[Reprinted from THE JOURNAL OF GENERAL PHYSIOLOGY, May 20, 1940,
Vol. 23, No. 5, pp. 575–584]

THE COMPOSITION OF FLUIDS AND SERA OF SOME MARINE ANIMALS AND OF THE SEA WATER IN WHICH THEY LIVE

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(Received for publication, February 23, 1940)

Knowledge of the chemical composition of body fluids and blood sera of the common marine invertebrates of North America is surprisingly scarce, although for European species the data are more complete (Bialaszewicz, 1933; Bethe and Berger, 1931; Robertson, 1939). When, recently, we had the opportunity of analyzing numerous species we found a parallel series of analyses had been completed by Homer W. Smith but never published. Professor Smith has kindly permitted us to include his data here and thus to make the present report considerably more complete than it otherwise could have been.[1]

Material and Methods

For all the species studied, care was taken to avoid contamination of the fluids by sea water or by other fluids of the animal, and to use only fresh intact animals in good condition. After drying the outside surface of each animal the fluids were allowed to drip into clean containers or were removed by pipette or hypodermic syringe. Where clotting occurred, the fluids were vigorously whipped to remove the clot. In a few cases

* The fluids studied by W. H. C. were collected at Mt. Desert Island Biological Laboratory, Salsbury Cove, Maine, during the summers of 1938 and 1939 and analyzed at Rutgers University. Preliminary reports were made by Cole and Kazalski (1939) and by Parker and Cole (1940). For assistance in preparing the samples we are indebted to Miss Barbara Parker and for analyzing them to Dr. J. D. Neuss. For financial assistance during 1939–40 we are indebted to the Permanent Science Fund of the American Academy of Arts and Sciences.

[1] The fluids studied by H. W. S. from echinoderms, worms, *Homarus*, and *Myxine* were collected and analyzed at the Mt. Desert Island Biological Laboratory during the summer of 1927 and those of *Homarus*, *Callinectes*, and *Limulus* in New York during the winter of 1927–28. Professor H. W. Smith expresses his indebtedness to the staff of the New York Aquarium for supplying live and acclimatized material; to the Elizabeth Thompson Science Fund during 1927–28 for financial assistance; and to Herbert Silvette for aid in the analyses.

the clots were analyzed separately in addition to the serum. If not analyzed immediately, about 1 ml. of toluene was added to each 100 ml. of fluid as a preservative.

Those analyses marked HWS (Table I) were made by the methods described by Smith (1929), while in those marked WHC the methods were as follows: chloride by the method of Wilson and Ball (1928) with the addition of nitrobenzene as suggested by Caldwell and Moyer (1935); sulfate by the microgravimetric determination of $BaSO_4$ using the filter stick technique; calcium by the method of Clark and Collip (1925), except that the precipitate of calcium oxalate was separated by filtration instead of centrifugation; magnesium by the method of Greenberg and Mackey (1932) using the calcium-free filtrate; sodium by the colorimetric method of Salit (1932) except that the precipitate was separated by filtration instead of centrifugation; potassium by the colorimetric method of Shohl and Bennett (1928) using filtration to separate the precipitate. Studies on the copper, nitrogen, and hemocyanin content of *Homarus*, *Cancer*, and *Limulus* will be reported later.

Where both CO_2 and pH determinations were made (HWS) the body fluid was removed without exposure to air into a syringe sealed with paraffin oil. In the case of small animals the analyses were made of pooled samples of blood from several specimens. In larger species (*Asterias*, *Solaster*, *Echiurus*, *Homarus*, and *Limulus*) individual animals were used. Those animals analyzed by HWS from "sea water" and "harbor water" respectively in New York had been in the New York Aquarium for several months and were more or less acclimatized. Although the sea water of the aquarium is constant in composition, the harbor water is continually pumped from off shore and varies in composition with the tide. The pH of the fluids studied by WHC was measured by the glass electrode and that of fluids studied by HWS by the quinhydrone electrode.

The freezing points were determined by the Beckman thermometer on pooled samples which had been in the refrigerator not more than 24 hours (WHC), or by a Heidenhain thermometer immediately after collection (HWS).

