THE BEHAVIOR OF ISOLATED HEARTS OF THE GRASSHOPPER, CHORTOPHAGA VIRIDIFASCIATA, AND THE MOTH, SAMIA WALKERI, IN SOLUTIONS WITH DIFFERENT CONCENTRATIONS OF SODIUM, POTASSIUM, CALCIUM, AND MAGNESIUM

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Insect tissue is able to maintain its activities in very abnormal surrounding media. However, there is an obvious need to determine the exact tolerance of insects to different ions. This information may lead to the formation of an adequate insect physiological solution. It is the purpose of this investigation to determine the tolerance of isolated insect hearts to different concentrations of sodium, potassium, calcium, and magnesium ions, as well as to changes in the osmotic pressure of the surrounding medium. These experiments were performed on a representative insect from each of the two groups described by Florkin, Duchâteau, and Leclercq (1949). Those selected were the adult grasshopper, *Chortophaga viridifasciata*, which typifies insects with a high sodium and a low potassium index (the percentage of a cation in relation to the sum of the cations is its index), and the pupae of the moth, *Samia walkeri*, which typify those with a low sodium and high potassium index.

Florkin, Duchâteau, and Leclercq (1949) divided insects into two groups, distinguished by differences in the concentration of sodium and potassium in the blood: those in which the sodium index is high and that of potassium low, and those in which the reverse relationship exists. They postulated that insects with a high potassium and low sodium index, such as the moth, *Samia walkeri*, have developed in the Tertiary in connection with the development of higher plants. Insects having a high sodium index were found to be either the ancestral forms or those whose development was independent of the evolution of plants. They also stated that the blood of insects with a low sodium and high potassium index has a higher magnesium contant than that of the more primitive insects.

Most saline solutions used in insect work are variations of frog Ringer solution, and completely fail to duplicate the concentration of ions found in insect blood. There have been two principal methods used in the formulation of insect saline solutions. Lewis and Robertson (1916) adapted the classical Ringer-Locke solution to insect material. They tried to formulate a medium which would have nutrient components in addition to protective, non-toxic qualities. They took the mineral salt concentration of Ringer-Locke solution and added protein broth and glucose as nutrients. The second method was used by Levy (1928) who stated that he based the composition of his saline solution on the available data concerning the salt content of insect blood. However, he failed to add magnesium, although the high content of this ion is one of the characteristics which differentiates insect blood from that of other animals. Davenport (1949), in his studies on the pharmacology of the heart of the grasshopper, *Stenopelmatus*, used frog Ringer solution. He claimed to obtain better results with this solution than with saline solutions devised especially for insect material.

Evidence of the varied composition of different saline solutions may be obtained by comparing the following solutions: Yeager's (1939), Pringle's (1938), and Griffith's and Tauber's (1943). All three solutions were made especially for the American cockroach, *Periplaneta americana*, and yet they have very dissimilar ion concentrations and osmotic pressures.

Material and Methods

The method for the preparation of glassware was that of Gese (1950) with the exception that no permanganate bath was used.

Nymphs of the grasshopper, *Chortophaga viridifasciata*, were collected in the field during the fall of 1952. They were placed in cages, kept at room temperature, and fed on a diet of fresh lettuce. Only adult grasshoppers were used for the experiments. Pupae of the cynthia moth, *Samia walkeri*, were collected in the fall of 1952, and were kept in the refrigerator to prevent development.

The blood of each species was analyzed for cation concentration and osmotic pressure. Blood was taken from the grasshopper by removing a metathoracic leg with a sharp scissors at the joint where the coxa articulates with the thorax; and from the cynthia pupa, by puncturing the cuticle at the tip of an antenna with a glass needle. In both cases, the blood was allowed to drop into a depression of a porcelain spot-plate, while pressure was exerted on the abdomen.

Each analysis of the cation content of grasshopper blood was made on 0.3 to 0.5 ml. of pooled blood. It was carefully measured and placed into a vycor crucible. The pipette was rinsed twice with glass-distilled water, and the rinsings were added to the blood in the crucible. It was then placed in a muffle oven and maintained at 500°C. for 18 to 24 hours, at which time the hemolymph was converted to a white ash. When cool, the ashed sample was dissolved in 5 ml. of 0.1 N HCl. This solution was transferred to a small beaker of the Beckman flame photometer. Transmission readings of the sodium, potassium, calcium, and magnesium content were made, after having first adjusted the photometer with standard solutions containing known amounts of each ion.

