The pH of pure Mt. Desert sea water, measured many times during the three-month season of 1939, averaged 8.10, with an average variation of 0.10. Extreme variations were +0.19 and —0.30, each of which were found only once. The pH of the body fluids and sera was consistently below that of sea water and varied somewhat more than that of sea water in the case of the echinoderms and *Venus*. Less variation appeared in the lobster, crab and *Limulus*. It seems safe to conclude that the crustacea and *Limulus* control the pH of their blood more effectively than the echinoderms and *Venus* control the pH of their body fluids. For none of the fluids did the pH change significantly after thorough whipping. The crustacean and *Limulus* sera sometimes showed a slight increase (0.05 to 0.15).

As has been found for these and other invertebrate fluids by other workers, the inorganic chemical composition varies considerably even when the animals are kept in sea water of constant composition. Further analyses are needed to delimit individual variations for each ion. However, it seems quite clear from the data in Table I that all the fluids contain about the same concentration of sodium, chlorine and potassium ions as does the sea water. The fluids of the echinoderms, *Venus* and *Limulus* contain also the same amount of calcium as does the sea water. The sera of the lobster and crab, however, contain significantly more calcium than the sea water. The echinoderm and *Limulus* fluids show the same magnesium content as sea water, but the sera of the crustacea and fluid of the clam contain significantly less magnesium than sea water, especially the lobster. For the sulfate ion, the fluids of echinoderms and *Venus* are similar to sea water, but the sera of crustacea and *Limulus* have a significantly lower sulfate content than that of sea water, especially the serum of the lobster. It may be tentatively concluded that the lobster accumulates calcium from the sea water to a significant extent, and similarly excludes magnesium and sulfate ions. The analysis of lobster serum reported by Cole and Kazalski (1939) showed more K and Ca ions than does the present analysis. It is likely that some of the difference is due to normal variation in the composition of lobster serum. However, it is believed that the new values are more accurate, due to improvement by Dr. Neuss of the techniques for determining K and Ca.

Reference

Cole, W. H. and L. A. Kazalski, 1939, Bull. Mt. Desert Is. Biol. Lab., 1939, p. 40.

FURTHER STUDIES ON PERFUSING SOLUTIONS FOR THE LOBSTER HEART

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Using the same procedures described by Cole and Kazalski (1939) for perfusing the heart of *Homarus americanus*, additional solutions were tested (1) to delimit the ranges of concentrations of potassium

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and calcium ions, within which the heart shows normal behavior; and (2) to determine the effects of a small change in osmotic pressure.

Attempts were made to buffer the solutions so that changes in pH of the fluid surrounding the heart would be minimized. Buffers containing phosphates could not be used because of the precipitation of calcium and magnesium phosphates. Small amounts of albumin and gelatin (up to 0.05%) were not effective and larger amounts caused slight opalescence of the solutions. Excellent buffering capacity was given by boric acid and NaOH. Even with old hearts (after 10 or more hours' beating in perfusion solution) the pH of the fluid in the cardiac chamber was no more than 0.1 lower than the entering solution. Accordingly to each liter of solution were added 17.57 ml. of 0.508 M boric acid and 0.956 ml. of 0.508 M NaOH. No harmful effects of the borate ion on the heart were observed.

The new analyses of lobster serum presented by Parker and Cole above indicated smaller amounts of potassium and calcium, and slightly larger amounts of magnesium and sulfate. Therefore, solution No. 35, prepared according to the new analysis, was tried (see Table I). It contained 9 millimoles (mM) of K, 20 mM of Ca; 12 mM of Mg and 6 mM of SO_4 contrasted to 15 K, 25 Ca, 8 Mg and 4 SO_4 as in the "normal" solution, No. 2, used in 1938. Whenever used on the heart it caused a slightly decreased rate and amplitude compared to No. 2, and hearts did not beat as long as in No. 2. To decide which ion was responsible, solutions Nos. 36, 37 and 38 were tested and found as satisfactory as No. 2, indicating that the increased Mg and SO_4 was unimportant and that the decreased K or Ca was also unimportant provided the larger amount of the other ion was present. For all subsequent tests, solution No. 36 was used as the reference standard to which the other solutions were compared. Since No. 45 also caused decreased rate and amplitude, it may be concluded that 2 potassium ions per 100 sodium ions are necessary for normal behavior of the heart provided there are 5 calcium ions present; if less than 5 calcium ions are present 3 potassium ions are necessary. Within these narrow limits calcium can replace potassium and vice versa. If calcium is increased or decreased even slightly beyond the limits (solutions Nos. 35 and 46), the rate and amplitude of the heart beat are changed. Increases and decreases of potassium can be somewhat larger before causing any change in the beat (solutions Nos. 40 and 43). Table I shows the effects of other solutions containing smaller and larger amounts of potassium (solutions Nos. 42, 41, 39 and 47) and smaller amounts of calcium (Nos. 44 and 45).

Decreasing the osmotic pressure by 25% without changing the ionic ratios (solution No. 48) caused increased tone and rate. Adaptation soon occurred, however, and beating continued for several hours. Return to the standard solution was followed by diastolic arrest from which recovery was very slow and incomplete. An increase of 25% in the osmotic pressure (solution No. 49) caused decreased rate which gradually caused diastolic arrest from which recovery was impossible.

Reference

Cole, W. H. and L. A. Kazalski, 1939, Bull. Mt. Desert Is. Biol. Lab., 1939, p. 40.

TABLE 1. COMPOSITION AND EFFECTS OF PERFUSING SOLUTIONS ON THE LOBSTER HEART. TOTAL MOLARITY WAS 0.508 EXCEPTING NOS. 48 AND 49 WHICH WERE 0.381 AND 0.635 RESPECTIVELY. pH = 7.3 − 7.5. TEMPERATURE = 17.7 ± 0.2° C; RATE OF FLOW = 10 ml./MINUTE

Solution No.	Millimoles per liter					No. of ions per 100 Na		Effects on heart
	NaCl	KCl	$CaCl_2$	$MgCl_2$	MgSO	K	Ca	
42	468	3	25	6	6	0.64	5.34	Inc. tone, dec. rate and ampl.
41	467	4	25	6	6	0.86	5.35	Inc. tone, dec. rate and ampl.
39	465	6	25	6	6	1.29	5.38	Dec. rate and ampl.
40	464	7	25	6	6	1.51	5.39	Dec. rate and ampl.
35	467	9	20	6	6	1.93	4.28	Sl. dec. rate and ampl.
37	462	9	25	6	6	1.95	5.41	Normal
38	461	15	20	6	6	3.25	4.34	Normal
36	456	15	25	6	6	3.29	5.48	Normal
2	452	15	25	4	4	3.32	5.53	Normal
44	471	15	10	6	6	3.18	2.12	Dec. rate and ampl.
45	466	15	15	6	6	3.22	3.22	Sl. dec. rate.
46	451	15	30	6	6	3.32	6.65	Sl. inc. rate.
43	446	25	25	6	6	5.61	5.61	Sl. dec. rate and ampl.
47	441	30	25	6	6	6.80	5.67	Inc. tone and rate.
48	342	11	19	4.5	4.5	3.22	5.55	Inc. tone and rate. Adaptation.
49	570	19	31	7.5	7.5	3.33	5.44	Dec. rate.