mental or control, could be determined. The general picture for the period under consideration was first, the completion of gametogenesis, then followed by a slow involution of the gonads, and finally the preliminary stages of the 1939 cycle. (cf. Matthews (1938) for a description of the normal gonadal cycle).

From these results it is clear that the involution of the sexual cycle of *Fundulus* is not accelerated by decreasing the daily ration of light, even though in nature gonadal involution is coincidental with a decrease in day-lengths. Likewise, an increase in daily illumination does not check or depress gonadal involution. Whether or not a precocious gametogenesis can be induced in *Fundulus* by photoperiodic manipulations is not conclusively determined by the failure to secure an accelerated cycle through a rapid increase in the length of daily illumination. Suffice to say, increased lighting between July 22 and August 27 was unable to induce an acceleration of gametogenesis at this time of the year, and with the gonads in the states above described.

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A PERFUSING SOLUTION FOR THE LOBSTER HEART AND THE EFFECTS OF ITS CONSTITUENT IONS ON THE HEART

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A satisfactory solution for the crayfish heart having been obtained and tested[1], it appeared desirable to find a similar one for the lobster. The composition of lobster serum and of perfusing solutions for the lobster heart reported in the literature do not agree with the analyses of mixed lobster serum recently obtained[2], (van't Hoff, quoted by Rogers, 1927; Hogben, 1925; Macallum 1909-10; Clark, 1927; Bethe and Berger, 1931). Our analyses were made on the mixed serum from 18 live, large lobsters, *Homarus americanus* collected from Mount Desert Island and shipped on ice by express to New Brunswick, New Jersey. The blood was collected (36 hours after removal from sea water) from a ventral puncture near the abdominal-thoracic junction. It dripped into a paraffined vessel and was constantly whipped to defibrinate. After removal of the fibrin by centrifugation, a small amount of toluene was added, and the serum stored at low temperature until ready for use. The pH of the serum before centrifugation was 7.45 according to the quinhydrone electrode. Later determinations on 10 indi-

vidual lobsters by the glass electrode within 30 minutes of removal from sea water, varied from 7.34 to 7.52, with an average pH of 7.44. Depression of the freezing point of the mixed serum was —1.829°. Later measurements on 3 lobsters just removed from sea water varied from —1.7481° to —1.7510° with an average of —1.7495°. This indicates that the lobster loses considerable water from its blood during the first few hours out of sea water, even though kept at low temperatures.

The analyses for Na and K were made by the method of Hillebrand and Lundell (1929); for Ca by that of Kramer and Tisdall (1921); and for the other ions by those of Rieman and Neuss (1937). The averages of duplicate determinations, expressed in milligrams per 100 grams of serum were as follows: Cl, 1575; SO_4, 40.2; Na, 987.0; K, 53.8; Ca, 97.2 and Mg, 20.4. The maximum errors in the analyses were 3.0% for K; 1.0% for Na, Ca, Mg and SO_4; and 0.1% for Cl. In terms of molarity the concentrations of the salts were roughly as follows: NaCl, 0.430; KCl, .014; $CaCl_2$, .024; $MgCl_2$, .004 and $MgSO_4$, .004. The carbonate was estimated to be equivalent to .002M $NaHCO_3$. These values agree best with those of Bethe and Berger (1931) and differ widely from those of Macallum (1909-10), especially as to Ca. Since the analyses did not include phosphates or organic constituents, and since the $\triangle°$ of a solution made according to that composition was only —1.6576°, the molarity was increased to 0.5. The solution adopted as best fitting the lobster was, therefore, as follows: NaCl, .452M; KCl, .015; $CaCl_2$, .025; $MgCl_2$, .004 and $MgSO_4$, .004. Carbonate could not be used to adjust the pH, so small amounts of NaOH were added to bring the pH to 7.4. Although this solution was not very well buffered, its pH would decrease only 0.1 over a period of several hours if protected from the air. The freezing point depression was —1.777°. In experiments on the lobster heart described below that solution (No. 2) was used as "normal" and all other solutions were compared with it.

