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THE CALCIUM AND CHLORIDE CONTENT OF LOBSTER SERUM AS AFFECTED BY DILUTION OF THE ENVIRONMENTAL SEA WATER

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Analyses for the brief inorganic ions in certain invertebrate sera (Parker and Cole, 1940; Cole, 1940) indicated that the concentrations of some or all of the ions in the sera of arthropods varied differentially with the concentration of those ions in the environmental sea water. It appeared desirable, therefore, to study more fully the effect of dilution of sea water on the concentrations of the ions in the sera of animals living in such water for different periods of time. During the summer of 1940 the calcium and chloride content of the serum of the lobster, *Homarus americanus* was determined for individuals living in full strength sea water and later transferred to 75 per cent sea water.

Animals weighing about one pound, freshly collected from their natural habitat, were used. From 5 to 10 ml. of blood were collected by a capillary glass tube inserted into the sinus under the soft exoskeleton of the ventral thoracic-abdominal junction. Upon removal of the tube, the puncture closed by clotted blood within a few minutes. Loss of blood upon return to water was negligible. Subsequent bleedings could be made from the same opening without evident injury to the animal. Some individuals were bled daily in that way for a week. After thorough clotting of the blood, the serum was removed for immediate analysis. Chloride was determined by the method of Wilson and Ball (1928) with the addition of nitrobenzene as suggested by Caldwell and Moyer (1935); calcium by the method of Clark and Collip (1925).

More than two bleedings within a week seemed to cause significant decreases in the protein content of the blood and slight decreases in the calcium content of the serum. Studies on the regeneration of blood and the effect of frequent bleeding will be made later.

In Table I are presented the data from twelve animals bled when removed from the sea and again when removed from 75 per cent sea water in which they had been living from 11 to 118 hours. Three of the animals, Nos. 3, 5 and 12 were bled a third time after having been returned to full strength sea water. The analyses of the full

strength sea water and of sera of the twelve animals living in it were so nearly alike respectively that they have been averaged in the table. (The chlorinity of Frenchman's Bay sea water is $504 \pm$ millimoles of chloride per liter of water.) The ratios of the concentrations in the sera to those in the environmental sea water (columns 7 and 8) give a measure of the net distribution of the ions across the absorbing and excreting membranes. Ratios between 0.9 and 1.1 should be taken to indicate uniform distribution between the inside and outside since individual variations and analytical errors give a latitude of ± 0.1 .

TABLE 1

Concentrations (millimoles per liter) of total chloride and calcium in the environmental sea water and in the sera of *Homarus americanus* living in full strength sea water and in 75% sea water for different periods of time, and the ratios of serum concentrations to sea water concentrations (C_1/C_0)

Animal Number	Time in Medium	Sea Water		Serum		Cl_i/Cl_0	Ca_i/Ca_0
		Cl mM/l.	Ca mM/l.	Cl mM/l.	Ca mM/l.		
1-12	several years	504 ± 9	11.91 ± 0.5	477 ± 39	18.97 ± 1.5	0.95* ± 0.04	1.59* ± 0.19
1	8.5 hrs.	380	8.25	313	29.30	0.82	3.55
2	12.0 hrs.	341	8.25	330	19.80	0.97	2.40
3	13.0 hrs.	389	7.29	399	18.71	1.02	2.56
3	60.0 hrs.	504	11.91	478	19.42	0.95	1.63
4	19.0 hrs.	341	8.25	264	19.64	0.77	2.38
5	20.0 hrs.	359	9.34	302	22.36	0.84	2.39
5	24.0 hrs.	504	11.91	462	20.34	0.92	1.70
6	22.0 hrs.	389	7.29	329	18.67	0.85	2.56
7	24.0 hrs.	330	7.94	381	19.68	1.15	2.48
8	37.0 hrs.	387	7.69	420	13.42	1.09	1.74
9	46.0 hrs.	389	7.29	407	13.26	1.05	1.81
10	70.0 hrs.	387	7.69	427	13.36	1.10	1.74
11	85.0 hrs.	394	6.95	438	12.54	1.11	1.80
12	118.0 hrs.	394	6.95	439	11.81	1.11	1.70
12	96.0 hrs.	504	11.91	524	17.00	1.04	1.42

* The slight differences between these values and those reported earlier (0.99 and 1.45; Cole, 1940) are within the individual variations and analytical errors. (See text.)

In the sera of lobsters living in full strength sea water the chloride ratio is 0.95 ± 0.04 and the calcium ratio is 1.59 ± 0.19 , indicating that serum chloride is only 5 per cent less than sea water chloride, but that serum calcium is 59 per cent greater than sea water calcium. At the end of 8.5 hours in 75 per cent sea water the serum contains 18 per cent less chloride but 255 per cent more calcium than the surrounding water contains, representing 13 per cent decrease of chloride and 196 per cent increase in calcium. During the next sixty hours in diluted sea water the ratios slowly tend towards those

for full strength sea water, the chloride content increasing to a concentration 75 per cent higher than in the surrounding sea water. Up to the end of 118 hours no further changes of significance occur, so that the net results are an increase in the chloride concentration of 15 per cent and in the calcium concentration of 16 per cent over the respective concentrations in the surrounding diluted sea water. Upon return to full strength sea water, however, the ratios go back to their original values.

These results show that the net movements of chloride and calcium ions through the absorbing and excreting membranes of the lobster are significantly different for the two ions. Chloride at first moves outward slightly, while calcium moves inward markedly; later chloride moves inward slightly and calcium moves outward slightly until new equilibria between serum and sea water concentrations of those ions are established and maintained. In full strength sea water and in diluted sea water the lobster is capable of expending energy against a concentration gradient, the amount of that energy being determined by the change in concentration of the environmental sea water and by the duration of exposure to the new environment. An interpretation of the mechanisms involved must await determination of the concentrations of the sodium, potassium, magnesium and sulphate ions as well as of the proteins in the blood of animals in diluted sea water.

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