*	
		Mean	33.8	

* Thickness of column of solution 0.8 cm.

† Centrifugal force about 41,000 times gravity.

Octopus or *Eledone* gives as much. On the other hand, there are small forms from which we were unable to get pure blood. This was the case with *Dentalium entale*. Other, somewhat larger, forms such as *Æolis*

TABLE X

Conchifera: Gastropoda

Pigment dissolved in plasma. Type of pigment, hemocyanin. Solvent 1 per cent NaCl; thickness of column of solution 1.2 cm. Standard species for mixture test, *Helix pomatia* L.; centrifugal force 22,000–60,000 times gravity.

Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
				per cent
Paludinidæ:				
Paludina vivipara (L.)				
Upsala, Sweden	10		93.0	
	20		99.2*	
	undil.		97.4‡	
Paludina contecta, Millet				
Germany	4		99.6†	
	4	Helix pomatia	100.3	±2
Littorinidæ:				
Littorina littorea (L.)				
Kristineberg, Sweden	10		99.1*(137.0)	
	2		97.3*(126.8)	
	4	Helix pomatia	96.3	±1
Fasciolaridæ:				
Neptunea antiqua (L.)				
Kristineberg, Sweden	15		107.3†	
	20	Helix pomatia	107.7†	±8
Buccinidæ:				
Buccinum undatum L.				
Kristineberg, Sweden	8		101.4*(131.6)	
	4	Helix pomatia	101.9	±1
Turbinellidæ:				
Busycon canaliculatum (L.)				
Woods Hole, Mass.	20		101.3(138.8)	
	20	Helix pomatia	96.9	±0

TABLE X—*Continued*

Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
				per cent
Limnæidæ:				
Limnæa stagnalis (L.)				
Upsala, Sweden	undil.		94.4	
	"		104.1(60.2)	
	"		96.8	
	"	Helix pomatia	96.6	±3
Pupillidæ:				
Achatina fulva Brug.				
Africa	10		95.7(63.1, 15.1)	
	10	Helix pomatia	107.4(64.3, 17.3)	±1
Helicidæ:				
Helix pomatia L.				
Upsala, Sweden	12	Paludina contecta	102.3	
	9	Littorina littorea	95.4	
	12	Neptunea antiqua	99.5	
	9	Buccinum undatum	100.8	
	20	Busycon canaliculatum	96.6	
	15	Limnæa stagnalis	99.2	
	9	Achatina fulva	105.7	
	12	Helix arbustorum	98.0†	
	12	Helix nemoralis	101.3†	
	10	Helix hortensis	101.3*	
	12	Limax maximus	110.0	
	10	Arion ater	99.9†	
	12	Nereis virens	102.7	
	12	Planorbis corneus	99.4	
	10	Planorbis corneus	103.2	
	12	Sepia officinalis	103.2	
	12	Octopus vulgaris	99.5	
	15	Octopus vulgaris	104.1	
	10	Octopus vulgaris	101.4	

Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
				per cent
Helix arbustorum L.				
Upsala, Sweden	5		95.3*	
	5		94.3*	
	12	Helix pomatia	96.3†	±2
Helix nemoralis (Müll.)				
Upsala, Sweden	8		101.7*	
	7		100.2*	
	6		99.5*	
	6		95.2	
	12	Helix pomatia	100.8†	±0
Helix hortensis (Müll.)				
Upsala, Sweden	10		99.2*	
	10		98.5*	
	10	Helix pomatia	101.1*	±0
Limacidæ:				
Agriolimax agrestis (L.)				
Upsala, Sweden	10		95.9*	
	10		104.2	
Limax maximus L.				
Länna, Sweden	12		97.0†	
	12	Helix pomatia	111.8†	±2
Arion ater L.				
Länna, Sweden	10		93.7†(59.3)	
	9		110.5(63.2)	
	9		91.4(60.1)	
	10	Helix pomatia	99.9(61.1)†	
	10	Lumbricus terrestris	(59.2)†	±3
Arion subfuscus Drap.				
Länna, Sweden	5		(64.3)	
		Mean	100.1 (16.2)	±1.9
			(61.9)	
			(133.6)	

* Thickness of column of solution 0.8 cm.
† Solvent pH 5.
‡ Visible light, K3-filter, maximum of light absorption in the visible spectrum, 600 mμ.

papillosa, Dendronotus arborescens and *Patella vulgata* gave enough blood but did not show any of the absorption bands characteristic of the respiratory proteins.