Protein and non-protein nitrogen were determined as follows. The protein was precipitated from the blood by the addition of trichloracetic acid. It was separated by centrifugation. The nitrogen content of each fraction was then determined by the micro Kjeldahl procedure. Details of this method are given by Ludwig (1951).

The osmotic pressure of the blood of the grasshopper was measured by Barger's vapor pressure method. The blood was equilibrated against known solutions of NaCl. Details of this procedure are given by Ludwig (1951). The freezing point depression of the blood was obtained by freezing the isotonic NaCl solution in a Beckman freezing point apparatus. That of the blood of the cynthia moth was measured directly by freezing 15 ml. of blood in a Beckman apparatus. The osmotic pressure (in atmospheres) of the blood of each species was calculated from its freezing point depression.

The effects of various solutions on the heart beats of each species were determined by the following procedure. The hearts were dissected by the method of Davenport (1949) who severed the thorax from the abdomen, and with fine scissors separated the abdominal tergites from the sternites. The dorsum was pinned, ventral side up, to a paraffin tray with fine glass pins, and the heart was exposed by removing the internal organs lying above it. The hearts were bathed with the experimental solution which was continuously oxygenated. The apparatus used was similar to that devised by Yeager and Hager (1934). The temperature was kept constant at 25°C. The hearts were observed through a binocular dissecting microscope; and by use of a stop watch, the rate of heart beat was determined. Readings were taken every 5 minutes for the first 3 hours. The adequacy of a solution to support heart beats was determined by the regularity of the beats during this time period. By regularity is meant a uniform amplitude of beats and a relatively constant rate of beating. A solution was also evaluated by the ability or inability of the immersed hearts to beat for 24 hours. When the experiment was continued overnight, the paraffin tray with the hearts was submerged in the test solution contained in a 1 liter beaker. The solution was aerated by means of a capillary tube leading from the aerating pump, the tube being placed close to the surface so that there would be no strong currents detrimental to the hearts.

Solutions of the four chloride salts isotonic to the blood of the insect were prepared and their effects tested. The molality of the potassium, calcium, and magnesium chloride corresponding to that of isotonic sodium chloride was determined from the table of "G" values given in Heilbrunn (1952).

After ion determinations had been made on the blood, the ratios in milligrams per cent of these ions to one another were determined. Isotonic solutions of sodium, potassium, calcium, and magnesium chloride were then mixed in such a way that the ions in the resulting solutions were in the same ratio as found in the blood. However, since the osmotic pressure of insect blood is determined largely by a high concentration of organic materials (Ludwig, 1951), these ions were in greater concentration than that found in the blood. Before this could be done, however, it was necessary to determine the amount (in grams) of ions found in each of the isotonic solutions of their chloride salts.

The effects of osmotic pressure on the heart beat of the grasshopper and the cynthia moth were determined by taking an isotonic solution found favorable to each insect and varying its osmotic pressure. Hypotonic solutions were prepared by diluting the isotonic solution; and hypertonic solutions, by adding a determined amount of the proper mixture of the chloride salts.

OBSERVATIONS

The concentration of sodium, potassium, calcium, and magnesium ions, as well as the osmotic pressure of the blood of *Chortophaga viridifasciata* and of

Samia walkeri, is listed in Table I. The blood the grasshopper has a high sodium index of 68. (The index of a cation is the per cent of that ion in terms of the total cation concentration, expressed as milliequivalents per liter.) The blood of the moth has a sodium index of less than 1. Ratios of sodium to potassium, potassium to calcium, and calcium to magnesium were calculated from data contained in this table. These ratios for the grasshopper are Na to K, 19 to 1; K to Ca, 1.2 to 1; Ca to Mg, 1 to 4.5. Ion determinations on the blood of the cynthia moth pupa made with the Beckman flame photometer were found to agree with those of Gese (1950), and her results, therefore, were used as a basis for the ion concentrations of this insect. Calculations of ion ratios for the pupal

TABLE I

Composition of the Blood of C. viridifasciata and S. walkeri

Determination	*C. viridifasciata*	*S. walkeri**
	mg. per cent	*mg. per cent*
Sodium	250.66	5.97
Potassium	13.52	164.60
Calcium	11.40	37.71
Magnesium	51.15	79.08
Protein nitrogen	253.40	628.58
Non-protein nitrogen	140.00	441.20
	Osmotic pressure	
Freezing point depression	0.89°C. (calc.)	1.11°C. (obs.)
NaCl equivalent	0.25 molal (obs.)	0.33 molal (calc.)
Atmospheres	10.7	13.36

* Values of ion concentrations from Gese (1950).

blood of the cynthia moth are Na to K, 1 to 28; K to Ca, 4 to 1; Ca to Mg, 1 to 2.

