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THE COLLOID OSMOTIC PRESSURES OF INVERTEBRATE BODY FLUIDS

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SUMMARY

Colloid osmotic pressures of the body fluids of twenty invertebrate species were measured directly. The results, which are generally lower than predicted values for the same species, pertain to several physiological questions: (1) they do not quantitatively explain the frequently observed hyperosmoticity of body fluids in species believed to be osmoconformers, indicating that the condition cannot be merely a consequence of a Gibbs-Donnan equilibrium; (2) the excess of hydrostatic over colloid osmotic pressure is very small. This result supports the hypothesis that the oxygen transport function of bloods with extracellular haemocyanins and haem proteins is limited by their colligative properties; (3) the pressure relationships and the absence of colloid osmotic activity in urine indicates that filtration contributes to urine formation in several species.

INTRODUCTION

Dissolved macromolecules in animal body fluids may modify the balance of water and solutes across several exchange sites. Many marine and estuarine invertebrates which are categorized as osmoconformers are not, in fact, isosmotic with the ambient medium. Instead, as emphasized by Pierce (1970) and Oglesby (1973), these animals are consistently hyperosmotic by an increment of 5–30 mOsm, regardless of the external osmotic concentration. Pierce (1970) pointed out that the presence of impermeable charged proteins in body fluids, separated by biological membranes from freely permeable ions in seawater, must result in a hyperosmotic condition inside the animal. He suggested that the observed hyperosmoticity is a passive consequence of a Gibbs-Donnan equilibrium. It is not clear, however, that the observed increment of 5–30 mOsm can be fully explained by the osmotic pressure of proteins in body fluids.

In addition to the total flux of water and ions between the animal and its environment, colloid osmotic pressures may influence the relative volumes of separate fluid compartments within the animal. The relationship between forces determining fluid balance within animals, commonly known as the Starling hypothesis, was formulated in the context of the vertebrate circulatory systems. Invertebrate circulatory systems are so different in design that the principles of fluid balance may be poorly expressed by the Starling hypothesis. Members of many phyla have a single internal fluid

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compartment consisting largely of open sinuses that are continuous with the intercellular spaces. The critical relationships may be those between extracellular and intracellular fluids rather than vascular and extravascular fluids. Others, such as the annelids, have two separate compartments of circulating body fluid, but the volumetric ratio between the two is quite different from that in vertebrates. The volume of blood, which must have higher hydrostatic and colloid osmotic pressures, may be only one-tenth that of coelomic fluid, which penetrates into the interstitial spaces (Oglesby, 1969; Mangum *et al.* 1975). Therefore, the likelihood of reduced tissue hydration by fluid movement into blood is remote.

Finally, the respiratory function of body fluids in many invertebrates may be affected by the presence of oxygen-carrying proteins outside of cells, where they contribute to the colloid osmotic pressure of the fluid. The extracellular disposition of these molecules requires a balance between the oxygen-carrying capacity of the fluid and the possible disruption of internal fluid balance. Thus *in vivo* concentrations of haemocyanins and extracellular haem proteins must represent an adjustment between the advantages of a high oxygen-carrying capacity and the work of maintaining an excess of hydrostatic over colloid osmotic pressure, if filtration from the blood is to occur.

The importance of colloids in the fluid balance of invertebrate animals has not been directly investigated. Redfield (1933) suggested that the extracellular respiratory pigments may serve to maintain the colloid osmotic pressure of invertebrate body fluids and that non-respiratory proteins do not occur in sufficient quantities to make an appreciable contribution. More recent measurements indicate great variability in protein concentration, even within an individual. The concentration of haemocyanin in the spider crab *Maia squinado*, for example, varies during the moult cycle by two orders of magnitude (Zuckerkandl, 1960). Such changes may be accompanied by fluctuations in blood pressure as well, but no information on the subject is available.

The colloid osmotic pressure of invertebrate body fluids has often been estimated but, to our knowledge, it has never been measured. The recent development of osmometers (Prather, Brown & Zweifach, 1972) suitable for use with small fluid volumes ($< 50\ \mu l$) permits direct determinations of this important parameter.