DISCUSSION

Discussion of the data will be facilitated by considering first the animals living in "full strength" sea water, and secondly, those living in diluted sea water or harbor water. Normal or full strength sea water may be arbitrarily defined as water containing 450 millimoles or more of chloride ions per liter.

Animals from Sea Water of "Normal" Concentration (≡ Chlorinity of 450 or More; Tables I and II)

The freezing points of the fluids of worms, echinoderms, and of *Venus* agreed well with that of sea water, indicating an isotonic condition. This conclusion agrees with other data in the literature, for a discussion of which the reader is referred to Krogh (1939).

Botazzi (1897), Macallum (1909–10), Schlieper (1935), and Pearse (1932) have reported that in the crab, *Limulus*, and the lobster the body fluid is hypertonic to sea water, while isotonicity has been reported by

Fredericq (1903–04), Garrey (1905), and Dailey, Fremont-Smith, and Carroll (1931). Since in our experience six different pooled samples each of *Homarus* and *Limulus* collected in three summers were consistently hypertonic we conclude that this is the normal condition. Our data are less extensive for the crabs, *Callinectes* and *Cancer*, but these also indicate hypertonicity.

The pH of all body fluids was distinctly below that of sea water. There was evidence that the pH of the sera of lobster, crabs, *Limulus*, and *Myxine* varied less among different individuals than did the pH of the blood and body fluids of the echinoderms, worms, and *Venus*. Furthermore, the amount of CO_2 present in fluids of the worms, Arthropoda, and *Myxine* was considerably greater than in the echinoderm fluids (excepting *Strongylocentrotus*), the CO_2 content of which was almost identical with that of sea water.

In accordance with procedure preferred by oceanographers, based primarily on the ease and accuracy of the chloride determination, the electrolyte composition is presented in terms of chlorinity; *i.e.*, millimoles of ions per liter divided by the millimoles of chloride ions per liter of fluid (Table I). For sea water at Salsbury Cove and New York the values obtained by HWS and WHC agreed closely with those reported by Clarke (1911) and Thompson (1936) for pure sea water. The small differences between the values of Thompson and those in Table I for potassium (HWS) (0.0179 *vs.* 0.0188 and 0.0204), and for sulfate (HWS) (0.0516 *vs.* 0.0629 and 0.0609) are perhaps due to the normal variation in the concentration of those ions in near-shore sea water, which has not yet been thoroughly mixed with open ocean water. Such variation could be caused by varying amounts of decomposition of small animals and plants, the former giving rise to excess sulfate and the latter to excess potassium.[2] The consistently low values for magnesium (WHC) for Maine sea water and for the fluids of animals from Maine (0.0977 *vs.* 0.0687, 0.0663, etc.) were probably due to some unknown systematic error in the analytical procedure for magnesium. The data of HWS are in closer agreement with the composition of Atlantic sea water. For the diluted sea waters from Delaware Bay and New York Harbor, the concentrations of potassium, calcium, and sulfate relative to that of chlorine were significantly different from those of pure sea water, a result to be expected in harbor waters (Clarke, 1911).

Although the actual ionic concentrations of the fluids give important information, the data are more easily interpreted by considering the ionic

[2] The marked accumulation of potassium by marine plants may be illustrated by *Valonia* (Osterhout, 1926–27).

concentrations of the fluids in relation to those in the external medium. The ratio of concentration inside to that outside furnishes evidence concerning the equilibria established with respect to the several ions. Such ratios are given in Table II, where the subscripts *i* and *o* refer to concentrations in the fluid and in the surrounding sea water respectively. Similar comparisons have previously been made for some of the ions in several invertebrates (Duval, 1925; Bethe and Berger, 1931; Dailey, Fremont-Smith, and Carroll, 1931; Bogucki, 1932; Bateman, 1933). In view of the latitude of analytical error and of variation between individuals, ratios between 0.9 and 1.1 should probably be taken to indicate uniform distribution between the internal and external medium. Ratios less than 0.9 indicate exclusion, and ratios greater than 1.1 indicate accumulation of ions relative to the external medium.

