# A PERFUSING SOLUTION FOR THE LOBSTER (HOMARUS) HEART AND THE EFFECTS OF ITS CONSTITUENT IONS ON THE HEART

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## INTRODUCTION

As indicated in the report on a perfusion solution for the crayfish heart (Cole, Helfer, and Wiersma, 1939), the inorganic composition of perfusing solutions for invertebrate hearts should agree closely with that of the serum of the animal used if normal behavior of the heart is to continue. Data have been accumulated on the serum of the lobster *Homarus americanus* (Cole and Kazalski, 1939; Parker and Cole, 1940; Cole, 1939–40 and 1941), which demonstrate that the concentrations of the common inorganic ions—sodium, potassium, calcium, magnesium, chloride and sulfate—vary differentially with the concentrations of those ions in the environment. Since many of the marine invertebrates studied in the laboratory are collected from shore and bay habitats, the sea water of which varies in inorganic composition from the average of open ocean water (Clarke, 1911; Thompson, 1936) parallel analyses of the environmental sea water should accompany those of the sera, if physiological solutions are to be prepared. To show clearly the differences, ratios of ionic concentrations in the serum to those in sea water, referred to total salt concentration of sea water (measured easily by chlorinity or millimoles of chloride per liter) may be used (Cole, 1939–40 and 1941).

The calcium content of lobster serum varies more than that of the other ions. It is believed that the variation may be correlated with the time since the last molt of the animal, as shown in the crab *Callinectes* by Hecht (1914). The value for calcium given in Table I is the average of nineteen individual sera analyzed during three summers (1938–40).

## RESULTS

A perfusing solution for the lobster heart made according to preliminary analysis of lobster serum, but with an increased total salt concentration to

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give isotonicity with serum (No. 2, Table I), was satisfactory for normal beating of the heart for as long as 26 hours.[1] Decreases and increases in the osmotic pressure of the solution up to 15 per cent (Nos. 2 *a* and 2 *b*) caused no significant changes in the rate or character of the heart beat for several hours. Environmental sea water and van't Hoff's solution, however, were both unsatisfactory for perfusion, since normal rate and amplitude of the heart beat continued only a few minutes, even though beating at decreased rate and amplitude might go on for 3 hours. Subsequent analyses of lobster serum, showing less potassium and calcium, resulted in the preparation of solution 35 containing less potassium and calcium, and more magnesium and sulfate, which caused decreased rate and amplitude of the beat. Solution 36, with the same amount of potassium and calcium as in No. 2, and the same amount of magnesium and sulfate as in No. 35, was satisfactory.

It should be noted that these solutions and the ones used later contained only the inorganic salts—sodium chloride, potassium chloride, calcium chloride, magnesium chloride, and magnesium sulfate. All organic and the minor inorganic constituents of the serum were absent. This may have been why the absolute amounts of calcium and potassium had to be increased above the amounts in serum. In other words, the missing constituents in inorganic perfusing solutions may be replaceable by increased amounts of potassium and calcium. On the other hand, the numbers of potassium and calcium ions per one hundred sodium ions in the best perfusing solutions (Nos. 2 and 36), while larger than the content of each ion in serum, show lower ratios to each other than in the serum. In the latter, the ratio of calcium to potassium per one hundred sodium ions is 2.0, while in the solutions it is 1.7.

Obviously, as for other hearts, critical ratios between the sodium, potassium, and calcium ions in a perfusing solution determine whether or not the lobster heart can continue normal beating. In order to determine those ratios and the specific effects of each ion, forty-two other solutions were tested. Some of the solutions and their effects are displayed in Table I.

Solutions of single components all caused arrest of the heart. Systolic arrest resulted from potassium chloride within 30 seconds, from urea within 5 minutes and from sodium chloride within 5 minutes (solutions 15, 52, and 1). Diastolic arrest resulted from magnesium sulfate within 6 seconds, from magnesium chloride within 15 seconds, from calcium chloride within 30 seconds, and from glucose within 50 seconds (solutions 51, 50, 26, and 53). None of these effects were irreversible, since recovery of the normal beat occurred within a few minutes, the order being just the reverse of the order of arrest.

[1] Details of the procedure used in preparing the animals and of testing perfusing fluids on the hearts *in situ* are fully described by Cole and Kazalski (1939) and by Cole, Helfer, and Wiersma, (1939). In the former report and in another by Cole and Parker (1940) preliminary results of perfusing the lobster heart are presented.