According to some general remarks in the text-books of physiology (Winterstein, 1925), the blood of the lamellibranchians should, as a rule, contain hemocyanin. We have examined the following species, some of which are fairly large and easy to bleed, viz., *Unio pictorum, Anodonta cygnea, Dreissensia polymorpha, Cyprina islandica, Lævicardium norvegicum, Dosinia exoleta, Tellina crassa, Solen ensis, Solen siliqua, Mya arenaria, Ostrea edulis, Mytilus edulis, Volsella modiolus, Pecten opercularis.* In none of them were we able to detect the presence of any respiratory protein. The blood of the mussels has often a blueish opalescence accompanied by a slight light absorption which might have misled the earlier observers, but it is caused by the presence of coarse

TABLE XI

Conchifera: Lamellibranchiata

Pigment enclosed in corpuscles. Type of pigment, erythrocruorin. Solvent, 1 per cent NaCl; centrifugal force about 260,000 times gravity.

Origin of blood	Dilution of blood	Thickness of column of solution	$s_{20°} \times 10^{13}$
		cm.	
Arcidæ:			
Arca pexata Say			
Woods Hole, Mass.	5	0.2	3.40
	6	0.4	3.39
	6	0.4	4.09
	6	0.6	3.24*
	20	1.2	3.20
		Mean	3.46

* Solvent buffer pH 6.8; determined by Mrs. I.-B. Eriksson-Quensel.

suspended particles of unequal size settling rapidly when run in the ultracentrifuge and not by a blood pigment. A few lamellibranchians possess red corpuscles. We have had the opportunity to study only one species of this type, the blood-clam *Arca pexata.* All the other Mollusca have their respiratory protein dissolved in the blood plasma. Hemocyanin is the most common pigment but erythrocruorin occurs in a few species. Chlorocruorin and hemerythrin have not been found in this group. Tables VIII–XIII contain the determinations of sedimentation constants.

TABLE XII

Conchifera: Cephalopoda: Decapoda

Pigment dissolved in plasma. Type of pigment, hemocyanin. Solvent, 1 per cent NaCl. Thickness of column of solution 1.2 cm. Standard species for mixture test, *Sepia officinalis* L. Centrifugal force about 75,000 times gravity.

Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
				per cent
Loliginidæ:				
Loligo vulgaris Lam.				
Naples, Italy.....	10		56.4	
	10	Sepia officinalis	55.1	±1
	15	Sepia officinalis	55.6	±1
Sepiolidæ:				
Sepiola oweniana d'Orb.				
Kristineberg, Sweden	12		56.3	
	12		56.4	
	20	Sepia officinalis	55.4‡	±0
Rossia oweni Ball				
Kristineberg, Sweden	10		54.6	
	15		57.4	
	15	Sepia officinalis	55.7‡	±1
	12	Octopus vulgaris	55.1†	
Sepiidæ:				
Sepia officinalis L.				
Naples, Italy.....	20		54.8*	
	25		57.1	
	20		55.9§	
	20		56.5	
	20		55.2†	
	20	Loligo vulgaris	55.4	
	20	Loligo vulgaris	55.0	
	20	Sepiola oweniana	55.2‡	
	20	Rossia oweni	55.4‡	
	20	Nereis virens	60.4‖	
	20	Lumbricus terrestris	58.4	
	20	Homarus vulgaris	56.1	
	20	Helix pomatia	60.1‖	
	20	Octopus vulgaris	55.6	
		Mean	56.2	±0.8

* Solvent pH 5.0.
† Solvent pH 6.8.
‡ Solvent pH 8.0.
§ Centrifugal force 115,000 times gravity.
‖ Centrifugal force 45,000 times gravity.