The protein and non-protein nitrogen in the blood of cynthia pupae are approximately 3 times as concentrated as in the grasshopper, the sum averaging 393.4 mg. per cent in the latter, and 1069.8, in the former species.

The blood of the grasshopper was found to be isotonic with 0.25 M NaCl which has a freezing point depression of 0.89°C. Its osmotic pressure was calculated to be 10.7 atmospheres at 0°C. The blood of the cynthia pupa has a freezing point depression of 1.11°C. Its osmotic pressure is 13.36 atmospheres at 0°C.

From these data, it was calculated that KCl, $CaCl_2$, and $MgCl_2$ solutions isotonic with the blood of the grasshopper have the following molalities; 0.25, 0.18, and 0.17, respectively. Corresponding values for the cynthia moth are 0.33, 0.23, and 0.23.

The effects of isotonic solutions of pure chlorides of sodium, potassium, calcium, and magnesium on the heart beats of the grasshopper and the cynthia pupa were determined. In isotonic NaCl, the hearts of the grasshoppers quivered or twitched for an average of 5.7 minutes. After the twitching had ceased, the beats became faint and irregular, and the beating completely stopped after 25.5 minutes. In isotonic $CaCl_2$, the hearts beat for an average of 19 minutes, but the beats were very faint and barely perceptible. The hearts failed to beat in isotonic KCl, $MgCl_2$, or in distilled water. In isotonic NaCl, the hearts of the cynthia pupa beat for an average of $2\frac{1}{2}$ hours. During the first 2 hours the beats were regular and vigorous, but during the last 30 minutes, they became progressively fainter. In isotonic KCl, the hearts beat for an average of 68

TABLE II

Ratio of Na^+ to K^+ Necessary for Maintaining the Heart Beat of C. viridifasciata.
(Isotonic Chloride Solutions Used)

Amount of each solution used			Ratio of Na^+ to K^+	Beats/min.	Time persisted
NaCl	KCl	$CaCl_2$			
ml.	*ml.*	*ml.*	*mg. per cent*		*hrs.*
—	1.00	1.15	0 to 1	19.3	3+
1.75	1.00	1.15	1.0 to 1	24.6	3+
3.48	1.00	1.15	2.0 to 1	37.3	3+
5.22	1.00	1.15	3.0 to 1	41.4	24
6.48	1.00	1.15	3.7 to 1	38.2	24
11.28	1.00	1.15	6.5 to 1	38.7	24
32.26	1.00	1.15	18.5 to 1	38.5	24
Bělăr's solution*			34.1 to 1	40.5	24

* Bělăr's (1929) solution contains 9 gm. NaCl, 0.2 gm. KCl, 0.2 gm. $CaCl_2$ to each liter of solution. It was modified by additional amounts of salts to bring the osmotic pressure to 1 ½ times its original value. Glucose and $NaHCO_3$ omitted.

minutes. In isotonic $CaCl_2$ they beat for an average of 53.2 minutes. The beats were vigorous but were infrequent and irregular. In isotonic $MgCl_2$, the hearts beat for an average of 36.6 minutes, but were infrequent and very faint. In distilled water, they beat for an average of 45.6 minutes. At the start the beats were normal, but they became progressively slower and fainter. From these data it can be seen that for the grasshopper, sodium is the least toxic ion, followed by calcium. For the cynthia moth, the order of ions in relation to increasing toxicity is sodium, potassium, calcium, and magnesium.

The ratio of ions similar to that in grasshopper blood was obtained by using the following amounts of isotonic salt solutions: 32.26 ml. of NaCl, 1 ml. of KCl, 1.15 ml. of $CaCl_2$, and 8.69 ml. of $MgCl_2$. When this solution was tested on the hearts, irregularities were seen. Certain salts were then omitted, and different combinations tried, until it was found that by eliminating $MgCl_2$

altogether, an excellent physiological solution was obtained. Hence, it may be concluded that magnesium is not necessary to maintain heart beats.

