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A PERFUSING SOLUTION FOR THE CRAYFISH HEART AND THE EFFECTS OF ITS CONSTITUENT IONS ON THE HEART[1]

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A SATISFACTORY solution for nerves of the crayfish, *Cambarus clarkii*, was developed by van Harreveld (1936) based upon chemical analyses of blood. Since its composition varied considerably from solutions reported by others to be satisfactory for crayfish, especially with respect to calcium, it seemed desirable to test it on the crayfish heart, to compare it with those other solutions, and to study the effects of constituent ions on the heart.

TABLE 1

COMPOSITION AND CATION RATIOS OF SOME OF THE SOLUTIONS USED ON THE CRAYFISH HEART

Solution	Total Molarity	NaCl Molar	KCl Molar	$CaCl_2$ Molar	$MgCl_2$ Molar	$NaHCO_3$ Molar	Cation Ratios When Na=100*		
							K	Ca	Mg
Crayfish blood (van Harreveld)..	Δ° C.= 0.85	....	0.0051	0.0137	0.0028	....	......	......	......
van Harreveld.....	0.2288	0.2051	0.0054	0.0135	0.0026	0.0022	2.63	6.58	1.27
Lindeman.........	0.1768	0.1709	0.00375	0.00216	....	....	2.19	1.26	......
C...............	0.1992	0.1880	0.00402	0.00720	....	....	2.14	3.83	......
Hoffmann.........	0.228	0.2200	0.004	0.002	....	0.002	1.82	0.91	......

* Omitting the Na in $NaHCO_3$.

The composition of van Harreveld's solution is given in Table 1. The pH varied from 7.2 to 7.8, and the depression of the freezing point was −0.86° C. which agrees well with the depression for crayfish blood (−0.85° C.; van Harreveld, 1936). This solution was first compared with the one reported by Lindeman (1929); then with a solution having intermediate amounts of sodium and calcium chlorides (solution C); with double Ringer's solution, which Hoffmann reported he used, although the compositions given are incorrect (Hoffmann, 1912–13); with isotonic single salt and glucose solutions, and with thirty-one other salt mixtures, the ionic ratios of which covered many significant possibilities.

[1] Nearly all the experiments herein reported were done at the Kerckhoff Laboratories in the fall and winter of 1937. To Drs. T. H. Morgan, C. A. G. Wiersma, and to other members of the staff the senior author expresses sincere thanks for the privileges and courtesies extended to him.

Since the extensive report of Plateau (1880) on the heart of decapods, there have been published many accounts of attempts to perfuse the heart of crustacea and of a few other invertebrates in order to compare the cardiac behavior of vertebrates with that of invertebrates. The work of Carlson (1905, 1906, 1906–7) on *Limulus* is perhaps the best known. A great variety of perfusing solutions has been used, and, as might be predicted, conflicting and equivocal results have been obtained, so that the question of how the invertebrate heart differs from the typical vertebrate heart is still largely unanswered. One probable reason for confusion has been the frequent use of unbalanced and improperly antagonized perfusing solutions. In the experiments reported here it has become clear that for the crayfish heart a favorable perfusing solution must correspond, in its inorganic composition at least, to the crayfish blood. It is likely that the same thing will be found true of other invertebrates. A critical review of the literature will, therefore, be postponed until proper solutions have been developed for representative species.

METHODS

Crayfish of both sexes collected in the vicinity of Pasadena and varying in body length from 6 to 10 cm. were prepared for perfusion of the heart as follows. The larger part of the carapace between the eyes and the cervical grooves was removed. The stomach, "liver," and reproductive glands were then removed, with the animal held vertically, head downwards.

Alimentary or liver juices were found to be highly injurious if they came in contact with the heart. The cavity was thoroughly washed with the physiological solution, and the ganglionic nerves were severed by cutting along each side of the ganglionic chain directly under the pericardial region. The anterior section of the pericardium was then cut away, thus exposing the pericardial cavity. A small spring pinch clamp 8 mm. long, made of rustless steel wire, was fastened to the midanterior margin of the heart. The animal was securely fastened in a vertical position head upwards. A thread attached the pinch clamp to a heart lever using a fine human hair as a marker on a lightly smoked drum. The tip of a glass tube with bore of about 1.5 mm. and a funnel-shaped opening at the upper end, was fastened about halfway down in the pericardial cavity at one side of the heart. The experimental solution flowed into that tube at a constant rate (6 ± 1.0 ml./min.) and at room temperature ($22° \pm 2.0°$ C.), filling the pericardial cavity and overflowing around the top of the latter. This method of perfusion was found to be far preferable to methods in which the crayfish is held in a horizontal position. Different perfusing solutions at the same rate of flow could quickly be substituted for van Harreveld's solution and compared with it. Clock time and frequency of beat, measured by a stop watch, were recorded on the smoked paper along with the heart record.