TABLE XIII

Conchifera: Cephalopoda: Octopoda

Pigment dissolved in plasma. Type of pigment, hemocyanin. Solvent, 1 per cent NaCl; thickness of column of solution 1.2 cm. Standard species for mixture test, *Octopus vulgaris* Lam. Centrifugal force 65,000 times gravity.

Origin of blood	Dilution of blood	Mixed with blood of	$s_{20°} \times 10^{13}$	Mixture test deviation from standard
Octopodidæ:				*per cent*
Octopus vulgaris Lam.				
Naples, Italy.....	20		50.6	
	20		49.5	
	15		48.4	
	15	Rossia oweni	48.7*	
	20	Sepia officinalis	49.7	
	20	Eledone cirrosa	50.0	
	17	Homarus vulgaris	50.5	
	15	Planorbis corneus	51.0	
	20	Helix pomatia	53.4†	
	15	Helix pomatia	54.5†	
Eledone moschata (Lam.)				
Naples, Italy.....	10		48.9*	
Eledone cirrosa (Lam.)				
Kristineberg, Sweden..	20		46.4	
	20	Octopus vulgaris	49.8	±0
		Mean	50.1	

* Solvent buffer pH 6.8.

† Centrifugal force 35,000 times gravity.

Six different sedimentation constants were found. The hemocyanin of the gastropods has the value 100.1×10^{-13}; the erythrocruorin of the same class 33.8×10^{-13}; the hemocyanin of the Amphineura, the Decapoda and the Octopoda 60.9, 56.2 and 50.1×10^{-13} respectively, and the erythrocruorin of the lamellibranchians 3.46×10^{-13}. The constants 133.6, 61.9 and 16.2×10^{-13} were observed for association and dissociation states of the gastropod hemocyanin. At the natural pH of the blood of the gastropods the dissociation product of constant 61.9, which is probably one-half the normal molecule, almost always appears. It is sometimes accompanied by the component of constant 16.2 (one-eighth of the normal molecule) and very seldom by the association product of constant 133.6. This latter molecule seems to be limited to the marine gastropods. It has been observed in the blood of *Littorina*, *Buccinum* and *Busycon*.

TABLE XIV

Eleutherozoa: Holothurioidea

Pigment enclosed in corpuscles. Type of pigment, erythrocruorin. Solvent, 1 per cent NaCl; thickness of column of solution 0.4 cm. Centrifugal force 250,000 times gravity.

Origin of blood	Dilution of blood	$s_{20°} \times 10^{13}$
Cucumariidæ:		
Thyone briareus Les.		
Woods Hole, Mass.	6	2.60
	6	2.10
	6	2.95
	9	2.64
		Mean 2.57

Echinoderma

Only one representative of this group possessing respiratory blood pigment has been studied, viz., the sea cucumber, *Thyone briareus*. The protein, which is of the erythrocruorin type, is contained in blood corpuscles. The sedimentation constant is 2.57×10^{-13}. Table XIV gives the determinations.

Tunicata

No respiratory protein is recorded from these animals. We have examined the blood of *Ciona intestinalis* in the ultracentrifuge. It did not show any light absorption due to respiratory proteins.

TABLE XV

Cyclostomata: Hyperoartia, Hyperotreta

Pigment enclosed in corpuscles. Type of pigment, erythrocruorin. Solvent, 1 per cent NaCl + CO. Centrifugal force 225,000–305,000 times gravity.

Origin of blood	Dilution of blood	Thickness of column of solution	$s_{20°} \times 10^{13}$
		cm.	
Petromyzontidæ:			
Lampetra fluviatilis (L.)			
Älvkarleby, Sweden	9	0.4	1.98
	7	0.3	2.09
Myxinidæ:			
Myxine glutinosa L.			
Kristineberg, Sweden	6	0.2	2.33
	6	0.4	2.20
			Mean 2.15

TABLE XVI

Pisces: Selachii, Dipnoi, Teleostei

Pigment enclosed in corpuscles. Type of pigment, hemoglobin. Solvent, 1 per cent NaCl + CO. Centrifugal force 225,000–285,000 times gravity.