The heart was prepared for perfusion as follows: pieces of the carapace and of the body wall between the eyes and the cervical grooves were removed. The oesophagus, stomach, liver, reproductive glands and the major part of the intestine were then removed, while the animal was held vertically head downwards to avoid alimentary or liver juices coming in contact with the pericardium. The cavity was thoroughly washed by the solution (No. 2), and the ganglionic nerves were severed by cutting along each side of the ganglion chain under the pericardial region. The anterior wall of the pericaridum was then punctured on each side exposing the pericardial cavity containing the heart. A small pinch clamp 8 mm. long, made of rustless steel wire (B. & S. gauge No. 25) on the principle of the familiar test tube clamp, was fastened to the mid-anterior

region of the pericardium. The animal was securely fastened in a vertical position, and a fine thread was attached from the clamp to a delicately balanced heart lever. A glass tube with a bore of about 2.0 mm. at its tip and a funnel-shaped opening at the upper end was fastened half way down in the pericardial chamber at one side of the heart. The perfusing solution flowed into the tube at a constant rate (10 ± 2 cc. per minute) and temperature (17.0 ± 0.4° C.), filling the chamber and overflowing around the top. Different solutions could quickly be substituted at the same rate of flow and compared with each other. Clock time and frequency of beat, measured by a stop watch, were recorded on the kymographic record along with the heart movements.

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. The frequency at that time varied from 63 to 135 per minute for the 45 lobsters used, the average being 87. In a large percentage of animals the beating of the heart was extremely regular and the amplitude of successive beats was constant. A few hearts were irregular both as to frequency and amplitude and rarely ever became regular afterwards.

It was first found that sea water and van't Hoff's solution were very poor perfusing media. With the former hearts might beat for as long as one hour; with the latter up to 4 hours. On solution 2, however, hearts continued "normal" beating for as long as 26 hours, although showing a very gradual decline in frequency and amplitude. It is believed that injury to the heart by the operation or by the clamp is the most common cause of failure of hearts perfused primarily by solution 2, if failure occurs before 5 hours. Since the solution contains no nutritive materials and none of the organic constituents of lobster blood, it is not surprising that the heart stops eventually. Such arrest occurs in diastole without any significant change in tone. Solution 3 was prepared with amounts of KCl, $CaCl_2$ and Mg salts intermediate between van't Hoff's and solution 2. Compared to the latter it caused slightly decreased rate and amplitude, although the heart would beat for as long as 6 hours. Obviously it was a much better solution than van't Hoff's, but not as good as No. 2.

Contradictory results were often obtained during the early experiments until it was discovered that the heart's behavior to solutions might depend upon previous treatment by very poor solutions. For instance, perfusion with solution No. 2 might cause diastolic arrest if preceeded by a half hour's perfusion with van't Hoff's solution. Recovery to normal would occur during the next hour on No. 2, indicating that the effect of van't Hoff's was temporary. Other solutions, to be mentioned later, might cause irreversible injury. It was, therefore, necessary to use fresh hearts or to be sure that previous per-

fusions were not very harmful, in order to avoid errors of interpretation. In general old hearts appeared to be more sensitive to changes in ionic content than fresh ones, although the type of response remained the same, unless serious injury of the heart had occurred.

Varying the pH of solution 2 from 7.0 to 8.0 caused no significant changes in frequency, tone or amplitude. At pH = 6.8 and 8.2, however, slight increases in tone and rate with decreases in amplitude almost always appeared. The pH of all solutions was, therefore, kept between 7.3 and 7.5 to avoid any effects due to the hydrogen ion.

To determine the relative roles of each cation and the best mixture of them, 30 other solutions of significant combinations were tested and compared to solution 2. The compositions and effects on heart are displayed in Table 1. In addition, single solutions of the salts, glucose and urea were tested (at 0.5 molarity for the former and 1.0 for the latter two).

The results of perfusion by those solutions may be briefly described as follows. Arrest of the heart in systole or near systole was caused by NaCl, urea or KCl single solutions; in less than 15 minutes by NaCl, less than 5 minutes by urea and less than 30 seconds by KCl. Arrest in diastole was caused by single solutions of $MgSO_4$, $MgCl_2$, $CaCl_2$ and glucose, all in from 6 to 50 seconds respectively. Recovery from those solutions occurred in reverse order of effectiveness for arrest, in from 30 seconds to 4 minutes. The binary mixture of KCl and $CaCl_2$ (No. 8) allowed the heart to beat for 25 minutes before arrest in three quarter's systole, although marked irregularity and increased rate appeared at the end of 3 minutes. The binary mixtures of KCl and $CaCl_2$ (No. 9), KCl and $MgCl_2$ (No. 22), KCl and $MgSO_4$ (No. 23) all caused arrest in systole or three-quarters systole, which is a typical potassium effect. Binary mixtures of NaCl and $CaCl_2$ (Nos. 10, 13, 27, 7 and 28) showed that even small amounts of Ca prevent the quick injury of pure NaCl, although almost the full amount of Ca is necessary to allow normal beating for any considerable time (see Sol. 7). Slight excess of Ca (No. 28) causes increased tone, decreased frequency and systolic arrest in about 30 minutes. Mixtures of NaCl and $MgCl_2$ or $MgSO_4$ (Nos. 20-21) caused slightly increased rate and tone and could be used for as long as 10 minutes before irregularity or arrest in systole. Never as good as the NaCl — $CaCl_2$ mixtures, they were somewhat better than pure NaCl, however.