RESULTS

During the first few minutes of perfusion the frequency, tone, and amplitude of the heart beat varied considerably, but by the end of 15 minutes they usually became relatively constant. Of eighty-two animals used for perfusion by van Harreveld's solution, the normal rate at the end of 15 minutes varied from 62 to 118 beats per minute with an average of about 93. The heart beat of seven intact animals observed through Cellophane windows, between 20° and 23° C., varied from 75 to 136, with an average of 116 per minute. In a large percentage of the preparations, the beat was extremely regular, and the amplitude of successive beats was constant. A few animals showed constant but different amplitudes of alternate beats or rhythmic beats of more than two amplitudes or a continuous irregularity of rate and amplitude. Such hearts rarely ever became typical afterward.

Hearts perfused only with van Harreveld's solution continued normal beating for many hours (usually from 8 to 10 hours; occasionally from 9 to 14 hours, and a few from 18 to 20 hours). With occasional stimulation by lentin (carbamylcholine chloride) two hearts continued beating for 24 and 29 hours. There is little doubt that the other tissues of the animal were dead within 3 hours. It is believed that injury to the heart by the operation and by the pinch clamp is the most common cause of failure of hearts perfused only by that solution, if failure occurs before 5 hours. Since the solution contains no nutritive materials, no hemocyanin, and none of the other organic constituents of crayfish blood, it is not surprising that the heart stops eventually. Such arrest occurs in diastole and is characterized during the later hours by slowly decreasing frequency and amplitude without any significant change in tone.

Fifteen hearts were perfused only with Lindeman's solution, and in each case the frequency exceeded that found for van Harreveld's by from 10 to 50 per cent. Beating continued for much shorter periods of time (usually less than 2 hours; occasionally from 2 to 3 hours). Arrest was characterized by slight increase in tone, as well as by decreased frequency and amplitude. If hearts were first perfused by van Harreveld's solution and then changed to Lindeman's, marked increase in frequency and amplitude always occurred, followed by decreases in each, up to arrest in tonic diastole or one-half systole. If the solution was replaced by van Harreveld's before the frequency had decreased too much, the heart quickly recovered its normal characteristics. Obviously Lindeman's solution is much less satisfactory for the heart than van Harreveld's. The former contains no magnesium, is not buffered by bicarbonate, has a molarity 23 per cent less than the latter, and contains only one-fifth the latter's ratio of calcium ions to sodium ions. Results to be described later show that the absence of magnesium is relatively unimportant and that the lack of buffer action and the lower osmotic pressure are minor faults. The chief factor, therefore, in rendering Lindeman's solution unsatisfactory is the incorrect calcium content.

Solution C, with intermediate amounts of sodium and calcium and an intermediate molarity, also caused increased frequency and amplitude as compared with van Harreveld's, but to a lesser degree than Lindeman's. Furthermore, there was considerable adaptation, in that the frequency and amplitude returned to normal in some cases, and beating continued for from 2 to 4 hours. Double frog Ringer's solution (or Hoffmann solution, cf. Table 1), with the same molarity as van Harreveld's but with smaller amounts of potassium and calcium than Lindeman's, was found to be very unsatisfactory, causing marked increase in frequency and amplitude at first, followed by rapid decrease up to arrest in systole within 30 minutes. Varying the pH of van Harreveld's solution from 6.4 to 8.4 caused no significant changes in the frequency, tone, or amplitude of the heart beat. Below or above those limits changes in tone and amplitude usually occurred, although a detailed study of such changes was not made.

Contradictory results were often obtained during the early experiments, until it was discovered that the heart's behavior in the solutions might depend upon previous treatment. For example, if a beating heart which had been perfused with double Ringer's solution for a certain period was returned to van Harreveld's solution, temporary arrest in diastole might occur, although recovery to normal would usually follow within the next hour. Subsequently, therefore, all results were checked on fresh hearts treated previously only with van Harreveld's solution.