Origin of blood	Dilution of blood	Thickness of column of solution	$s_{20°} \times 10^{13}$
		cm.	
Rajidæ:			
Raja clavata L.			
Kristineberg, Sweden	—	0.6	4.21
	—	0.4	4.34
Lepidosirenidæ:			
Protopterus annectens Owen			
Senegal	2	0.3	4.52
	4	1.2	4.15
Salmonidæ:			
Salmo irideus Gibb.			
Harbo fish-culture, Sweden	4	0.4	4.14
	4	0.4	4.11
Cyprinidæ:			
Cyprinus carassius			
Åkerlänna, Sweden	5	0.4	4.38
	10	1.2	4.25
	6	0.3	4.63*
Anguillidæ:			
Anguilla anguilla (L.)			
Älvkarleby, Sweden	10	0.4	4.21
	10	0.4	4.04
Esocidæ:			
Esox lucius L.			
Upsala, Sweden	—	1.2	4.40
	—	1.2	4.05
Gasterosteidæ:			
Gasterosteus pungitius L.			
Upsala, Sweden	10	1.2	4.45
Percidæ:			
Lucioperca sandra Cuv.			
Upsala, Sweden	—	1.2	4.48
Tautoga onitis (L.)			
Woods Hole, Mass.	10	0.4	4.12
	10	0.4	4.27

TABLE XVI—*Continued*

Origin of blood	Dilution of blood	Thickness of column of solution	$s_{20}° \times 10^{13}$
		cm.	
Pleuronectidæ:			
Pleuronectes platessa L.			
Kristineberg, Sweden	—	0.4	4.26
Triglidæ:			
Prionotus carolinus (L.)			
Woods Hole, Mass.	10	0.4	4.24
	10	0.4	4.49
Blennidæ:			
Opsanus tau (L.)			
Woods Hole, Mass.	10	0.4	4.21
	10	0.4	4.46
			Mean 4.29

* Frozen for 41 days.

Acrania

A representative of this group, viz., *Branchiostoma lanceolatum,* was tried. We did not find it possible to draw enough blood from it for an ultracentrifugal test.

Vertebrata

The above measurements show that nowhere among the invertebrates has the respiratory protein hemoglobin characterized by the sedimentation constant 4.4×10^{-13} and the active hemin group been met with. In view of this fact it became of great importance to study the red blood pigment of the various classes of the vertebrates in order to find out whether hemoglobin is the only respiratory blood protein of the higher animals. Tables XV–XX contain the determinations of the sedimentation constants.

The blood pigment from the lowest class of the vertebrates, the Cyclostomata, has a sedimentation constant of 2.15×10^{-13} or less than half that of hemoglobin. Sedimentation equilibrium measurement by Mrs. I.-B. Eriksson-Quensel indicates that this pigment which we have classed among the erythrocruorins consists of a mixture of molecules of weight 17,500 and 34,500.

The mean values of the constants for Mammalia, Aves, and Pisces, viz., 4.29, 4.31, and 4.29×10^{-13}, are identical within the limits of error. The pigments from the reptiles and the amphibians very often show a second component of constant 7.14×10^{-13} probably corresponding to a double molecule. This association product seems to increase

TABLE XVII

Amphibia: Urodela, Anura

Pigment enclosed in corpuscles. Type of pigment, hemoglobin. Solvent, 1 per cent NaCl + CO; centrifugal force 195,000–290,000 times gravity.

Origin of blood	Dilution of blood	Thickness of column of solution	$s_{20°} \times 10^{13}$
		cm.	
Salamandridæ:			
Salamandra maculosa Laur.			
Germany	—	1.2	4.89(7.66)
	—	1.2	4.96(6.24)
	10	0.6	4.60*
	—	0.6	4.83*
Bufonidæ:			
Bufo viridis Laur.			
Germany	—	1.2	4.72(7.54)
	—	1.2	4.83(7.27)
Bufo valliceps Wiegm.			
Honduras	—	0.6	4.82†
	—	0.6	4.73*(7.73, 12.53)
Ranidæ:			
Rana temporaria L.			
Upsala, Sweden	—	1.2	4.28
	—	1.2	4.62
			4.73(7.29) Mean

* Centrifugal force 365,000 times gravity.
† Second computation incalculable.

on standing. Sometimes a third component about 12×10^{-13} (probably a triple molecule) was observed in old samples of reptile blood. The constant of the normal component is 4.71×10^{-13} or slightly higher than that for the mammals, birds, and fishes.