TABLE I

Compositions and effects of perfusing solutions on the lobster heart; pH = 7.3 to 7.5; temperature = 16.6 to 17.7°C.; rate of perfusion = 10 ml. per minute. Solutions efficiently buffered by adding 17.57 ml. of 0.5 M boric acid and 0.956 ml. of 0.5 M NaOH to each liter.

| Solution | Millimoles per liter | | | | | | No. of ions per 100 Na | | Effects on heart |
|---|---|---|---|---|---|---|---|---|---|
| | Cl | Na | K | Ca | Mg | $SO_4$ | K | Ca | |
| Sea Water—Maine | 500 | 458 | 8.5 | 11.4 | 52 | 32 | 1.86 | 2.49 | Normal, 10 min. |
| Lobster serum | 472 | 454 | 9.3 | 18.6 | 9.0 | 5.0 | 2.05 | 4.10 | Not tested |
| Van't Hoff solution | 554 | 454 | 10.0 | 10.0 | 35.0 | 17.0 | 2.20 | 2.20 | Normal, 10 min. |
| No. 2 | 525 | 452 | 15.0 | 25.0 | 8.0 | 4.0 | 3.32 | 5.53 | Normal, 26 hrs. |
| No. 2*a* | 446 | 384 | 12.8 | 21.0 | 6.8 | 3.4 | 3.33 | 5.47 | Normal, 20 hrs. |
| No. 2*b* | 604 | 520 | 17.3 | 28.7 | 9.2 | 4.6 | 3.32 | 5.51 | Normal, 19 hrs. |
| No. 36 | 533 | 456 | 15.0 | 25.0 | 12.0 | 6.0 | 3.29 | 5.48 | Normal, 26 hrs. |
| No. 37 | 533 | 462 | 9.0 | 25.0 | 12.0 | 6.0 | 1.95 | 5.41 | Normal, 20 hrs. |
| No. 38 | 528 | 461 | 15.0 | 20.0 | 12.0 | 6.0 | 3.25 | 4.34 | Normal, 22 hrs. |
| No. 6 | 525 | 452 | 15.0 | 25.0 | 8.0 | 0 | 3.32 | 5.53 | Normal, 3 hrs. |
| No. 5 | 525 | 460 | 15.0 | 25.0 | 0 | 0 | 3.26 | 5.44 | Normal, 2 hrs. |
| No. 35 | 528 | 467 | 9.0 | 20.0 | 12.0 | 6.0 | 1.93 | 4.28 | Dec. rate and ampl. |
| No. 1 | 525 | 525 | 0 | 0 | 0 | 0 | 0 | 0 | Arrest in systole |
| No. 15 | 525 | 0 | 525 | 0 | 0 | 0 | — | 0 | Arrest in systole |
| No. 26 | 526 | 0 | 0 | 263 | 0 | 0 | 0 | — | Arrest in diastole |
| No. 50 | 526 | 0 | 0 | 0 | 263 | 0 | 0 | 0 | Arrest in diastole |
| No. 51 | 0 | 0 | 0 | 0 | 525 | 525 | 0 | 0 | Arrest in diastole |
| No. 52 urea 1 M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Arrest in systole |
| No. 53 glucose 1 M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Arrest in diastole |
| No. 8 | 500 | 484 | 16.0 | 0 | 0 | 0 | 3.30 | 0 | Arrest in systole |
| No. 7 | 526 | 474 | 0 | 26.0 | 0 | 0 | 0 | 5.49 | Arrest in systole |
| No. 21 | 504 | 496 | 0 | 0 | 4.0 | 0 | 0 | 0 | Arrest in systole |
| No. 20 | 500 | 492 | 0 | 0 | 8.0 | 4.0 | 0 | 0 | Arrest in systole |
| No. 9*a* | 520 | 0 | 120 | 200 | 0 | 0 | Ca/K = 1.67 | | Arrest in systole |
| No. 18*a* | 530 | 470 | 0 | 26.0 | 8.0 | 4.0 | 0 | 5.53 | Arrest in diastole |
| No. 16*a* | 504 | 480 | 16.0 | 0 | 8.0 | 4.0 | 3.33 | 0 | Arrest in systole |
| No. 41 | 533 | 467 | 4.0 | 25.0 | 12.0 | 6.0 | 0.86 | 5.35 | Dec. rate and ampl. |
| No. 40 | 533 | 464 | 7.0 | 25.0 | 12.0 | 6.0 | 1.51 | 5.39 | Dec. rate and ampl. |
| No. 46 | 538 | 451 | 15.0 | 30.0 | 12.0 | 6.0 | 3.32 | 6.65 | Increased rate |
| No. 43 | 533 | 446 | 25.0 | 25.0 | 12.0 | 6.0 | 5.61 | 5.61 | Dec. rate and ampl. |
| No. 47 | 533 | 441 | 30.0 | 25.0 | 12.0 | 6.0 | 6.80 | 5.67 | Inc. tone and rate |
| No. 3 | 526 | 442 | 12.0 | 17.0 | 29.0 | 10.0 | 2.72 | 3.85 | Dec. rate and ampl. |
| No. 45 | 523 | 466 | 15.0 | 15.0 | 12.0 | 6.0 | 3.22 | 3.22 | Decreased rate |
| No. 44 | 518 | 471 | 15.0 | 10.0 | 12.0 | 6.0 | 3.18 | 2.12 | Dec. rate and ampl. |
| No. 48 | 400 | 342 | 11.0 | 19.0 | 9.0 | 4.5 | 3.22 | 5.55 | Inc. tone and rate |
| No. 49 | 666 | 570 | 19.0 | 31.0 | 15.0 | 7.5 | 3.33 | 5.44 | Decreased rate |
| No. 29 LiCl | 525 | Li 452 | 15.0 | 25.0 | 8.0 | 4.0 | 3.32 | 5.53 | Arrest in systole |
| No. 30 NaBr | 525 | Br 452 | 15.0 | 25.0 | 8.0 | 4.0 | 3.32 | 5.53 | Dec. rate and ampl. |
| No. 31 NaI | 525 | I 452 | 15.0 | 25.0 | 8.0 | 4.0 | 3.32 | 5.53 | Arrest in diastole |