To determine the relative roles of each cation and the best mixture of them, various significant combinations, the majority of which are displayed in Table 2, were tested. In all of the solutions the pH was practically the same owing to the buffering action of

TABLE 2

IONIC RATIOS, MOLARITIES, AND CHARACTERISTICS OF SOLUTIONS USED ON THE CRAYFISH HEART

Solution	NaCl	KCl	$CaCl_2$	$MgCl_2$	$NaHCO_3$	Total M	Characteristics
van Harreveld's....	0.205	0.0054	0.0135	0.0026	0.0022	0.2288	"normal"
1.......	0.217	0.0057	....	0.0026	0.0022	0.2275	No Ca
2.......	0.210	....	0.0138	0.0027	0.0022	0.2287	No K
3.......	0.207	0.0054	0.0136	....	0.0022	0.2282	No Mg
4.......	0.208	0.0046	0.0114	0.0026	0.0022	0.2288	−15% K
5.......	0.204	0.0063	0.0134	0.0026	0.0022	0.2285	+17% K
7.......	0.201	0.0098	0.0132	0.0025	0.0022	0.2287	+84% K
8.......	0.195	0.0051	0.0238	0.0025	0.0022	0.2286	+82% Ca
9.......	0.191	0.0093	0.0234	0.0024	0.0022	0.2283	+84% K, +74% Ca
10.......	0.188	0.0122	0.0232	0.0024	0.0022	0.2280	+125% K, +74% Ca
11.......	0.206	0.0054	0.0125	0.0026	0.0022	0.2287	−7% Ca
12.......	0.203	0.0054	0.0147	0.0025	0.0022	0.2278	+9% Ca
13.......	0.188	0.0100	0.0260	0.0024	0.0022	0.2286	+85% K, +92% Ca
14.......	0.214	0.0027	0.0068	0.0027	0.0022	0.2284	−50% K, −50% Ca
17.......	0.202	0.0053	0.0160	0.0026	0.0022	0.2281	+18% Ca
18.......	0.205	0.0054	0.0128	0.0026	0.0022	0.2280	−5% Ca
19.......	0.1025	0.0027	0.0067	0.0013	0.0022	0.1154	$\frac{1}{2}$ v.H.
20.......	0.410	0.0108	0.0270	0.0052	0.0022	0.4552	2×v.H.
21.......	0.207	0.0027	0.0136	0.0026	0.0022	0.2281	−50% K
22.......	0.209	0.0041	0.0103	0.0026	0.0022	0.2282	−24% K, −21% Ca
23.......	0.208	0.0055	0.0103	0.0026	0.0022	0.2286	−21% Ca
24.......	0.212	....	0.0139	....	0.0022	0.2281	Na+Ca only
25.......	0.220	0.0057	....	....	0.0022	0.2279	Na+K only
26.......	....	0.058	0.145	....	0.0022	0.2052	Ca+K only
27.......	0.198	0.0053	0.0200	0.0025	0.0022	0.2280	+53% Ca
28.......	0.196	0.0080	0.0200	0.0024	0.0022	0.2286	+48% K, +48% Ca
29.......	0.194	0.0104	0.0200	0.0023	0.0022	0.2289	+92% K, +48% Ca
30.......	0.182	0.0240	0.0180	0.0022	0.0022	0.2284	+344% K, +33% Ca
31.......	0.187	0.0246	0.0123	0.0024	0.0022	0.2285	+350% K
32.......	0.191	0.0201	0.0125	0.0024	0.0022	0.2282	+270% K
33.......	0.198	0.0052	0.0130	0.0100	0.0022	0.2284	+284% Mg
34.......	0.189	0.0050	0.0125	0.0196	0.0022	0.2283	+650% Mg

the sodium bicarbonate, the concentration of which was constant. Also, with the exception of solutions 19 and 20, osmotic pressure was essentially the same, since the total molarities were relatively constant.

The absence of magnesium from van Harreveld's solution (solution 3) produced no significant effect for short periods up to 2 hours; after that time, decreases in frequency and increases in amplitude occurred at a greater rate than with the whole solution. Obviously, the magnesium ion plays a relatively minor role in sustaining and regulating the heart beat of crayfish. Absence of calcium (solutions 1 and 25), however, caused quick arrest in systole; absence of potassium (solutions 2 and 24) or of sodium (solution 26) caused quick arrest in diastole. Conversely, isotonic calcium chloride or magnesium chloride caused arrest in diastole, while isotonic sodium chloride or potassium chloride or sodium bicarbonate caused arrest in systole. These results, in some respects, are similar to those reported by Walzl (1937) for the oyster but differ considerably from those of Lindeman (1928) on the crayfish. Since the latter's solution was so incorrectly balanced for calcium, the results obtained by changing from it to other solutions were undoubtedly abnormal results of hearts treated earlier with unbalanced solutions as previously described.