The difference is believed to be real. Tests carried out at high speed (72,000 r.p.m. corresponding to a centrifugal force 380,000 times gravity) have not given any indication of a composite character of the 4.7 component. Sedimentation equilibrium measurements will be required to decide whether this deviation is due to a real difference in molecular weight or merely to a slightly different shape of the hemoglobin molecule of the reptiles and amphibians. Some recent determinations of sedimentation constants and molecular weights carried out by K. O. Pedersen on horse blood hemoglobin have shown that this molecule tends to dissociation at high dilutions. We have observed such a phenomenon even more strikingly in one case of cat's blood hemoglobin. Here dissociation into half molecules took place even at moderate dilutions.

TABLE XVIII

Reptilia: Testudinata, Squamata

Pigment enclosed in corpuscles. Type of pigment, hemoglobin. Solvent, 1 per cent NaCl + CO; centrifugal force 220,000–290,000 times gravity.

Origin of blood	Dilution of blood	Thickness of column of solution	$s_{20°} \times 10^{13}$
		cm.	
Testudinidæ:			
Chrysemys picta (Schneid.)			
N. America	—	0.6	4.47(6.97)
	—	0.6	4.38(6.67)
Anguidæ:			
Anguis fragilis L.			
Germany	—	0.3	4.96
	30	0.6	4.47*
	30	0.6	5.00*
Lacertidæ:			
Lacerta vivipara Jacq.			
Germany	—	0.6	4.20(6.96)
	—	0.6	4.90(7.07)
	10	0.6	4.67(7.30)
	20	0.6	4.50†
Chamæleontidæ:			
Chamæleon chamæleon (L.)			
S. Europe	—	0.4	4.57
Colubridæ:			
Coluber longissimus (Laur.)			
S. Europe	5	0.3	4.67
	5	0.3	4.75
	10	0.6	5.04
	20	0.6	4.72
	20	0.6	4.80
	20	0.6	4.66*
	60	2.2	4.98
			Mean 4.69(6.99)

* Centrifugal force 380,000 times gravity.
† Second computation not calculable.

SEDIMENTATION RATIOS

In order to check the above determinations and bind together the higher constants into a system of relative values, a number of ultracentrifugal runs were carried out on mixtures containing two blood pigments of different sedimentation constants. Such relative measurements are possible only in the case of rapid sedimentation where the

TABLE XIX

Aves: Gallinacei, Columbæ, Lamellirostres, Striges, Pici, Passeres

Pigment enclosed in corpuscles. Type of pigment, hemoglobin. Solvent, 1 per cent NaCl + CO. Centrifugal force, 230,000–380,000 times gravity.

Origin of blood	Dilution of blood	Thickness of column of solution	$s_{20°} \times 10^{13}$
		cm.	
Phasianidæ:			
Gallus gallus L.			
Upsala, Sweden	30	0.3	4.13
	30	0.3	4.31
Columbidæ:			
Columba livia L. v. domestica			
Upsala, Sweden	—	1.2	4.46
	—	1.2	4.33
Anatidæ:			
Anas platyrhyncha L. v. domestica			
Upsala, Sweden	40	0.6	4.41
	40	1.2	4.34
Strigidæ:			
Syrnium aluco L.			
Upsala, Sweden	—	0.6	4.28
	—	0.6	4.39
Picidæ:			
Picus viridis L.			
Upsala, Sweden	40	0.4	4.37
	80	1.2	4.23
Paridæ:			
Parus major L.			
Upsala, Sweden	30	1.2	4.35
	15	0.6	4.21
Corvidæ:			
Corvus cornix L.			
Upsala, Sweden	20	0.6	4.45
	44	1.2	4.25
			Mean 4.32

diffusion is low and the boundaries therefore fairly sharp. This condition is not fulfilled for the pigments contained in corpuscles. The individual values obtained from these runs have already been used. In Table XXI we have recorded the observed sedimentation ratios as well as the ratios calculated from the mean values of the different types of the sedimentation constant.