Results from solutions containing no Mg, or SO_4 (Nos. 5, 6) and from those in which the Mg or SO_4 content was decreased (Nos. 14, 17-19, 23-24) indicated quite clearly that neither ion is of particular importance for the heart. Solution 6 with no SO_4 could be used for several hours without any significant effect, and solution 5 with no Mg was satisfactory for almost

as long although the frequency and tone were usually increased slightly.

Solutions containing no Ca (Nos. 16, 8, 17, 20, 21) all produced increased frequency and decreased amplitude with occasional slightly increased tone and irregularity followed by arrest in three quarters systole. The presence of some Mg seemed to improve the solution (16, 17). Absence of K (Nos. 18-19) also caused increased frequency followed by eventual arrest in diastole just as in the binary mixtures of Na and Ca. Absence of Na (Nos. 9, 22-25) regularly caused quick arrest, in systole when K was present (9, 22-23) and in diastole when only Mg and Ca were present (24-25). Doubling the Mg (No. 4) caused only a slightly increased frequency and amplitude, allowing the heart to beat normally otherwise for many hours.

Solutions in which Li replaced Na (No. 29), and Br or I replaced Cl (Nos. 30, 31) were all unsatisfactory, but in different degrees. I caused very quick arrest in diastole; Li an increased frequency and tone followed by arrest in one half systole within 6 minutes; Br appeared satisfactory for a few minutes (up to 12) causing only a slightly decreased rate and amplitude.

Halving the concentration of solution 2, which reduced the osmotic pressure by approximately one half, (No. 33), caused marked increased frequency and decreased amplitude accompanied by slightly increased tone. Arrest occurred, however, in diastole in about 20 minutes. Using the solution developed for the crayfish (No. 32) with some less K, more Ca and less Mg at about one half the osmotic pressure, likewise caused increased frequency and tone, and decreased amplitude. Arrest followed within 15 minutes in diastole. If the concentration of the crayfish solution was almost doubled (No. 34), no significant effect was noticeable for about one hour. Hypertonic solutions were not used.

From all the results it became clear that solution 2 which agrees very closely with the inorganic content of the serum of the lobster, is the best solution for the heart, allowing it to beat normally for from 18 to 26 hours. Compared to the solutions used by Hogben (1925) and by Zoond and Slome (1928-29) it differs markedly in the content of Ca and K. The numbers of Ca and K ions per 100 Na ions in their solutions were only 0.8 and 1.0 respectively, which obviously were so low that beating could continue for only a short time (up to 3 hours; Hogben). Zoond and Slome reported that the heart of Palinurus "will maintain a normal rhythm almost indefinitely on a suitable mixture of sodium and calcium chlorides," the optimum ratio being 100 cc. of 0.6 M NaCl plus 5 cc. of 0.5 M $CaCl_2$. Expressed in terms of moles this ratio is also altogether too low. What is meant by "almost indefinitely" and "long periods of time" is not explained in the report. Hogben stated that

TABLE 1

Compositions and physiological effects of various solutions on the lobster heart. Total molarity was 0.5 excepting Nos. 32 and 33. pH = 7.3-7.5. Temp. = 16.6-17.4°C. Rate of flow = 10 cc./min.

