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A Physiological Solution for Freshwater Crustaceans.

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Contrary to what we know of the nerve muscle preparation of the frog, the irritability of the nerve of a freshwater crustacean quickly disappears on bathing it in an isotonic sodium chloride solution. For marine crustaceans seawater may be used successfully as a physiological solution. For *Astacus fluviatilis*, Hoffmann's[1] formula, a solution of about twice the concentration of the Ringer solution for the frog, has generally been employed. With such solutions the results with nerve muscle preparations have not been very satisfactory (Hoffmann, Segaar[2]). With the crustacean heart, however, the results have been better (Potioné,[3] Lindemann[4]). An attempt was made to find a better physiological solution for the freshwater crayfish.

Two species of freshwater crustaceans, *Astacus trowbridgii* and *Cambarus clarkii* were investigated. The freezing point of the blood was determined in order to know the molecular concentration. The amount of the physiologically important ions, Ca, K and Mg was estimated, and finally the pH was determined. As even large specimens do not give more than .5 to 1 cc. of blood, the blood of 8 to 10 crayfishes was collected and mixed, and all the estimations except the pH were done with this. The figures obtained in this way are thus averages, for our purpose not a disadvantage. The blood was collected by sucking it out of the pericardial cavity which surrounds the heart ventricle, with a paraffined hypodermic syringe. The blood for the Ca, K and Mg determination and for the freezing point was cooled to 0° and stirred for about 15 minutes to prevent clotting. After centrifuging a clear serum was obtained. The blood for the pH determination was collected in a small amount of a 1% potassium oxalate solution under a layer of liquid paraffin, to avoid loss of CO_2

The freezing point determination of the serum was made with an ordinary Beckmann thermometer. For the estimation of the

[1] Hoffmann, P., *Z. f. Biol.*, 1914, **63**, 411.
[2] Segaar, J., *Z. f. vergl. Physiol.*, 1929, **10**, 120.
[3] Potioné, H., *Z. f. vergl. Physiol.*, 1926, **3**, 528.
[4] Lindemann, V. F., *Physiol. Zool.*, 1928, **1**, 576; 1929, **2**, 395.

Ca, K and Mg ions the procedure of Kramer and Tisdall[5] was used. To determine the Ca, the modification by Clark and Collip[6] was chosen. The K determination was done exactly as described by Kramer and Tisdall. For the Mg determination their method was slightly modified. A precipitate of ammonium-magnesium-phosphate was formed and washed in the way described by Tschopp,[7] and the phosphate in the precipitate was estimated with the colorimetric method of Kuttner and Cohen.[8] Determining the Ca, K and Mg in a solution of known concentration, the methods for Ca and K were found to be accurate within 5%, that for Mg within 10%. The pH determination was done with a glass electrode.

In Tables I and II figures obtained from the blood of *Astacus trowbridgii* and of *Cambarus clarkii* are given. For both species 3 different groups of animals obtained at different times were examined. The blood of each group was taken more than once, with an interval of at least one week. The large variations in the figures obtained in different groups of one species and in different blood

TABLE I.
Astacus trowbridgii.

	Freezing point depression	Ca mg./cc.	K mg./cc.	Mg mg./cc.
Group I	.79	.43	.23	.066
	.81	.48	—	.072
	.80	.45	.19	.075
Group II	.88	.54	.22	.076
	.84	.45	.16	.077
Group III	.86	.49	.17	.071
	.81	.44	.16	.067

(The figures in Tables I and II are averages of duplicate determinations.)

TABLE II.
Cambarus clarkii.

	Freezing point depression	Ca mg./cc.	K mg./cc.	Mg mg./cc.
Group I	.71	.46	.18	.042
	.72	.44	.16	.044
Group II	.84	.54	.21	.072
	.75	.52	.17	.060
Group III	.82	.55	.19	.069
	.74	.42	.16	.077

5 Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **46**, 339; 1921, **47**, 475.
6 Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.
7 Tschopp, E., *Helvetica chimica acta*, 1927, **10**, 843.
8 Kuttner, Th., and Cohen, H. R., *J. Biol. Chem.*, 1927, **75**, 517.

samples of the same group have been observed by other authors in the blood of crustaceans and various causes have been suggested. The molting seems to have an important diminishing influence on the molecular concentration of the blood of crustaceans living in freshwater (Bethe and Berger,[9] Schwabe,[10] Scholles,[11] Huf[12]). The production of eggs also influences the composition of the blood very definitely (Schwabe[10]). Starvation has been mentioned as causing a lower molecular concentration of the blood. But even without these special conditions the blood of these animals does not seem to be very constant. Huf detected variations in the Cl of the blood of 10% in one specimen of *Astacus fluviatilus* in the course of one to 2 weeks. According to Scholles repetitive bleeding causes only a small diminution of the molecular concentration of the blood of *Eriochier sinensis* living in fresh water. Therefore, one cannot be surprised at the differences in the various figures of the Tables I and II, as no attempt has been made to examine animals living under exactly the same circumstances and at the same periods of their lives. The animals of the first group of both species had lived in a freshwater aquarium and were fed for many weeks before blood was taken for the first time. The first figures of Groups II and III from *Astacus trowbridgii* as well as from *Cambarus clarkii* were obtained from blood samples taken as soon as the animals were brought in. Almost all these figures are higher than those of Group I and higher than the other figures of Groups II and III obtained one week later. This is understandable since all the factors mentioned above as influencing the composition of the blood cause a lowering of the molecular concentration. It seems reasonable to consider the first figures of the Groups II and III of both species as those from animals living most nearly in the normal state. For Astacus and for Cambarus a freezing point depression of about .85°, and a Ca, K and Mg

TABLE III.
pH of the Blood.