We find that the animals examined here may be classified into four

TABLE I

*Freezing Points, pH, and Composition of Some Marine Animal Fluids and of the Sea Water in Which the Animals Lived**

Species and habitat	Fluid	pH		$-\Delta^\circ$		mM/liter Cl		Chlorinity ratios in mM/liter										mM/liter CO_2
								Na/Cl		K/Cl		Ca/Cl		Mg/Cl		SO_4/Cl		
		HWS	WHC	HWS	WHC	HWS	WHC	HWS	WHC	HWS	WHC	HWS	WHC	HWS	WHC	HWS	WHC	HWS
Sea water, Salsbury Cove—Maine		—	8.10	—	1.759	483	492	0.861	0.931	0.0188	0.0173	0.0195	0.0197	0.1040	0.0687	0.0629	0.0516	2.15
Sea water—New York		—	—	1.850	—	525	—	0.859	—	0.0204	—	0.0293	—	0.0985	—	0.0609	—	2.15
Cucumaria frondosa—Maine	Coelomic	7.30	7.80	—	1.750	487	501	0.862	0.910	0.0199	0.0147	0.0192	0.0178	0.103	0.0663	0.0612	0.0509	2.14
Cucumaria frondosa—Maine	Ambulacral	—	7.75	—	1.749	—	494	—	0.962	—	0.0158	—	0.0180	—	0.0628	—	0.0514	—
Asterias vulgaris—Maine	Coelomic	7.20	7.54	—	1.762	488	505	0.846	0.911	0.0196	0.0164	0.0192	0.0178	0.104	0.0608	0.0615	0.0503	2.13
Chirodota laevis—Maine	Coelomic	7.00	—	—	—	488	—	0.861	—	0.0198	—	0.0209	—	0.113	—	0.0619	—	2.24
Solaster endica—Maine	Coelomic	6.90	—	—	—	488	—	0.861	—	0.0198	—	0.0196	—	0.102	—	0.0615	—	2.40
Echinarachnius parma—Maine	Coelomic	6.90	—	—	—	488	—	0.857	—	0.0189	—	0.0192	—	0.101	—	0.0611	—	2.24
Strongylocentrotus drobachiensis—Maine	Coelomic	7.20	7.84	—	1.776	488	510	0.861	0.904	0.0199	0.0188	0.0196	0.0173	0.0996	0.0608	0.0594	0.0496	6.00
Amphitrite brunnea—Maine	Blood	6.80	—	—	—	477	—	0.851	—	0.0273	—	0.0200	—	0.115	—	0.0646	—	5.0
Glycera dibranchiata—Maine	Blood	7.40	—	—	—	483	—	—	—	0.0199	—	0.0207	—	0.127?	—	0.0569	—	5.0
Echiurus pallasii—Maine	Coelomic	7.60	—	—	—	480	—	0.917	—	0.0263	—	0.0191	—	0.0885	—	0.0640	—	8.00
Venus mercenaria—Maine	Mantle	—	7.90	—	1.760	—	514	—	0.856	—	0.0144	—	0.0185	—	0.0486	—	0.0496	—
Homarus americanus—Maine	Serum	7.61	7.45	—	1.811	498	472	0.934	0.962	0.0172	0.0197	0.0214	0.0362	0.0095	0.0191	—	0.0106	5.12
Homarus americanus—New York	Serum	7.55	—	1.880	—	495	—	0.937	—	0.0181	—	0.0383	—	0.0186	—	0.0214	—	5.50
Cancer borealis—Maine	Serum	—	7.81	—	1.825	—	479	—	0.960	—	0.0213	—	0.0240	—	0.0457	—	0.0392	—
Callinectes hastatus—New York	Serum	7.55	—	1.932	—	480	—	0.958	—	0.0281	—	0.0410	—	0.0198	—	0.0238	—	7.0
Limulus polyphemus†	Serum	7.24	7.47	1.880	1.783	463	478	0.909	0.956	0.0311	0.0207	0.0346	0.0195	0.0584	0.0605	0.0445	0.0442	7.0
Myxine glutinosa—Maine	Serum	7.63	—	—	—	448	—	0.897	—	0.0203	—	0.0118	—	0.0502	—	0.0134	—	3.7
Delaware Bay water		—	8.01	—	1.336	—	319	—	0.878	—	0.0232	—	0.0169	—	0.0984	—	0.0514	—
Venus mercenaria—Delaware Bay	Mantle	—	7.65	—	1.369	—	373	—	0.799	—	0.0182	—	0.0298	—	0.0799	—	0.0515	—
Venus mercenaria—Delaware Bay	Blood	—	7.68	—	1.386	—	374	—	0.826	—	0.0184	—	0.0302	—	0.0778	—	0.0508	—
Limulus polyphemus—Delaware Bay	Serum	—	6.98	—	1.378	—	308	—	0.984	—	0.0282	—	0.0205	—	0.0799	—	0.0183	—
New York Harbor water		—	—	0.651	—	177	—	0.904	—	0.0252	—	0.0225	—	0.0910	—	0.0640	—	—
Limulus polyphemus—New York Harbor	Serum	7.46	—	1.01	—	265	—	0.981	—	0.0249	—	0.0208	—	0.0194	—	0.0169	—	10.8
Callinectes hastatus—New York Harbor	Serum	7.49	—	1.61	—	411	—	0.985	—	0.0204	—	0.0397	—	0.0730	—	0.0876	—	5.6
Homarus americanus—New York Harbor	Serum	7.56	—	0.945	—	270	—	0.859	—	0.0296	—	0.0518	—	0.0200	—	0.0148	—	5.1