MATERIALS AND METHODS

With two exceptions the animals studied were collected in East Jutland. The horseshoe crab *Limulus polyphemus* was shipped to Denmark from Woods Hole, Massachusetts, U.S.A., and the terrestrial decapod *Deckenia imitatrix* was brought from Kenya. Marine species were maintained in a recirculating system of natural seawater at 18–21 ‰ salinity and 6 °C. Freshwater species were maintained in aerated pond water at 19–21 °C, and the land crab in moist sand at 23–26 °C.

Measurements of colloid osmotic pressure

Most of the fluid samples were withdrawn into 1 ml syringes following direct needle puncture. A 50 μl syringe was used to collect from the nephridial sac of dissected *Arenicola marina*, and the urine of *Limulus polyphemus* was obtained by cannulating the excretory opening with polyethylene tubing. Unless the sample

contained cellular debris, in which case they were first centrifuged, they were applied directly to the osmometer.

The osmometer, described in detail by Prather *et al.* (1972), consists of an Amicon UM-10 membrane which separates a sample chamber from a small volume of saline (Ringer's for the arthropods and the freshwater molluscs; seawater for the remaining species). It was attached directly to a Statham P23Db pressure transducer, whose signal was amplified by a Brush amplifier and a Radiometer recorder (REA 310). The temperature (15 °C) in a water jacket enclosing the apparatus was contolled thermostatically with a recirculating water bath.

The osmometer was calibrated empirically. The measurements were calibrated to zero at the beginning and at the end of a set of determinations. If the zero line was not repeatable, the data were discarded. Each set of measurements was calibrated with a measured column of saline (10 cm). The linearity of the response was established initially by calibrations to 5, 10 and 20 cm saline. The data were read to the nearest subdivision (0·1 in) on the recorder tracing paper, without interpolation. Therefore the observations take the form of discrete variates in increments of approximately 0·1 mmHg, the exact value depending on the calibration.

Measurements of hydrostatic pressure

Pressures in circulating body fluids were measured (1) directly, by cannulating vessels, sinuses, the pericardial space or the heart of *Limulus polyphemus*, or (2) indirectly, by measuring the pressure transmitted from the contracting heart to a catheter placed in the gut, which surrounds the heart of *Anodonta cygnea*. They were measured by Statham pressure transducers (P23BB), and the signal was amplified and displayed on a Brush Mark 260 recorder.

RESULTS AND DISCUSSION

Relation of measurements to predictions of colloid osmotic pressure

The data obtained by direct measurement are given in Table 1; a summary of the values in the literature is given in Table 2. The values in Table 2 were predicted from protein concentration, blood oxygen-carrying capacity or osmotic pressure of purified respiratory pigments. The discrepancies between measured and predicted values for the same species are not surprising in cases where the predictions were based on gross protein concentration or on blood oxygen-carrying capacity. These calculations assume that the mean molecular weight of the proteins is the same as that in mammalian blood, or that the haem:protein ratio is the same as that of Haemoglobin A. Both assumptions are untrue (Chew *et al.* 1965; Waxman, 1971). An explanation of the discrepancy between direct measurements on blood and those on purified proteins at equivalent protein concentrations is less obvious. In the early experiments of Roche & Combette (1937), it may have been due to dissociation of macromolecules into a larger number of osmotically active particles. These authors noted that their data on the osmotic pressure of lugworm haemoglobin yielded an estimate of molecular weight which was erroneously low by almost two orders of magnitude.

In any event, the comparison of Tables 1 and 2 emphasizes the large departure of predicted values, regardless of the procedure.