TABLE XX

Mammalia: Insectivora, Rodentia, Carnivora, Ungulata, Primates

Pigment enclosed in corpuscles. Type of pigment, hemoglobin. Solvent, 1 per cent NaCl + CO. Centrifugal force 210,000–330,000 times gravity.

Origin of blood	Dilution of blood	Thickness of column of solution	$s_{20°} \times 10^{13}$
		cm.	
Erinaceidæ:			
Erinaceus europæus L.			
Upsala, Sweden	20	0.3	4.42
	20	0.3	4.57
Leporidæ:			
Oryctolagus cuniculus L.			
Upsala, Sweden	20	0.3	4.27
	20	0.3	4.44
Felidæ:			
Felis domestica Schreber			
Upsala, Sweden	30	0.3	4.26
	30	0.3	4.17
Canidæ:			
Canis familiaris L.			
Upsala, Sweden	25	0.3	4.29
	60	0.6	4.11
Equidæ:			
Equus caballus L.			
Upsala, Sweden		0.6	4.40
			4.42*
Bovidæ:			
Bos taurus L.			
Upsala, Sweden	30	0.3	4.18
	30	0.3	4.36
Cercopithecidæ:			
Cercopithecus sp.			
W. Africa	40	0.6	3.94
	20	0.3	3.86
	27	0.3	4.22
Hominidæ:			
Homo sapiens			
	—	0.6	4.54
	20	0.3	4.42
			Mean 4.29

* Mean of four determinations by K. O. Pedersen.

The observed and calculated ratios agree within the limits of experimental error. From the observed ratios and the sum of all the absolute values of sedimentation constants a set of relative values may be calculated. In Table XXII these constants together with the absolute values found by us and those determined by other investigators are given.

TABLE XXI

Observed and calculated sedimentation ratios for respiratory pigments dissolved in the blood of the invertebrates, and showing two boundaries when subjected to the mixture test.

Species	Sedimentation Ratio		Deviation from calc. ratio
	Observed	Calculated	
			per cent
Helix pomatia/Nereis virens	1.72	1.75	−1.7
Helix pomatia/Sepia officinalis	1.72	1.75	−1.7
Helix pomatia/Octopus vulgaris	1.86	1.99	−7.0
Helix pomatia/Planorbis corneus	2.92	2.95	−1.0
Lumbricus terrestris/Nereis virens	1.08	1.08	±0
Lumbricus terrestris/Arenicola marina	1.07*	1.08	−0.9
Nereis virens/Planorbis corneus	1.67	1.68	−0.6
Sepia officinalis/Octopus vulgaris	1.12	1.14	−1.8
Sepia officinalis/Homarus vulgaris	2.44	2.43	+0.4
Rossia oweni/Octopus vulgaris	1.13	1.14	−0.9
Octopus vulgaris/Planorbis corneus	1.51	1.48	+2.0
Octopus vulgaris/Homarus vulgaris	2.29	2.13	+7.5
Planorbis corneus/Homarus vulgaris	1.33	1.44	−8.3
Calocaris macandreæ/Homarus vulgaris	1.50	1.44	+4.2
Homarus vulgaris/Pandalus borealis	1.41	1.39	+1.4
Homarus vulgaris/Palæmon fabrici	1.39	1.39	±0
Homarus vulgaris/Eupagurus bernhardus	1.29	1.39	−7.8
			mean dev. −0.95

* Determined by Inga-Britta Eriksson-Quensel.

DISCUSSION OF RESULTS

One of the most striking points borne out by the above investigation is, perhaps, the fact that low sedimentation constants were always found for the pigments enclosed in red blood corpuscles. Only four different values were observed, viz., 2.1, 2.6, 3.5 and 4.4×10^{-13}, probably corresponding to molecules of mass $1/2 \times 34{,}500$, $1 \times 34{,}500$, a mixture of

TABLE XXII

Absolute and relative values of the seven highest sedimentation constants of blood pigments.