Table II shows the various solutions used in determining the ratio of sodium to potassium necessary for maintaining the heart beat of the grasshopper. When the ratio of sodium to potassium is less than 3 to 1, there is some irregularity in the heart beat; but when the ratio is 3 to 1, or when more sodium is present, the beats are regular and persist for 24 hours. The ratio of sodium to potassium can go up to at least 34 to 1 without any appreciable effect.

The ratios of potassium to calcium necessary for maintaining the heart beat of the grasshopper were determined, and the results are shown in Table III. On the basis of milligrams per cent, when this ratio is 1 to 1, 2 to 1, or 3 to 1,

TABLE III

Ratio of K^+ to Ca^{++} Necessary for Maintaining the Heart Beat of C. viridifasciata. *(Isotonic Chloride Solutions Used)*

Amount of each solution used			Ratio of K^+ to Ca^{++}	Beats/min.	Time persisted
NaCl	KCl	$CaCl_2$			
ml.	*ml.*	*ml.*	*mg. per cent*		*hrs.*
32.26	—	1.15	0 to 1	23.3	3+
32.26	1.00	1.38	1 to 1	39.0	24
32.26	2.00	1.38	2 to 1	42.0	24
32.26	3.00	1.38	3 to 1	43.5	24
32.26	4.00	1.38	4 to 1	27.7	3+
32.26	1.00	2.76	1 to 2	42.2	24
32.26	1.00	4.14	1 to 3	43.8	24
32.26	1.00	5.52	1 to 4	27.4	3+

the resulting physiological solutions are excellent. However, if the potassium is increased so that the ratio is 4 to 1, irregularities are seen in the heart beat. Very slight irregularities are seen in the beats of hearts treated with solutions having a potassium to calcium ratio of 1 to 2 and 1 to 3; the beating persists for 24 hours. However, when the ratio is decreased so that the potassium to calcium is 1 to 4, the beats are very irregular. Therefore, a good physiological solution for the grasshopper must have this ratio 1 to 1, or it may be increased to a value of 3 to 1.

A solution with its sodium, potassium, calcium, and magnesium content in the same ratio as found in the blood of the cynthia pupa contains the following amounts of isotonic salts: 1 ml. of NaCl, 16.17 ml. of KCl, 5.23 ml. of $CaCl_2$, and 17.95 ml. of $MgCl_2$. However, irregularities in the beats were obtained with this solution. When no $MgCl_2$ was present the solution was found to be superior to one containing this salt; however, it was not an excellent solution. When the potassium content was decreased to one-half its original value, the resulting solution was satisfactory.

Observations made through the dorsal body wall of the cynthia pupa, showed that the heart beat *in situ* is irregular; therefore, as was expected, the beats in all the experimental solutions used were also irregular. Hence, the ratios of ions required to maintain normal heart beats were determined, for the most part, by the ability of the hearts to beat in the solutions for 24 hours.

Table IV lists the various solutions used to determine the ratios of sodium to potassium necessary for maintaining the heart beat of the cynthia pupa. It was found that if the ratio of sodium to potassium, was 1 to 13.8 or if the sodium content was high, the irregularities were very slight and the hearts would beat for 24 hours. Therefore, it may be concluded that with the cynthia pupa, only a small amount of sodium is required to have a good physiological solution.

TABLE IV

Ratio of Na^+ to K^+ Necessary for Maintaining the Heart Beat of S. walkeri.
(Isotonic Chloride Solutions Used)

Amount of each solution used			Ratio of Na^+ to K^+	Beats/min.	Time persisted
NaCl	KCl	$CaCl_2$			
ml.	*ml.*	*ml.*	*mg. per cent*		*hrs.*
—	8.08	5.23	0 to 1	11.0	5+
0.50	8.08	5.23	1 to 27.6	10.8	5+
1.00	8.08	5.23	1 to 13.8	13.6	24
4.04	8.08	5.23	1 to 3.4	13.2	24
8.08	8.08	5.23	1 to 1.7	12.9	24
16.00	8.08	5.23	1.2 to 1	13.4	24
64.60	8.08	5.23	4.7 to 1	12.9	24
18.54	0.59	0.70	18.4 to 1	15.2	24
Bêlâr's solution*			34.1 to 1	13.5	24

* See footnote below Table II.

However, if a great deal of sodium is added, at least as much as 34 parts of sodium to 1 of potassium, there is no appreciable effect on the heart beat.