Table 1. *Measurements of colloid osmotic pressure and hydrostatic pressure of invertebrate body fluids*

Species	Fluid	Colloid osmotic pressure (mmHg) mean	±S.E.	N	Hydrostatic pressure (mmHg)
Cnidaria					
Metridium senile (L.)					
Control	Gastrovascular	0·04	0·03	6	0·05–7·34 (Batham & Pantin, 1950)
6–8 h desiccation		0·07	0·02	6	
Annelida					
Aphrodita aculeata L.	Coelomic	0·18	0·05	6	
Arenicola marina (L.)	Coelomic	0·06	0·03	10	10–70 (Chapman & Newell, 1947)
	Blood	1·53	0·23	11	
	Nephridial	0		6	
Nephtys hombergi Savigny	Coelomic	0·23	0·02	10	
	Blood	1·17	0·20	4	
Mollusca					
Gastropoda					
Acera bullata Müller	Blood	0·25	0·03	4	
Buccinum undatum L.	Blood	0·99	0·12	7	
Lamellibranchia					
Anodonta cygnea (L.)	Blood	0·23	0·02	12	7·3–55·0
	Pericardial	0·17	0·02	12	
Mya arenaria (L.)					
Control	Blood	0·13	0·03	9	1·8 (Trueman, 1966)
	Pericardial	0·07	0·03	9	
	Mantle	0	0	6	
12 h desiccation	Blood	0·39	0·07	4	
	Pericardial	0·56	0·08	4	
	Mantle	0	0	4	
Mytilus edulis L.					
Control	Blood	0·08	0·01	18	
	Pericardial	0·11	0·02	18	
	Mantle	0	0	12	
18 h desiccation	Blood	0·20	0·05	9	
	Pericardial	0·41	0·07	8	
	Mantle	0	0	6	
Arthropoda					
Crustacea					
Carcinus maenus (L.)	Blood	1·73	0·12	7	2·7–10·3 (Picken, 1936) 0·5–13 (Blatchford, 1971)
Crangon crangon (L.)	Blood	2·95	0·16	6	
Deckenia imitatrix Hilgendorff	Blood	1·86	0·37	8	
Eupagurus bernhardi (L.)	Blood	2·09	0·15	10	
Hyas araneus (L.)	Blood	2·02	0·10	4	
Cancer pagurus (L.)	Blood	2·62	0·66	4	
Portunus depurator (L.)	Blood	1·20	0·25	5	
Xiphosura					
Limulus polyphemus (L.)					
Unpaired observations	Blood	0·69	0·06	15	
	Urine	0		6	
Paired observations	Postbranchial blood	0·72	0·08	6	4–59
	Prebranchial blood	0·52	0·05	6	< 1–6

Table 1 (*cont.*)

Species	Fluid	Colloid osmotic pressure (mmHg) mean	±S.E.	*N*	Hydrostatic pressure (mmHg)
Echinodermata					
Asterias rubens L.	Coelomic	0		4	
Echinocardium cauda-tum (Pennant)	Coelomic	0·13	0·04	6	
Urochordata					
Ciona intestinalis (L.)	Blood	0·37	0·08	7	0·1–2·2 (Kriebel, 1968)

Table 2. *Estimates of the colloid osmotic pressure of invertebrate body fluids*

Species	Colloid osmotic pressure (mmHg)	Method	Source
Annelida			
Aphrodita hastata	trace	Calculated from protein content	Florkin & Blum (1934)
Arenicola marina (coelomic fluid)	0		
Sipunculoidea			
Phascolion strombi	0		
Mollusca			
Aplysia	0·02		
Octopus	3·24		
Arthropoda			
Homarus	1·15		
Annelida			
Arenicola	0·3	Calculated from protein content or O_2-carrying capacity	Eliassen (1953)
Glycera	6·4		
Mollusca			
Helix	0·01		
Planorbis	0·1		
Octopus	0·5		
Arthropoda			
Chironomus	22·8		
Homarus	0·5–0·8		
Nephrops	0·75		
Mollusca			
Anodonta cygnea		Calculated from protein content	Picken (1937)
Blood	2·8		
Pericardial fluid	2·5		
Limnaea stagnalis			
Blood	1·7		
Pericardial fluid	0·5		
Urine	2·5		
Arthropoda			
Chironomus	100	Calculated from oxygen carrying capacity	Jones (1970)
Mollusca			
Pila leopoldvillensis	12·7	Measured at 2 g haemocyanin/100 ml	Adair & Elliott (1968)
Annelida			
Arenicola marina	5·0–7·5	Extrapolated to 10·7–14·4 g haemoglobin/100 ml from measurements at 2·09–9·6 g/100 ml	Roche & Combette (1937)