* The total non-protein nitrogen in the sera in no case exceeded 20 mg. per cent and the urea nitrogen did not exceed half this value. Urea was almost entirely absent from *Homarus*, and of course was entirely absent in *Limulus* (Denis, 1922) the blood of which contains a high concentration of urease.

† Analyses by HWS were made on animals from sea water near New York; analyses by WHC on animals collected at Harpswell, Maine and kept in Salsbury Cove sea water for 3 months.

groups according to the composition of the body fluid compared to that of the external medium. The first group consists of the echinoderms and the clam, *Venus*, the fluids of which showed ionic ratios of 1.0 ± 0.1,

TABLE II

Ratios of Ionic Concentrations in Animal Fluids to Those in the Environmental Sea Water, Expressed in Millimoles of Ion Per Liter

Species and habitat	Fluid	Chlorinity of sea water in mM Cl/liter	$\frac{Na_i}{Na_o}$	$\frac{K_i}{K_o}$	$\frac{Ca_i}{Ca_o}$	$\frac{Mg_i}{Mg_o}$	$\frac{Cl_i}{Cl_o}$	$\frac{SO_{4_i}}{SO_{4_o}}$
* *Cucumaria frondosa*—Maine	Coelomic	488	1.003	0.965	0.957	0.991	1.013	0.993
* *Asterias vulgaris*—Maine	Coelomic	488	0.998	1.012	0.962	0.959	1.018	0.993
Chirodota laevis—Maine	Coelomic	483	1.010	1.060	1.085	1.100	1.010	1.007
Solaster endica—Maine	Coelomic	483	1.010	1.060	1.016	0.992	1.010	1.000
Echinarachnius parma—Maine	Coelomic	483	0.995	1.011	0.995	0.982	1.010	0.969
* *Strongylocentrotus drobachiensis*—Maine	Coelomic	488	1.008	1.097	0.981	0.944	1.023	0.981
Amphitrite brunnea—Maine	Blood	483	0.976	1.429	1.016	1.100	0.988	1.027
Glycera dibranchiata—Maine	Blood	483	—	1.055	1.064	1.228	1.000	0.927
Echiurus pallasii—Maine	Coelomic	483	1.057	1.385	0.975	0.850	1.023	0.994
Venus mercenaria—Maine	Mantle	492	0.961	0.871	0.979	0.740	1.045	1.004
Venus mercenaria—Delaware Bay	Blood	319	1.104	0.933	2.093	0.927	1.172	1.157
**Homarus americanus*—Maine	Serum	488	1.054	1.018	1.448	0.180	0.995	0.197
Homarus americanus—New York	Serum	525	1.028	0.838	1.231	0.181	0.943	0.330
Homarus americanus—New York Harbor	Serum	177	1.450	1.678	3.505	0.332	1.525	0.350
Cancer borealis—Maine	Serum	492	1.004	1.200	1.186	0.648	0.974	0.740
Callinectes hastatus—New York	Serum	525	1.020	1.262	1.279	0.184	0.914	0.356
Callinectes hastatus—New York Harbor	Serum	177	2.531	1.879	4.095	0.186	2.322	0.318
Limulus polyphemus—New York	Serum	525	0.933	1.346	1.039	0.522	0.882	0.644
Limulus polyphemus—Maine	Serum	492	0.989	1.112	0.949	0.828	0.972	0.824
Limulus polyphemus—New York Harbor	Serum	177	1.625	1.477	1.382	0.319	1.497	0.395
Limulus polyphemus—Delaware Bay	Serum	319	1.082	1.176	1.170	0.783	0.966	0.343
Myxine glutinosa—Maine	Serum	483	0.968	1.000	0.564	0.450	0.928	0.197