Table V shows the effect of different ratios of potassium to calcium on the pupal heart beat of the cynthia moth. The ratio of potassium to calcium, may vary from 1 to 1 to a limit of 3 to 1 without any harmful effects. If this ratio is increased or decreased beyond these limits, the resulting solutions are toxic. If the ratio of potassium to calcium is decreased below unity, the solutions are very injurious to the heart. On the other hand, if the ratio is increased above 3 to 1, the resulting toxic effects are only slight.

The effects obtained by varying the osmotic pressure on the heart beat of the grasshopper are given in Table VI. When the osmotic pressure is increased to 1 ¼ times the isotonic value, the resulting solution is satisfactory. However, if the osmotic pressure is increased to 1 ½ times its isotonic value, slight irregularities are seen; when it is increased still further, great irregularities and slow heart beats are obtained. With these very hypertonic solutions the heart beats are vigorous, although often very infrequent. On the other hand, with hypotonic solutions the heart beats are very faint and also irregular.

TABLE V

Ratio of K^+ to Ca^{++} Necessary for Maintaining the Heart Beat of S. walkeri. (Isotonic Chloride Solutions Used)

Amount of each solution used			Ratio of K^+ to Ca^{++}	Beats/min.	Time persisted
NaCl	KCl	$CaCl_2$			
ml.	*ml.*	*ml.*	*mg. per cent*		*hrs.*
16.00	—	5.23	0 to 1	2.4	3+
18.54	0.59	0.70	1.1 to 1	15.2	24
16.00	8.08	5.23	1.9 to 1	13.4	24
Yeager's solution*			2.6 to 1	17.7	24
16.00	13.31	5.23	3.2 to 1	17.7	5+
16.00	16.17	5.23	3.9 to 1	20.8	5+
16.00	1.00	2.80	1 to 2.2	7.6	5
16.00	1.00	4.20	1 to 3.4	2.2	3

* Yeager's solution contains 10.93 gm. NaCl, 1.57 gm. KCl, 0.85 gm. $CaCl_2$, 0.17 gm. $MgCl_2$ to each liter of solution.

TABLE VI

Effect of Different Osmotic Pressures on the Heart Beat of C. viridifasciata

Osmotic pesssure	Beats/min.	Duration of beating
atm.		
0 (distilled water)		Failed to beat
2.7		5 min.
5.4	38.1	3+ hrs.
8.1	36.6	3+ hrs.
10.7 (isotonic)	38.5	24 hrs.
13.4	41.0	24 hrs.
16.1	31.5	3+ hrs.
18.8	7.2	2½ hrs.
21.4	6.7	2½ hrs.

The results of different osmotic pressures on the heart beat of pupae of the cynthia moth are listed in Table VII. The hearts will beat normally in solutions having three-fourths of the osmotic pressure of cynthia blood. However, if the osmotic pressure is further decreased, the heart beats become faint and irregular. If it is increased above that of the blood, the rate of beating slows considerably, and the beats become irregular. The beating, however, is vigorous in hypertonic solutions as compared with the faintness characteristic in hypotonic solutions.

To determine whether or not all the effects obtained by varying the ionic concentrations of solutions were caused directly by the addition or omission of these ions, pH readings were made on the various solutions used. The pH values were found to vary from 5.51 to 7.3, and were in the range of values found by Suchyta (1954) to give normal heart beats for the cockroach, *Periplaneta americana*. The effects, therefore, were probably caused solely by the ions studied.

TABLE VII

Effect of Different Osmotic Pressures on the Heart Beat of S. walkeri

Osmotic pressure	Beats/min.	Duration of beating
atm.		
0 (distilled water)	9.7	45.6 min.
3.34	16.7	80.0 min.
6.68	14.0	3+ hrs.
10.02	18.6	24 hrs.
13.36 (isotonic)	17.2	24 hrs.
16.70	10.3	3+ hrs.
20.04	10.0	3+ hrs.
23.38	2.9	2½ hrs.
26.72	2.1	2½ hrs.