Among the binary solutions of salts, considerable differences were found. Potassium chloride and calcium chloride (No. 9 *a*) caused arrest in systole in less than 1 minute, indicating the predominating effect of potassium. Solutions of potassium chloride and magnesium chloride or magnesium sulfate, and of calcium chloride and magnesium chloride or magnesium sulfate were similarly effective in causing arrest, the former group in systole and the latter in diastole. Sodium chloride and magnesium chloride or magnesium sulfate (Nos. 20 and 21) were slightly better than sodium chloride alone, causing arrest in systole within 5 minutes. Sodium chloride and potassium chloride (No. 8) caused arrest in systole within 10 minutes, while sodium chloride and calcium chloride (No. 7) allowed normal beating for 30 minutes. The order of antagonism of sodium was therefore: calcium > potassium > magnesium. Calcium and magnesium did not antagonize potassium in the absence of sodium.

Tertiary and quartenary salt solutions were better than binary solutions provided they contained sodium, potassium, and calcium in certain proportions. Omission of potassium or sodium caused arrest in diastole (solution 18 *a*); omission of calcium caused arrest in systole (solution 16 *a*); but omission of magnesium or sulfate or both made no difference for as long as 3 hours.

The critical ratios between the sodium, potassium, and calcium ions were determined by a series of solutions in which the number of potassium and calcium ions per one hundred sodium ions were varied (solutions 3, 37, 38, 40, 41, 43–47). The results indicated that at least two potassium ions per one hundred sodium ions were necessary for normal behavior of the heart, provided there were five calcium ions present; three potassium and five calcium ions could be present, but three potassium ions were necessary if only four calcium ions were present. Within those narrow limits calcium could replace potassium and *vice versa*, without affecting the heart beat. If calcium or potassium were increased or decreased beyond those limits (solutions 35, 40, 46, and 47), the character of the heart beat was altered. For the crayfish (Cole, Helfer and Wiersma, 1939) the limits of the number of potassium and calcium ions per one hundred sodium ions were found to be from 1.8 and 6.8 to 2.6 and 6.6 respectively, showing narrower limits for calcium than in the lobster.

Decreasing or increasing the osmotic pressure of the perfusing solution by 25 per cent, without changing the ionic ratios of sodium potassium, and calcium, caused increased tone and rate or decreased rate respectively (solutions 48 and 49). Adaptation to the former soon occurred and beating continued for several hours, but there was no adaptation to the latter, which caused irreversible diastolic arrest.