Sample	*Cambarus clarkii*	*Astacus trowbridgii*
A	7.54	7.60
B	7.53	7.67
C	7.56	7.41
D	7.54	7.53
E	7.48	
F	7.63	

[9] Bethe, A., and Berger, E., *Pflügers Arch.*, 1931, **227**, 571.
[10] Schwabe, E., *Z. f. vergl. Physiol.*, 1933, **19**, 183.
[11] Scholles, W., *Z. f. vergl. Physiol.*, 1933, **19**, 522.
[12] Huf, E., *Pflügers Arch.*, 1933, **232**, 559.

concentration in the blood of respectively .55, .20 and .07 mg. per cc. must be considered as normal. The pH values, determined on the blood of these 2 species are collected in Table III. A pH of 7.55 can be taken as the average value for both species.

It is interesting that the composition of the blood of these 2 American freshwater crayfishes agrees in general with that obtained from *Astacus fluviatilis* studied in Europe (Berger,[13] Scholles).

A physiological salt solution based on the figures given above was prepared; containing 12 gm. sodium chloride, 1.5 gm. calcium chloride, .4 gm. potassium chloride, .25 gm. magnesium chloride, and .2 gm. sodium bicarbonate per liter.

The survival of the nerve in this solution was investigated. The nerve bundle in the meropodite of the claw was prepared, and the preparation bathed in the solution. At regular intervals the nerve was stimulated faradically. The contraction of the adductor muscle of the claw was taken as an indicator of the irritability of the preparation. As the muscles in the propodite remain in the shell, they are not exposed to the solution in which the preparation is lying, so the influence of the solution on the nerve only was investigated. After about 15 minutes' immersion, the threshold of the stimulation was found. The irritability of the nerve was then examined at regular times. In general the threshold stayed at the same level for many hours, then dropped rather suddenly. The time was estimated from the moment the preparation was immersed in the solution until the nerve needed a stimulus so strong that the part of it situated in the carpopodite, not exposed to the solution, was stimulated by current escape. The experiments were carried out in a constant temperature room at a temperature between 17 and 18°.

Of 12 preparations of *Cambarus clarkii,* bathed in the physiological solution, the average time during which the nerve kept its irritability was 26 hours; one preparation of this group remained irritable as long as 32 hours. For 12 preparations of *Astacus trowbridgii* these periods were 25 and 35 hours respectively. The same experiments were done with other solutions, namely an isotonic sodium chloride solution and the solution used by Segaar. The nerve in an isotonic salt solution remained irritable only for a short time. The average time for Cambarus was 50 minutes; the longest time 70 minutes. It is probable that the nerves of the crayfish bathed in an isotonic salt solution lose their irritability so soon because of the absence of the relatively thick layer of myeline

13 Berger, E., *Pflügers Arch.*, 1931, **228**, 790.

which in the frog nerve covers the nerve fibre. The solution of twice the ordinary strength, as the Ringer solution for the frog, was not as ineffective as would be expected from the description of Segaar. Using 12 preparations the average period of irritability in this solution was 6 hours; the longest 11 hours.

Adding Ca, K and Mg ions changes an isotonic sodium chloride solution, in which the crayfish nerve kept its irritability for only 50 minutes on the average, to solution in which the irritability is kept for 26 hours. Is it necessary to add all these ions to obtain this result or may some of them be left out? It turned out that it is particularly the Ca ion that improves the isotonic salt solution. Adding the proper amount of calcium chloride (1.5 gm. per liter) to the isotonic solution gives a fluid in which the nerve kept its irritability for an average of 21.5 hours; the longest time was 28 hours. Adding potassium and magnesium chloride in the right amount (.4 gm. and .25 gm. per liter respectively) did not improve the isotonic salt solution very much. The nerves of 12 preparations stayed irritable for an average of 95 minutes; the longest time was 160 minutes. As the first solution ($NaCl + CaCl_2$) maintained the irritability nearly as long as did the complete physiological solution, and as the second solution ($NaCl + KCl + MgCl_2$) maintained it only slightly longer than the isotonic salt solution, it is clear that the calcium ion is one of the important ions for maintaining the irritability of the nerve in crustaceans. However, some influence of the other ions (K or Mg or both) cannot be denied. The fact that the double Ringer solution keeps the irritability of the nerve for an average of 6 hours must be ascribed to the calcium chloride content of .22 gm. per liter, though this is only 1/7 of the amount in the physiological solution described above.