* These ratios were calculated from averages of the values of HWS and WHC from Table I.

excepting the unexplained low ratio of 0.74 for magnesium in the mantle fluid of *Venus*.

The second group includes the worms, two of which accumulated potassium (*Amphitrite* 1.4 and *Echiurus* 1.38), one accumulated magnesium (*Glycera* 1.2), and one excluded magnesium (*Echiurus* 0.85). Ratios for the other ions were 1.0 ± 0.1.

The third group includes the Arthropoda, all of which gave ratios of 1.0 ± 0.1 for the sodium and chloride ions, but excluded sulfate and magnesium; the lobster, *Homarus*, being especially efficient in this respect. As to the other ions, the following differences among the four species appear to be significant: *Homarus* serum contained on the average the same amount of potassium as the sea water but accumulated calcium; *Limulus* serum, on the other hand, contained the same amount of calcium but accumulated potassium; the crabs, *Cancer* and *Callinectes* accumulated both potassium and calcium. Ratios calculated from data on the European lobster, *Homarus vulgaris*, and crab, *Cancer pagurus*, reported by Robertson (1939) are closely similar to the ones given here, except for the calcium ratio in *Cancer* which is 1.47 instead of 1.19; and the potassium ratio in *Homarus* which is 1.45 instead of 0.98. Since a footnote suggested that the potassium content was "possibly too high" for *Homarus* the calculated ratio may also be too large.

The fourth group consists of the primitive chordate (*Cyclostomata*), *Myxine*, the serum of which showed ratios of 1.0 ± 0.1 for sodium, potassium, and chloride ions, but excluded about one-half of the calcium and magnesium ions and about 80 per cent of the sulfate ion. Although the freezing point depression was not recorded, the chlorinity of the serum indicated approximate isotonicity with sea water. Except for this difference *Myxine* serum resembled typical vertebrate sera much more closely than those of invertebrates.

The ratios for groups three and four showed unmistakable differential equilibria for at least three of the four ions: potassium, calcium, magnesium, and sulfate. In *Homarus*, for example, the average ratios were as follows: potassium, 0.928; calcium, 1.340; magnesium, 0.180; and sulfate, 0.264. The over-all distribution is, of course, the resultant of absorption and excretion but in the net indicates a physiologically preferential distribution across either the absorbing or excreting membranes. That such preferential capacities are physiologically significant among the invertebrates is sometimes overlooked, since the capacity of the fishes and higher aquatic vertebrates to regulate so efficiently the osmotic pressure of the body fluids, a capacity poorly developed among the invertebrates, overshadows the ionic composition of the lower forms. The unequal distribution of ions implies the expenditure of energy against a concentration gradient across the membranes involved. (Further evidence on this point may be found in the papers of Duval (1925), Bethe and Berger (1931), Dakin and Edmonds (1931), Bogucki (1932), and Bateman (1933).)