Solute and water balance with the external medium

The data in Table 1 do not support the hypothesis that the observed hyperosmotic concentrations (5–40 mOsm) of body fluids result primarily from a Gibbs–Donnan equilibrium. The highest colloid osmotic pressures, equivalent to less than 1 mOsm, are found in decapod crustaceans, which actively maintain a hyperosmotic blood at the experimental salinity (18–21 ‰). Colloid osmotic pressures in the annelids *Arenicola marina* and *Nephtys hombergi*, which remain hyperosmotic by 20–30 mOsm regardless of the external concentration (de Leersnyder & Glaçon, 1973; Oglesby, 1973), are considerably less than 1 mOsm. To explain this margin, the osmotic pressure of particles exhibiting ideal behaviour according to the Van't Hoff law would have to approach 360 mmHg; the actual osmotic pressure of 5–30 mM solutions of impermeable colloids, would of course, be much higher.

Both hydrostatic and colloid osmotic pressures respond to desiccation so that water loss to the external medium may be reduced. Since the gastrovascular cavity of the sea anemone *Metridium senile* serves as its hydraulic skeleton, hydrostatic pressure varies considerably with the phase of activity. So long as the animal is submerged, however, the hydrostatic pressure difference across the body wall remains at least at the 'average basal' level of 0·05 mmHg, which is apparently sufficient to maintain an erect posture, and it rises to levels as high as 7·34 mmHg during contractions of the columnar muscles (Batham & Pantin, 1950). The colloid osmotic pressure of gastrovascular fluid cannot be detected in 4 of the 6 animals sampled, and the mean value does not differ significantly from zero (Table 1). Fluid must move into the tissues surrounding the gastrovascular cavity, and it must exit through the epidermal tissues bathed by the ambient medium. When an animal is exposed to the air it becomes very flaccid and the erect posture is not maintained, suggesting that internal hydrostatic pressure falls below the basal value. After a period of air exposure a colloid osmotic pressure was detected in all of the same 6 animals, and the mean value is significantly higher than the basal hydrostatic pressure (Table 1). Thus the pressure difference favouring water loss is abolished under these conditions.

A similar but more pronounced response occurs in the two lamellibranchs *Mya arenaria* and *Mytilus edulis*. Cardiac arrest, which accompanies prolonged air exposure of *M. edulis* (Helm & Trueman, 1967), must induce a large reduction of the hydrostatic pressure of blood and thus a diminished water loss.

Fluid balance within animals

In most cases where measurements of both parameters are available for the same species, there is an excess of hydrostatic over colloid osmotic pressure of a particular fluid compartment (Table 1). Moreover, data for hydrostatic pressure in related species suggest the same relationship. For example, blood pressures in the gastropod heart range from 1·4–3·7 mmHg in an aquatic limpet (Jones, 1970) to 3·8–27 mmHg in a terrestrial snail (Dale, 1973). The only data for blood pressures in the annelids are those for the tropical earthworm *Glossoscolex giganteus* (Johansen & Martin, 1965), which is so big (> 500 g) that the figures may have little meaning for the speci

examined here. However, the similar molecular weights of glossoscolecid and arenicolid haemoglobins permit a reasonable estimate of the colloid osmotic pressure of *Glossoscolex* blood. Its oxygen-carrying capacity (Johansen & Martin, 1966) is 1–2 times that of *Arenicola marina* blood (Wolvekamp & Vreede, 1940). If the relationship between osmotic pressure and number of molecules also resembles that of *A. marina* haemoglobin (Roche & Combette, 1937), the colloid osmotic pressure of *Glossoscolex* blood is about 1·5–3·0 mmHg. A minimum estimate of average hydrostatic pressure in the *Glossoscolex* vascular system can be obtained by halving pulse pressure; it is at least 6·5 mmHg in the ventral vessel and 3·7 mmHg in the dorsal vessel. Therefore there is no evidence that the presence of extracellular proteins in body fluids actually poses a problem of tissue dehydration in this species or any of those examined, although the margin of hydrostatic over colloid osmotic pressure is often small.