Isotonic sodium chloride regularly caused an immediately increased amplitude and frequency for from 1 to 5 minutes, accompanied by a gradually increased tone and decreased amplitude. During the next few minutes the frequency slowly decreased while the tone increased and the amplitude decreased, until arrest usually occurred in systole in from 9 to 12 minutes, although a few hearts stopped in diastole. Hearts which had been arrested by other means could often be temporarily revived by isotonic sodium chloride (cf. Rogers, 1905). Isotonic glucose (0.45 molar) gave somewhat variable results. In ten hearts a markedly decreased frequency and a gradually increased tone were followed by arrest in systole or near systole within 5 minutes. In three hearts the frequency, the amplitude, and the tone decreased, and arrest occurred in diastole. In one heart the frequency and amplitude decreased, but the tone remained unchanged up to arrest in diastole. Similarly, mixtures of isotonic glucose and sodium chloride gave equivocal results as far as arrest was concerned. However, the typical sodium chloride effect of initially increased frequency and amplitude occurred in mixtures containing 25 per cent of the normal sodium chloride content or more, while a decreased frequency and amplitude occurred in mixtures containing less than this proportion.

By decreasing the molarity of van Harreveld's solution one-half, without altering the ionic ratios (solution 19), marked increases in frequency and amplitude with a slight increase in tone occurred, followed by gradual fatigue. Amplitude decreased faster than frequency, and the beat continued for as long as 20 minutes, when arrest in systole occurred. Recovery in van Harreveld's, however, was incomplete, indicating a certain amount of irreversible injury. Doubling the molarity of van Harreveld's solutions (solution 20) without changing ionic ratios caused quick arrest in diastole, with good recovery in van Harreveld's within a few minutes.

The other solutions tested showed the following qualitative results. The effects produced on the heart were in all cases roughly proportional to the amount of change in cation concentration, the greater effects being produced by the larger changes. Excess of sodium or deficiency of potassium (solutions 4, 14, 21, and 22) caused increased frequency and slightly increased amplitude; excess of potassium or deficiency of sodium (solutions 5, 7, 9, 10, 13, 29–32) caused decreased frequency and increased amplitude; excess of calcium (solutions 8–10, 12, 13, 17, 27–30) and magnesium (solutions 33 and 34) caused decreased frequency and amplitude; a deficiency of calcium (solutions 11, 14, 18, 22, and 23) caused increased frequency and amplitude.

Quantitatively, the most important ionic ratios are those of potassium and of calcium to sodium. As indicated earlier, the magnesium content is of little importance unless a large excess is present (284 and 650 per cent in solutions 33 and 34). Decreasing potassium by 50 per cent (solution 21) caused only a slightly increased rate; increasing it 17 per cent (solution 5) caused no effect. Small changes in calcium, however, caused noticeable effects (7 per cent decrease in solution 11, 21 per cent decrease in solution 23, and 9 per cent increase in solution 12). Table 3 gives the qualitative effects of increasing the number of potassium and calcium ions per 100 sodium ions in those solutions in which the magnesium and the sodium ions were not significantly changed. Expressed on the basis of 100 sodium ions, the normal content is 2.63 potassium ions and 6.58 calcium ions. The former can probably change by 0.75 (nearly 30 per cent), but the latter by only about 0.25 (about 4 per cent) without causing changes in the heart beat for several minutes. The limits of calcium are, therefore, very narrow; of potassium considerably wider; of sodium and magnesium still wider. The large increases in potassium (270 and 350 per cent in solutions 32 and 31) caused a marked decrease in frequency and a significant increase in amplitude, the latter being followed in from 2 to 5 minutes by a gradually decreasing amplitude and increasing tone. Feeble and slow contractions, however, might continue up to 10 minutes before arrest occurred in systole. In view of the fact that excess potassium "narcotizes" the peripheral nerves of crustaceans (Cowan, 1934), the continued beating of the crayfish heart in solutions 30, 31, and 32 may indicate at least temporary independence of nervous control.

TABLE 3

THE EFFECTS OF INCREASING K AND Ca RATIOS (RELATIVE TO Na=100), IN SOLUTIONS USED ON THE CRAYFISH HEART

Solution	K	Percentage of Change	Ca	Percentage of Change	Effects on Heart
2	0	....	6.37	....	Increased rate and tone on fresh hearts
21	1.31	−50	6.55	....	Slightly increased rate on fresh heart
v.H.	2.63	....	6.54	....	"Normal"
1	2.63	....	0	....	Increased rate; systolic arrest
23	2.64	....	4.94	−25	Increased rate
11	2.62	....	6.04	−8	Increased rate
18	2.63	....	6.23	−5	Slightly increased rate
v.H.	2.63	....	6.58	....	"Normal"
12	2.66	....	7.24	+10	Decreased rate
17	2.62	....	7.92	+20	Decreased rate
27	2.67	....	10.1	+53	Decreased rate
8	2.62	....	12.2	+82	Decreased rate and amplitude
5	3.09	+17	6.54	....	No real effect
7	4.85	+84	6.56	....	Decreased rate
32	10.5	+300	6.55	....	Decreased rate and increased amplitude
31	13.2	+400	6.58	....	Decreased rate; increased tone and amplitude

SUMMARY

1. Van Harreveld's solution is far more satisfactory for perfusing the crayfish heart (*Cambarus clarkii*) than Lindeman's or double Ringer's solution. Hearts have continued beating for as long as 20 hours in van Harreveld's.