DISCUSSION

Boné (1944), in his determinations of the Na/K ratio in the blood of 27 different species of insects, found phytophagous insects to have ratios of less than one, while the carnivorous insects have ratios greater than one. *Chortophaga viridifasciata*, however, is an exception to this rule since it is a phytophagous insect with a ratio of Na/K of 19. In view of this observation, the grouping of insects by Florkin, Duchâteau, and Leclercq (1949) may be more accurate than that of Boné. These authors in 1953 reviewed the present knowledge of the cation content of insect blood, and placed all insects thus analyzed into their two original groups. Their observation with respect to the concentration of magnesium in the blood of these two groups was found to hold true for the two species studied in the present work. The blood of the cynthia moth contains 79.08 mg. per cent of magnesium, while that of the grasshopper, only 51.15 mg. per cent. The Ca/Mg ratios of these two phytophagous insects are in accordance with the findings of Clark and Craig (1953) who stated that phytophagous insects possess Ca/Mg ratios of less than unity.

Vertebrate skeletal muscle undergoes a rhythmic twitching or quivering when immersed in an isotonic solution of a sodium salt. Carlson (1907) described the relation of the normal heart rhythm of the horseshoe crab, *Limulus polyphemus*, to the artificial rhythm produced by NaCl. He found rhythmic twitchings corresponding to contractions of vertebrate skeletal muscle in the majority of heart preparations when placed in NaCl solutions. This same phenomenon was also found in the present investigation with the hearts of grasshoppers. When immersed in an isotonic NaCl solution, they quivered for an average of 5.8 minutes. On the other hand, the pupal hearts of cynthia moths did not react in this fashion. Their beats were normal for 2 hours following immersion in isotonic NaCl, and there was no quivering at any time.

The action of potassium on the heart beat of the flies, *Phormia regina* and *Calliphora erythrocephala*, was studied by Levy (1928). He found that if the potassium concentration in his insect saline solution was increased to 2, 3, or 4 times its original value (the ratio of NaCl/KCl in Levy's solution is 9.0/0.7), there was no change in the heart beat. After a certain limit, however, it caused an increase in the tonus of the heart, and systolic arrest. In terms of milligrams per cent, the ratio of Na/K in Levy's solution was 10. When the potassium concentration was increased to 4 times its original value, the ratio became approximately 3. This limiting ratio of 3 is in accordance with that found in this investigation for the grasshopper.

Bergerard (1947) worked with different insects having a high potassium index, and tried to diminish the ratio of sodium to potassium by balancing the systolic effects of potassium with the addition of calcium. He was not able to obtain normal beating, however, when the ratio of Na/K was below 3. He thought that possibly he would have succeeded had he replaced part of the calcium with magnesium. In the present studies, however, normal heart beats of the cynthia moth were obtained when the ratio of Na to K was 1 to 13.8 which represents one-half of this ratio found in the blood. When the ratio was further decreased so as to equal that found in the blood (1 to 27.6), the beating became very irregular. The irregularities were not diminished by an additional amount of calcium. Magnesium was substituted for part of the calcium, according to the suggestion of Bergerard, but with no success. It is probable, therefore, that at least one-half of the potassium in the blood of the cynthia moth is bound with the proteins or other organic compounds.

Griffiths and Tauber (1943) found satisfactory activity of the crop of the cockroach, *Periplaneta americana*, with ratios of $KCl/CaCl_2$ between 0.50 and 1.00. Although these ratios were calculated on the basis of the chloride salts and not ion concentrations, all lie close to the range of ion ratios of potassium to calcium found favorable to the heart of the grasshopper and the cynthia moth. Expressed as ionic ratios in milligrams per cent, those found by Griffiths and Tauber range from a value of 1 to 1.3 to one of 1.4 to 1. In this investigation, it was found that the ratios of K to Ca may vary from 1 to 1 to as much as 3 to 1.

Magnesium, except in small amounts, was found to be toxic to the heart of both the grasshopper and the cynthia moth, and hence its presence is not required for the maintenance of normal heart beats in these forms. Lindeman (1928) came to the same conclusion when he studied the effects of various ions on the heart beat of the crayfish, *Cambarus clarkii*. This observation was also verified by Cole, Helfer, and Wiersma (1939). Fiszer (1950), in his studies on the heart of the cricket, *Gryllus domesticus*, found that magnesium causes diastolic arrest which is preceded by faint, irregular beats. Similar faint, irregular beats were found with the heart of the cynthia moth, whereas that of the grasshopper failed to beat in isotonic $MgCl_2$. Contrary to the results found in this investigation, Fiszer found that the heart of *Gryllus domesticus* beat regularly when the ratio of Mg/Ca is less than 9. In the present studies, the apparent toxicity of magnesium, in spite of its high content in the blood, may be explained by the hypothesis that the magnesium is not free, but is also found with the protein or other organic constituents.