Animals from Diluted Sea Water (Chlorinity Less Than 450; Tables I and II)

Venus, *Homarus*, *Callinectes*, and *Limulus* collected from brackish water showed significant differences from specimens living in sea water. All the fluids were hypertonic to the external medium, even the mantle fluid of *Venus*. The relative hypertonicity of the arthropods was much greater than in sea water. It should be noted that the compositions of the mantle

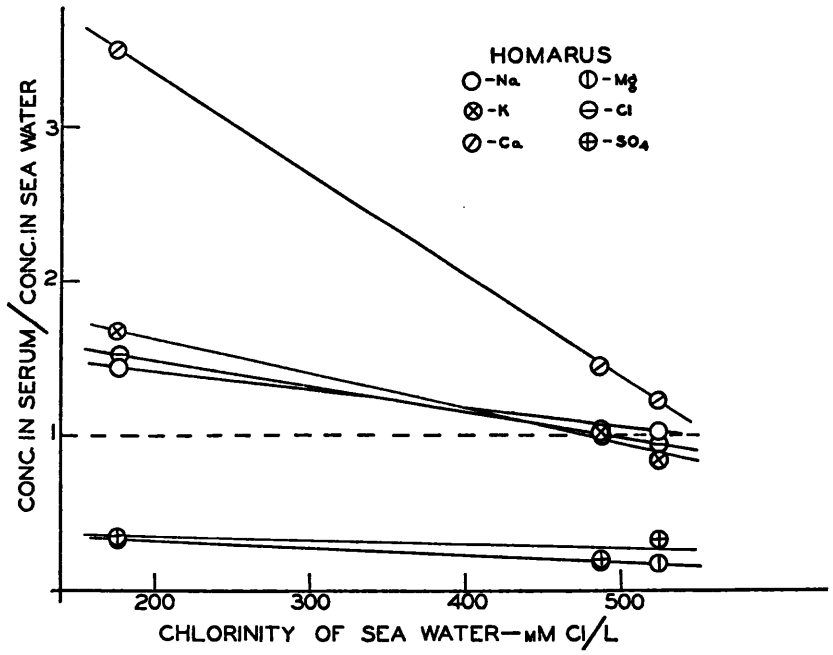

FIG. 1. Ratios of ionic concentrations in lobster (*Homarus*) serum to corresponding concentrations in environmental sea water plotted against concentration of chloride ions in the sea water.

fluid and of the blood of *Venus* were identical within the experimental error. Each contained more calcium than the Bay water. Since living conditions for the clam are less favorable in Delaware Bay than in pure sea water, it is likely that the increased calcium content is due to the solution of $CaCO_3$ from the valves, which was reported by Dugal (1939) in clams kept out of sea water for some time.

The increased hypertonicity of the Arthropoda was not caused by equally increased concentrations of the different ions. A graphical representation of the data for *Homarus* (Fig. 1) shows how the distribution of each ion changes independently when the animal moves from sea water into brackish

water. Ratios for magnesium and sulfate remain about the same in dilute sea water but the other ions accumulate in the following order: Ca>K>Cl>Na which differs from the order in sea water. Similar results are shown by *Callinectes* and *Limulus*. It has long been known (Krogh, 1939) that the fluids of marine invertebrates, isotonic to sea water become hypertonic to the external medium when the animals live in brackish water, but the differential accumulation of ions has not been reported. The possibility, however, was foreseen by Macallum (1926).

SUMMARY

1. The electrolyte composition, the pH, and freezing points of the fluids of several invertebrates and one primitive chordate are reported.

2. Fluids of the worms, echinoderms, and the clam *Venus* were isotonic with sea water; fluids of the Arthropoda were hypertonic to sea water.

3. The pH of all fluids was below that of sea water. In the Arthropoda and *Myxine* less individual variation in pH appeared than in the echinoderms and worms.

4. Ratios of ionic concentrations in the fluid to those in the sea water indicated (1) uniform distribution of ions between the internal and external media for the echinoderms and *Venus*; (2) differential distribution of potassium and magnesium in the worms; (3) differential distribution of sulfate, magnesium, potassium, and calcium in the Arthropoda; and (4) differential distribution of calcium, magnesium, and sulfate in *Myxine*.

5. The unequal distribution of ions implies the expenditure of energy against a concentration gradient across the absorbing or excreting membranes, a capacity frequently overlooked in the invertebrates.

6. The sera of the Arthropoda from diluted sea water showed higher concentrations of sodium, potassium, calcium, and chloride ions relative to the respective concentrations in the external medium than in normal sea water, and also showed different orders for those ions.

7. The increase in osmotic pressure of the sera of the animals moving into brackish water is caused by unequal accumulation of sodium, potassium, calcium, and chloride ions. Sulfate and magnesium ionic ratios do not change.

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