Standard species	$s_{20°} \times 10^{13}$				
	Mean absolute value				Relative value
	Present invest.	No. determinations	Other invest.	No. determinations	
Helix pomatia	100.1	58	100.1	39*	98.3
Lumbricus terrestris	61.7	13	60.9	14†	61.8
Nereis virens	57.0	61	57.4	15†	57.1
Octopus vulgaris	50.1	13	51.7	29†	51.4
Planorbis corneus	33.9	25	33.7	11†	33.9
Homarus vulgaris	23.5	35	23.1	11†	23.6
Pandalus borealis	16.9	19	16.1	18†	17.1

* Determinations by Inga-Britta Eriksson-Quensel and K. O. Pedersen.
† Determinations by Inga-Britta Eriksson-Quensel.

both, and $2 \times 34{,}500$ containing 1, 2, and 4 atoms of iron respectively. The first three values are represented in the invertebrates and in the lowest class of the vertebrates, the Cyclostomata, while the last value is found exclusively in the higher classes of the vertebrates. Among the amphibians and reptiles a higher component of constant 7.1×10^{-13}, probably corresponding to a molecule built up of two normal hemoglobin molecules, was sometimes observed. The reddish-brown corpuscles found in the cœlomic fluid of the sipunculoid worms contain an entirely different protein, the hemerythrin, which has, however, a sedimentation constant of the same order of magnitude as the hemoglobin of the higher vertebrates. From the standpoint of blood kinship it would seem reasonable to assume that the vertebrates have developed from some invertebrate group possessing blood corpuscles and characterized by a red respiratory pigment of the same sedimentation constant and molecular weight. As a matter of fact, the lowest class of the vertebrates, the Cyclostomata, possesses a respiratory blood protein which with regard to sedimentation constant resembles closely the pigment contained in the erythrocytes of the capitellide worms. It is of interest to note that H. E. Jordan (1933) arrives at a similar conclusion from the consideration of histological data.

The blood pigments which occur dissolved in the plasma are as a rule characterized by high sedimentation constants and consequently by high molecular weights. The only exception so far observed is the red pigment of the *Chironomus* larvæ, which has a constant identical with that

of the red pigment contained in the erythrocytes of the capitellide worms. It might seem tempting to put forward the hypothesis that the giant pigment molecules dissolved in the blood plasma play, to a certain extent at least, the rôle of blood corpuscles. On the other hand, it must be borne in mind that even the largest of these molecules, that of the Gastropoda hemocyanin, has a diameter of only 1/200 of that of a red blood corpuscle.

The remarkable constancy of the mass and shape properties of the molecules of the respiratory proteins within closed animal groups, as expressed by the identity of the sedimentation constants, is probably a measure of the similarity of the chemical processes which lead to the formation of these pigments. To a certain extent, therefore, identity of sedimentation constant may be taken as a criterion of biological kinship. From this point of view it is of interest to note that the hemocyanins of the very old arthropod genera *Limulus* and *Euscorpius* have the same sedimentation constant, viz., 34×10^{-13}. Only two other genera, the crustacean *Calocaris,* the blood of which contains hemocyanin, and the red-blooded snail *Planorbis* have the same constant.

One must, however, be very careful when trying to trace relationships between the various classes of animals from the properties of their blood pigments. The above investigation has shown that the number of different sedimentation constants is very restricted (so far only 14 have been observed) and that the same constant occurs in respiratory proteins containing different active groups. It is therefore not unprobable that the same constant may sometimes be represented in animal classes which could hardly be looked upon as nearly related. As an example of this kind, one might mention the erythrocruorin of *Lumbricus* and the hemocyanin of *Tonicella,* which have the same sedimentation constant, viz., 61×10^{-13}.

The observations about the behaviour of the blood pigments near the borders of the pH stability regions as well as the systematic study of the entire stability ranges and the determination of their molecular weights from sedimentation equilibrium measurements of some of these proteins, now being carried out by Mrs. I.-B. Eriksson-Quensel, seem to indicate that the blood protein molecules of high sedimentation constants break up into definite units at certain critical pH values and that these dissociation products are identical with the blood proteins of lower molecular weight as far as the mass and shape properties go. It seems, therefore, that only a few molecular masses are stable and that it would depend upon the composition of the molecule with regard to the various amino acids or other constituents and upon the actual pH of the solution whether one or the other of the different possibilities is realized.