It is apparent that insect tissues have an unusual tolerance to the cation content of the surrounding medium. A possible explanation may be an impermeability to these cations, and hence the tissue remains unaffected. This possibility should be the subject of future studies.

Drieux (1950), in his study of the effects of osmotic pressure on the heart beat of the bee moth, *Galleria mellonella*, varied the concentration of Levy's solution between 0.5 and 1.5 times its original value. He found that hypertonic solutions retard the beats and increase the amplitude, and that hypotonic solutions have an opposite effect. His results are similar to those found in this investigation.

It was noted that the blood of the cynthia moth has an osmotic pressure of 13.36 atmospheres, and its heart will beat normally in solutions having osmotic pressures varying from 10.02 to 13.36 atmospheres. The blood of the grasshopper, on the other hand, has an osmotic pressure of 10.7 atmospheres, and its heart beats normally in solutions having an osmotic pressure of 10.7 to 13.4 atmospheres. Therefore, although the osmotic pressures of their blood are very different, their hearts appear to be tolerant to solutions having approximately the same osmotic pressure. Furthermore, since the ratios of potassium to calcium necessary for maintaining normal heart beats of both the grasshopper and the cynthia moth are the same—1 to 1 to as much as 3 to 1—solutions which are favorable to the grasshopper are also favorable to the cynthia moth. The converse of this statement, however, does not hold, for cynthia hearts can beat normally when the ratio of sodium to potassium is reduced to 1 to 13.8, whereas grasshopper hearts must have a ratio which equals or exceeds 3 to 1. These facts help explain the apparently consistent results obtained by bathing insect tissues in solutions such as frog Ringer solution. Further studies should be made to determine a range of osmotic pressures and ion concentrations favorable to the majority of insects. This information would facilitate the problem of formulating a satisfactory insect saline solution.

SUMMARY

1. The blood of *Chortophaga viridifasciata* was analyzed. The average concentrations of inorganic cations expressed as milligrams per cent are: sodium, 250.66; potassium, 13.52; calcium, 11.40; and magnesium, 51.15. The osmotic pressure of the blood at 0°C. is 10.7 atmospheres. Protein and non-protein nitrogen, expressed as milligrams per cent, are 253.4 and 140.0, respectively.

2. The blood of *Samia walkeri* has an osmotic pressure of 13.36 atmospheres at 0°C. Its protein nitrogen is 628.58, and its non-protein nitrogen, 441.20 milligrams per cent.

3. The effects of isotonic chloride solutions of sodium, potassium, calcium, and magnesium and of distilled water on the heart beat of these two species were determined. The heart of the grasshopper failed to beat in isotonic solutions of KCl, $MgCl_2$, or in distilled water. For both insects, sodium was found to be the least toxic ion. In the case of the grasshopper, calcium ranks next in order. In the case of the moth, potassium ranks next after sodium and is followed by calcium and magnesium.

4. The ratio of sodium to potassium in milligrams per cent, necessary for maintaining the normal heart beat of *Chortophaga viridifasciata* is 3 to 1, but it may be increased to at least 34 to 1 without any appreciable effects. The ratio of potassium to calcium necessary for maintaining the normal heart beat of this insect is 1 to 1, and may be increased to as much as 3 to 1.

5. The ratio of sodium to potassium, in milligrams per cent, necessary for maintaining the normal heart beat of *Samia walkeri* was found to be equal to or to exceed 1 to 13.8. The sodium content may be increased so that the ratio of sodium to potassium is 34 to 1 without any toxic effects. The ratios of potassium to calcium required for normal heart beat in this insect may be 1 to 1, 2 to 1, or 3 to 1.

6. The hearts of the grasshoppers beat normally in isotonic solutions having an osmotic pressure of 10.7 atmospheres. They beat equally well in solutions having an osmotic pressure of 13.4 atmospheres. The hearts of the cynthia pupae beat normally in isotonic solutions having an osmotic pressure of 13.36 atmospheres. However, they also beat normally in solutions having an osmotic pressure of 10.02 atmospheres. Therefore, although the blood of the cynthia moth and of the grasshopper have different osmotic pressures, their hearts are tolerant to solutions having the same tonicity. Because of this, and since the ratios of potassium to calcium necessary for maintaining normal heart beats of both insects are the same, solutions favorable to the grasshopper may also be favorable to the cynthia moth.

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