The results in Table 1 also suggest that filtration from lugworm blood must occur and that it must play a role, however slight, in excretion. The process of filtration from coelomic fluid cannot be very important in the elaboration of urine, because coelomic fluid has no significant colloid content. Blood pressure must exceed that in nephridial fluid, which is released for a few seconds each minute when the sphincter muscle around the nephridiopore relaxes (Chapman & Newell, 1947). Some fraction of primary urine must be formed directly from blood, and another fraction must be formed by fluid movement from blood first into coelomic fluid and then into the nephridia. The nephridial lumen does not contain haem compounds (Mangum & Dales, 1965) and inulin injected into the blood of other annelid species is not excreted in urine (Boroffka, Altner & Haupt, 1970). The urine must be an ultrafiltrate, although the quantitative importance of ultrafiltration may be small.

The molluscan nephridium receives fluid from the pericardium rather than the circulatory system, and the wall of the heart has often been implicated as a site of ultrafiltration and primary urine formation. Indeed, specialized pores for ultrafiltration have been found in the auricle of a freshwater gastropod (Boer, Algera & Lommerse, 1973). As in the annelids, the very absence of extracellular haemoglobins (and haemocyanins) in the secondary fluid of many molluscs is evidence of ultrafiltration. The excretory role of the heart in lamellibranchs without extracellular haemoglobins is less easily decided. Although Pierce (1970) and Tiffany (1972) concluded that ultrafiltration cannot occur in several marine and freshwater species, their calculations of the effective filtration pressure were made from total osmolality. Their data include the osmotic activity of freely diffusible substances as well as that of molecules with restricted permeability, which are in fact the effective particles in osmotic balance between internal fluid compartments.

The data in Table 1 confirm their conclusions for the marine species, but the colloid osmotic pressures of blood and pericardial fluid in the fresh water mussel *Anodonta cygnea* are significantly different ($P < 0{\cdot}05$ according to Student's t test for paired observations). The direct measurements essentially confirm the indirect estimates of colloid osmotic pressure made by Picken (1937), although our values for hydrostatic pressure are lower. The average hydrostatic pressure in the ventricles of 15 intact animals was 1·8 mmHg, which exceeds the colloid osmotic pressure of blood. Ultrafiltration across the ventricular wall must occur.

Data for heart (postbranchial) and pedal (prebranchial) blood of 8 mussels do not

differ significantly ($P > 0.05$ according to Student's t test for paired observations), thus providing no evidence of a net change in colloid osmotic pressure as the blood flows past the tissues.

The absence of osmotically active colloids in urine and the excess of hydrostatic over colloid osmotic pressure in blood indicate that urine is formed as an ultrafiltrate in the horseshoe crab *Limulus polyphemus*. Although the data in Table 1 were collected from samples obtained without the application of external pressure, we noticed that slight compression of the tissues surrounding the excretory opening causes a conspicuous increase in urine flow. The exclusion of osmotically active colloids from urine is somewhat surprising in view of the evidence that inulin is excreted in crayfish urine (Kirschner & Wagner, 1965). The blood supply to the xiphosuran coxal gland is more extensive than that to crustacean excretory organs, however. The two organs may be quite different in function despite similarities in histological structure and embryonic origin.