Soln— No.	NaCl M	KCl M	$CaCl_2$ M	$MgCl_2$ M	$MgSO_4$ M	No ions per 100 Na K	Ca	Mg	SO_4	Effects on heart
2	.452	.015	.025	.004	.004	3.32	5.53	1.77	0.885	Normal beating for 26 hours
v.H.*	.454	.010	.010	.035	.017	2.2	2.2	11.45	3.74	Decreased rate & amplitude
3	.442	.012	.017	.019	.010	2.72	3.85	6.56	2.26	Slightly dec. rate & ampl.
6	.452	.015	.025	.008	.....	3.32	5.53	1.77	.....	No effect for 3 hours
5	.460	.015	.025	.....	.....	3.26	5.44	.....	.....	Slightly inc. rate & tone
4	.444	.015	.025	.012	.004	3.38	5.63	3.60	0.91	Slightly inc. rate & ampl.
18	.470	.....	.026	.004	.....	.....	5.53	0.85	.....	Arrest in diastole
19	.470	.....	.026	.....	.004	.....	5.53	0.85	0.85	Arrest in diastole
16	.480	.016	.....	.004	.....	3.33	.....	0.83	.....	Arrest in ¾ systole
8	.484	.016	.....	.....	.....	3.30	.....	.....	.....	Arrest in ¾ systole
17	.480	.016	.....	.....	.004	3.33	.....	0.83	0.83	Arrest in ¾ systole
20	.492	.....	.....	.004	.004	.....	.....	1.62	0.81	Arrest in ¼ systole
21	.496	.....	.....	.004	.....	.....	.....	0.81	.....	Arrest in ¼ systole
9	.....	.188	.312	.....	.....	Ca/K: 1.7		.....	.....	Arrest in systole or ¾ sys.
22	.....	.395	.....	.105	.....	Mg/K: 0.26		.....	.....	Arrest in systole
23	.....	.395	.....	.....	.105	Mg/K: 0.26		.....	.....	Arrest in ¾ systole
24	.....	.....	.431	.....	.069	Mg/Ca: 0.16		.....	.....	Arrest in ½ systole
25	.....	.....	.431	.069	.....	Mg/Ca: 0.16		.....	.....	Arrest in ¼ systole
10	.497	.....	.003	.....	.....	.....	0.6	.....	.....	Dec. rate & irregularity
11	.492	.....	.008	.....	.....	.....	1.63	.....	.....	Dec. rate & irregularity
12	.487	.....	.013	.....	.....	.....	2.67	.....	.....	Dec. rate & irregularity
13	.481	.....	.019	.....	.....	.....	3.95	.....	.....	Sl. dec. rate & irregularity
27	.476	.....	.024	.....	.....	.....	5.02	.....	.....	Some irregularity
14	.458	.015	.023	.....	.004	3.28	5.00	0.87	0.87	Normal beating for 10+ hours
7	.474	.....	.026	.....	.....	.....	5.49	.....	.....	No effect 1 hr.; then irreg.
28	.471	.....	.029	.....	.....	.....	6.16	.....	.....	Inc. tone & dec. rate
29	.452 Li	.015	.025	.004	.004	3.32	5.53	1.77	.885	Arrest in ½ systole
30	.452 Br	.015	.025	.004	.004	3.32	5.53	1.77	.885	Dec. rate & ampl.
31	.452 I	.015	.025	.004	.004	3.32	5.53	1.77	.885	Quick arrest in diastole
32	.205	.0054	.0135	.0026	.....	2.63	6.58	1.27	.....	Inc. rate & tone & dec. ampl.
33	.226	.0075	.0125	.0020	.....	3.32	5.53	0.89	.....	Inc. rate & tone & dec. ampl.
34	.410	.0108	.0270	.0052	.....	2.63	6.58	1.27	.....	No sig. effect for 1 hour

*van't Hoff's soln—

beating continued for "3 or 4 hours." Evidently this was his maximum period.

As indicated earlier in this report, the lobster heart may beat in very poor solutions for as long as 3 hours, providing minimum amounts of Ca and K be present and the total molarity is about 0.5. When returned to a solution which corresponds with the inorganic composition of the serum, however, quick arrest will probably occur, and recovery of the beat may not take place for several minutes. Such tests are bound to lead to incorrect interpretation, as admitted by Zoond and Slome in a footnote (p. 91) concerning the sensitivity of the heart to K. From the results reported herewith, it is demonstrated that for the lobster heart a solution containing the same proportion of all the major inorganic ions found in serum should be used on fresh preparations, in order to show the fundamental effects of each ion or any combination of ions. A simplified solution, such as Hogben's or Zoond and Slome's, changes the character of the heart, and may cause atypical results. In spite of their poor solutions, however, they found the same kind of arrest for Na, Ca, K and Mg as reported here.

Compared with the crayfish, the lobster heart is far less sensitive to changes in ionic content of the perfusing solution, but qualitatively it requires the same ions — Na, K, Ca and Cl, and is the most sensitive to changes in Ca content than to any other ion. Contrasted to the crayfish[1] it gave no response to adrenalin unless the dose was very large (1 to 100). In over 50 tests on 8 different lobsters acceleration was observed only once. Large doses caused decreased frequency and amplitude. The response to adrenalin will be studied again to clear up any doubts concerning it.

FOOTNOTES

[1]Unpublished results.

[2]We are indebted to Mr. Wilbur H. McComas, Rutgers University, for making the analyses.

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