Solutions in which lithium replaced sodium, and bromide or iodide replaced chloride were all unsatisfactory, but in different degrees (solutions 29–31). Iodide caused quick arrest in diastole, lithium caused increased rate and tone

followed by arrest in systole within 6 minutes, while bromide allowed normal beating for about 12 minutes, followed by decreased rate and amplitude, and by arrest in diastole within 30 minutes. Lithium chloride behaves like sodium and potassium chlorides in causing systolic arrest. The bromide and iodide ions, however, show a marked difference from the chloride ion, since their sodium salts cause diastolic instead of systolic arrest.

In the earlier experiments the pH of the solutions was adjusted to that of the serum (7.4 ± 0.1) by adding small amounts of 1.0 N sodium hydroxide. Varying the pH from 7.0 to 8.0 caused no significant changes in frequency, tone, or amplitude of the heart beat. At lower or higher values, increases in tone and rate accompanied by decreases in amplitude occurred. The buffering capacity of such solutions was insufficient to maintain a pH of 7.4 in the pericardial chamber for more than about 4 hours of perfusion. In later experiments, the solutions were more efficiently buffered by adding 18 ml. of 0.5 M boric acid and 1.0 ml. of 0.5 M sodium hydroxide per liter. The pH of pericardial fluid even after more than 14 hours of perfusion by solution 2 or 36, so buffered, remained at 7.4 ± 0.1. No harmful effects of the borate ion were apparent.

## DISCUSSION

For long continued beating of the lobster heart (20 hours or more) perfused *in situ*, the perfusing solution must correspond closely with the inorganic composition of the serum. Sea water is quite unsatisfactory for perfusion, since it contains 540 per cent more sulfate, 477 per cent more magnesium, 39 per cent less calcium, and 9 per cent less potassium than the serum does. A series of synthetic solutions gradually approaching the inorganic composition of the serum were proved to be increasingly satisfactory. Although hearts will beat for several hours on solutions not containing magnesium and sulfate, they will beat much longer (20 hours or more) if those ions are present. Sodium, potassium, calcium, magnesium, sulfate, and chloride ions are all necessary for prolonged normal beating; the critical number of ions being 100, 3, 5, 2–3, 1–2, and 116 respectively. It is not surprising, therefore, that perfusing solutions used by Hogben (1925) for *Homarus vulgaris*, and by Zoond and Slome (1928) for *Palinurus lalandii*, allowed normal beating for only 3 to 4 hours. For short periods of normal beating the lobster heart will tolerate improperly balanced solutions, but sooner or later the frequency, tone, or amplitude will be altered. Lithium cannot replace sodium; neither can iodide or bromide replace chloride.

Variations in the osmotic pressure of properly balanced solutions up to 15 per cent cause no significant change in the beat, although smaller variations will cause changes if the ionic ratios are not correct.

All of the results on perfusing the lobster heart agree qualitatively with

those on the crayfish heart (Cole, Helfer, and Wiersma, 1939), although the former is quantitatively less sensitive to variations in osmotic pressure, in potassium and in calcium content, but more sensitive to variations in pH than the crayfish heart. These differences may be associated with the differences between sea water and fresh water. The former has a more nearly constant pH, a larger potassium and calcium content than fresh water, but a more variable osmotic pressure, since bay and estuary waters may contain from 275 to 500 millimoles of chloride per liter.

## SUMMARY

1. An inorganic perfusing solution for the heart of the lobster *Homarus americanus*, to allow prolonged normal beating (20 hours or more) must agree closely with the inorganic composition of the serum, which varies differentially with that of the environmental sea water.

2. All of the chief inorganic ions of the serum are necessary—Na, K, Ca, Mg, Cl, and $SO_4$; the critical numbers of the ions being 100, 3, 5, 2–3, 116, and 1–2 respectively. Absence of Mg and $SO_4$ will be tolerated for several hours.

3. The pH of the solution must agree with that of the serum within 0.2.

4. The osmotic pressure of the solution must agree with that of the serum within 15 per cent.

5. Beating of the heart will continue for several hours on improperly balanced solutions but changes in frequency, tone, or amplitude will occur. Hearts adapted to such solutions will show different responses to physical and chemical stimuli of the solution than those perfused on properly balanced solutions.

6. Arrest in systole is caused by isotonic NaCl, KCl, LiCl, and urea, and arrest in diastole by isotonic $CaCl_2$, $MgCl_2$, NaBr, NaI, $MgSO_4$, and glucose.

7. Lithium cannot replace sodium; neither can bromide or iodide replace chloride ions.

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