Relation of blood pressure to osmotic pressure of extracellular respiratory pigments

The small margin of hydrostatic over colloid osmotic pressure in some species (Table 1) may be misleading because few studies have included extensive measurements of blood pressure in different regions of a circulatory system. Although the average margin in *Limulus polyphemus* is relatively great, hydrostatic pressures as low as 0·4 mmHg were recorded in the sinuses when the animal was resting. As indicated above, the difference between diastolic pressure and colloid osmotic pressure in the blood of large annelids may be very small. Blatchford's (1971) results for *Carcinus maenas* include blood pressures ranging from less than 0·5 mmHg in the branchial vein to 13 mmHg in the ventricle during locomotor activity. Picken's (1936) value for hydrostatic pressure in the ventral thoracic sinus, from which our blood samples were taken (Table 1), is 2·7 mmHg. Therefore the excess of hydrostatic over colloid osmotic pressure in this species is not very great.

The function describing the increase of colloid osmotic pressure with concentration of arthropod haemocyanins is not known, but it is almost certain to be exponential in form, like that for extracellular haemoglobin (Roche & Combette, 1937) and molluscan haemocyanin (Adair & Elliott, 1968). A small increase in number of haemocyanin molecules in crab blood may cause serious disturbances in fluid balance, and it may explain the very low oxygen-carrying capacity. The data also emphasize the adaptive advantage of the tendency towards subunit aggregation of the extracellular oxygen-carrying pigments, which reduces the number of osmotically active particles. The highest colloid osmotic pressures are found in the decapod crustaceans, whose haemocyanins have a molecular weight of about $400–900 \times 10^3$; lower colloid osmotic pressures are found in the annelids, in which the molecular weight of extracellular haem proteins is about 3×10^6; even lower colloid osmotic pressures occur in the molluscs, with haemocyanins weighing 9×10^6 daltons.

CONCLUSIONS

The present results support the generalization that extracellular oxygen-carrying pigments function in the maintenance of colloid osmotic pressures of invertebrate body fluids, but the function is not exclusive to them. For example, the colloid osmotic pressure of tunicate blood, which contains no known respiratory pigment, is more than half that of the horseshoe crab *Limulus polyphemus*, which contains a haemocyanin of considerable importance in oxygen transport. They also support the idea that the respiratory function of body fluids with extracellular pigments is limited by the number of protein particles, and that the limit is reduced by the aggregation of functional subunits into very high molecular weight compounds.

The results do not support the hypothesis that the Gibbs–Donnan equilibrium is fully responsible for the widespread hyperosmoticity of many marine and estuarine osmoconformers.

The relevance of our findings to excretory phenomena and to the fluid balance between separate compartments within animals requires two assumptions that should be made explicit: (1) We assume that the porosities of the biological membranes are similar to those used in the experimental apparatus. While the assumption is true of the glomerular capillaries in amphibians and mammals, membranes with both lower and higher porosities are believed to exist elsewhere in biological systems. Lower porosities in several regions of vertebrate circulatory systems and in the membranes of several cell types are well known (Landis & Pappenheimer, 1963). Higher porosities are believed to exist in the capillaries of two species of teleost fish (Hargens, Millard & Johansen, 1974). The excretion of injected inulin in crayfish urine suggests either an active secretion process or a high porosity of membranes in the antennal gland (Kirschner & Wagner, 1965). (2) We also assume that a low colloid osmotic pressure in one fluid relative to another results from ultrafiltration and not from permeation followed by active resorption of the osmotically active macromolecules. The only finding known to us that contradicts this assumption is very indirect evidence of haemoglobin resorption by the mammalian kidney. When various solutions of tetrameric haemoglobin were injected into blood, a test solution at one intermediate concentration was recovered from the glomerulus but not from the urine (Lippman, Ureen & Oliver, 1951). Regardless of the interpretation of this experiment, the finding does not entail the conclusion that high molecular weight haemoglobins and haemocyanins can be resorbed by invertebrate excretory organs.

In addition, we should reiterate the limitation that our conclusions on renal function are not quantitative. Because we have not measured rates of urine formation by the different mechanisms, we cannot conclude that an organ is primarily a filtration kidney or a secretion kidney. We can conclude only that pressure relationships dictate that urine in annelids, freshwater molluscs and xiphosurans is formed in part by ultrafiltration, the magnitude of which may be very small.

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