2. Moderate changes in pH (from 6.4 to 8.4) and slight changes in osmotic pressure do not affect the heart for several hours. Halving the osmotic pressure causes increased frequency and amplitude; doubling it causes quick arrest in diastole.

3. The heart beat is primarily controlled by the sodium ion, which in the absence of other cations causes too high a frequency and amplitude leading to quick fatigue.

4. Potassium, calcium, and magnesium retard the frequency, and a proper balance between them and sodium is necessary for continued beating.

5. The calcium ion is by far the most important antagonist to sodium. Its optimum concentration can be changed by only about 5 per cent without affecting the heart.

6. Increased frequency is caused by excess of sodium or by a lack of potassium, calcium, or magnesium, and decreased frequency by a lack of sodium or by an excess of potassium, calcium, or magnesium.

7. Increased amplitude is caused by excess of sodium or potassium, and decreased amplitude by excess of calcium or magnesium.

8. Arrest in systole is caused by isotonic NaCl, KCl, or $NaHCO_3$, and arrest in diastole by isotonic $CaCl_2$ or $MgCl_2$.

9. Isotonic glucose is more harmful than isotonic NaCl, usually causing arrest in systole or near systole, although a few hearts were arrested in diastole. Mixtures of isotonic glucose and sodium chloride containing 25 per cent or more of the normal NaCl content produced the typical sodium chloride effect of initially increased frequency and amplitude. Mixtures containing less than 25 per cent of the normal NaCl content caused decreased frequency and amplitude, the effect typical of pure glucose solutions.

10. The perfusion solution for the crayfish heart must correspond closely in its inorganic content with the blood serum of the crayfish, if normal behavior of the heart is to be maintained. Simplified solutions may profoundly alter the heart beat, even though it may continue for several hours.

11. The heart may adapt itself to an incorrect solution and continue beating for a considerable time, but its reactions to changes in the perfusing solution may be quite different from those secured following perfusion by a different initial solution.

LITERATURE CITED

CARLSON, A. J. 1905–6. Comparative physiology of the invertebrate heart. The physiology of the cardiac nerves in the Arthropods. Amer. Jour. Physiol., 15:127–35.

———. 1906. Comparative physiology of the invertebrate heart. V. The heart rhythm under normal and experimental conditions. *Ibid.*, 16:47–66.

———. 1906. Comparative physiology of the invertebrate heart. VI. The excitability of the heart during the different phases of the heart beat. *Ibid.*, pp. 67–84.

———. 1906. Comparative physiology of the invertebrate heart. VII. The relation between the intensity of the stimulus and the magnitude of the contraction. *Ibid.*, pp. 85–99.

———. 1906. Comparative physiology of the invertebrate heart. VIII. The inhibitory effects of the single induced shock. *Ibid.*, pp. 100–109.

———. 1905–7. The relation of the normal heart rhythm to the artificial rhythm produced by sodium chloride. *Ibid.*, 17:478–86.

COWAN, S. L. 1934. The action of potassium and other ions on the injury potential and action current in *Maia* nerve. Proc. Roy. Soc. London, B, 115:216–60.

HOFFMANN, P. 1912–13. Über den Herzschlag des Flufskrebses mit besonderer Berücksichtigung des systolischen Stillstandes. Zeitschr. f. Biol., 59:297–311.

LINDEMAN, V. F. 1928. The physiology of the crustacean heart. I. The effect of various ions upon the heart rhythm of the crayfish, *Cambarus clarkii*. Physiol. Zoöl., 1:576–92.

PLATEAU, F. 1880. Recherches physiologiques sur le cœur des crustacés décapodes. Arch. biol., 1:595–695.

ROGERS, C. G. 1905. The effect of various salts upon the survival of the invertebrate heart. Jour. Exper. Zoöl., 2:237–52.

VAN HARREVELD, A. 1936. A physiological solution for fresh water crustaceans. Proc. Soc. Exper. Biol. Med., 34:428–32.

WALZL, E. M. 1937. Action of ions on the heart of the oyster (*Ostrea virginica*). Physiol. Zoöl., 10:125–40.