A detailed investigation of the isoelectric points of the respiratory proteins by Dr. K. O. Pedersen (1933) of this laboratory has shown, however, that, as a rule, each species is characterized by a special value of the isoelectric point of its blood pigment. The chemical composition of the respiratory protein therefore varies from one species to the other even in cases where the sedimentation constants are identical. No relationship has so far been found between the position of the isoelectric point and the mass of the molecule. At the present time we are not able to predict what sedimentation constant a respiratory protein of known chemical composition would have, but it is believed that this will become possible with increasing knowledge of the constitution of the protein molecule.

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Summary

1. A systematic study of the sedimentation constants of the respiratory blood proteins throughout the animal kingdom has been carried out by means of the ultracentrifugal method.

2. Respiratory proteins enclosed in corpuscles have low sedimentation constants. The following values were found for erythrocruorin: capitellide worms and Cyclostomata, 2.1×10^{-13}; glyceride worms and lamellibranchians, 3.5×10^{-13}; holothurians, 2.6×10^{-13}. Hemoglobin characterized by the sedimentation constant 4.4×10^{-13} occurs only in

the five higher classes of the vertebrates, viz., Mammalia, Aves, Reptilia, Amphibia, Pisces. Among the reptiles and amphibians an association product of hemoglobin with sedimentation constant 7.1×10^{-13}, probably representing a doublet, was often found. This product was never observed in the other classes of the vertebrates.

3. Respiratory proteins dissolved in the plasma have, as a rule, high sedimentation constants. The only exception is the erythrocruorin of the *Chironomus* larvæ, which has the constant 2.0×10^{-13}. All polychæte worms and hirudineans with dissolved pigment have the constant 57.5×10^{-13} (erythrocruorin and chlorocruorin). A dissociation product of constant 11.7×10^{-13} (probably 1/16 of the normal molecule) is sometimes found. The oligochæte worms have a variety of erythrocruorin of slightly higher constant, 61.9×10^{-13}. The crustaceans show as a rule two sedimentation constants, 16.9 and 23.5×10^{-13}. The former one, which occurs both in erythrocruorins and hemocyanins, probably represents a molecular weight of one-half of the latter. Species characterized by the latter constant have hemocyanin and give a mixture of both constants in alkaline solution, thus demonstrating the dissociation into half molecules. One crustacean species (*Calocaris macandreae*) has a hemocyanin constant of 34.0×10^{-13}. This sedimentation constant is also characteristic of the hemocyanin of the scorpion (*Euscorpius carpaticus*). The xiphosuran (*Limulus polyphemus*) has a hemocyanin of the same constant, but its blood contains two more hemocyanin varieties of constants 16.5 and 59.1×10^{-13} and a dissociation product of constant 6×10^{-13}. The erythrocruorin of the gastropods has the same constant, 33.8×10^{-13}. The normal hemocyanin of the gastropods, on the other hand, is characterized by the constant 100.1×10^{-13}. It forms an association product of constant 133.6 $\times 10^{-13}$ and two dissociation products of constants 16.2 and 61.9×10^{-13} (probably 1/2 and 1/8 of the normal molecule respectively). The constant 60.9×10^{-13} is also found in the amphineuran hemocyanin (*Tonicella marmorea*). The cephalopods show two constants, 56.2 $\times 10^{-13}$ for the decapods and 50.1×10^{-13} for the octopods. The latter constant has not been observed in any other place and is the only sedimentation exclusively characteristic of an animal group.

4. The significance of the data collected in the present investigation cannot be fully understood until a sufficient number of pH-stability curves and sedimentation equilibrium measurements have been made on respiratory proteins. The few determinations of this kind so far available, however, seem to indicate that all native proteins form a closed system in which only a very limited number of mass and shape types

are stable. Reversible association and dissociation reactions take place easily when the pH of the solution is slightly changed. Within a well-defined animal group all species have as a rule respiratory pigments of the same sedimentation constant (or constants) and dissociate in a similar way. Biological kinship, therefore, is usually accompanied by identity in the sedimentation constants. On the other hand, owing to the small number of different constants possible, the same constant must of necessity occur in different animal groups.

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