EFFECTS OF pH AND OF VARIOUS CONCENTRATIONS OF SODIUM, POTASSIUM, AND CALCIUM CHLORIDE ON MUSCULAR ACTIVITY OF THE ISOLATED CROP OF PERIPLANETA AMERICANA (ORTHOPTERA)*

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In 1940, Griffiths and Tauber (13) published a preliminary account of attempts to devise a physiological salt solution for the isolated foregut of the American roach, *Periplaneta americana* L. Initially, they tested insect physiological solutions which were recommended by Bělař (2), Hobson (16), Yeager (25), TenCate (23), and Glaser (10). Only the solutions of Bělař and Hobson produced results which were at all satisfactory for the excised crop of the American roach. Secondly, amounts of the chlorides of sodium, potassium, and calcium were varied, and it was found that 14.6 gm. of NaCl, 0.45 gm. of KCl, 0.50 gm. of $CaCl_2$, and 0.19 gm. of $NaHCO_3$ in 1 liter of water gave the best results among many mixtures tested, when contractions of the cockroach foregut were used as a criterion of adequacy.

The foregut of the American roach, used for these tests, may be divided into several more or less distinct regions: an esophagus, a crop or ingluvies, and a gizzard or proventriculus. The crop is composed of three layers of tissue (Fig. 1). The innermost layer is the cuticula, which, in section, appears to be of varying thickness, to have a roughened inner margin, and to be lined as though it were stratified. The middle is composed of a single layer of large epithelial cells. Muscle tissue surrounds the outside of the crop. Irregularly placed longitudinal fibers lie inside the encircling transverse muscles. All fibers are distinctly striated, with well defined sarcomeres. The activity of these muscles fibers was studied in the present investigation. Only those contractions which resulted in change in length of the ingluvial region, and hence in movement of the attached lever were recorded in the work described here. Neither simple peristalsis, anti-peristalsis, nor proventricular activity appeared to influence the action of the lever.

Method

The apparatus used for recording muscular activity of the crop in various salt mixtures was the same as that previously described by Griffiths and Tauber (13). The

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method involved use of a glass tube through which the solution to be tested was passed, and in which the crop was suspended from the recording lever. This lever was a capillary glass tube so arranged that actual amplitude of movement was magnified seven times as it was recorded upon a slowly revolving kymograph drum which made one revolution in 16 hours. Solutions were all aerated with oxygen. All work was done at room temperature, usually between 22 and 26°C.

Test solutions were made up in distilled water redistilled from glass. Various mixtures of chemically pure sodium chloride, potassium chloride, sodium bicarbonate, calcium chloride, sodium dihydrogen phosphate, hydrochloric acid, and sodium hydroxide were used in the experimental solutions. Concentrated stock solutions

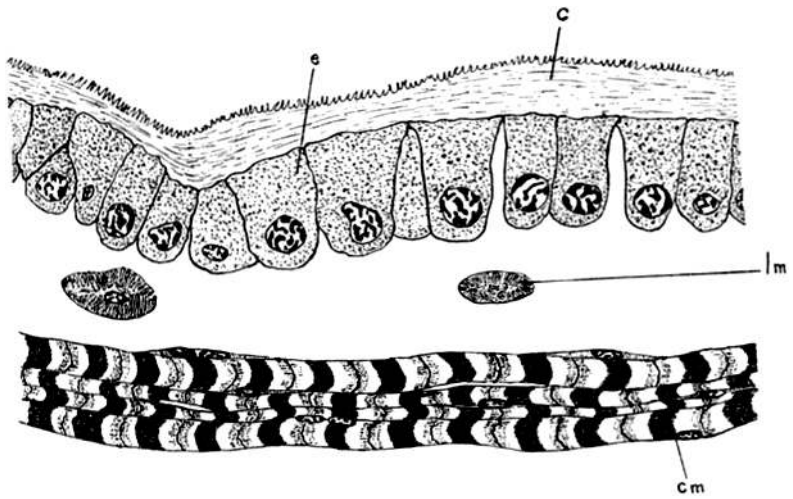

FIG. 1. Drawing of portion of cross-section of crop from *Periplaneta americana* c = cuticula; cm = circular muscle; e = epithelium; lm = longitudinal muscle.

were made of each of the above chemicals to facilitate preparation of dilutions to be tested.

Insects used in these investigations were adult specimens of the American cockroach, *Periplaneta americana* L. Only healthy individuals, between the ages of 15 and 100 days, were used. The roaches had all been laboratory-reared, at room temperature, on a diet of whole wheat bread, banana, raw beef steak, and Pablum.

Ten foreguts, five from male roaches and five from females, were tested in each solution. Experimental animals were usually starved for 48 hours and then were given access to a mixture of ground starch and carmine which had been dampened with water. This colored food was used to determine food intake of specimens used for experimentation on the following day.

Each isolated crop preparation was allowed to run until failure to maintain spontaneous contractions terminated the experiment. Since the speed of the kymograph drum was known, duration of activity could be calculated in hours. Every 20 min-

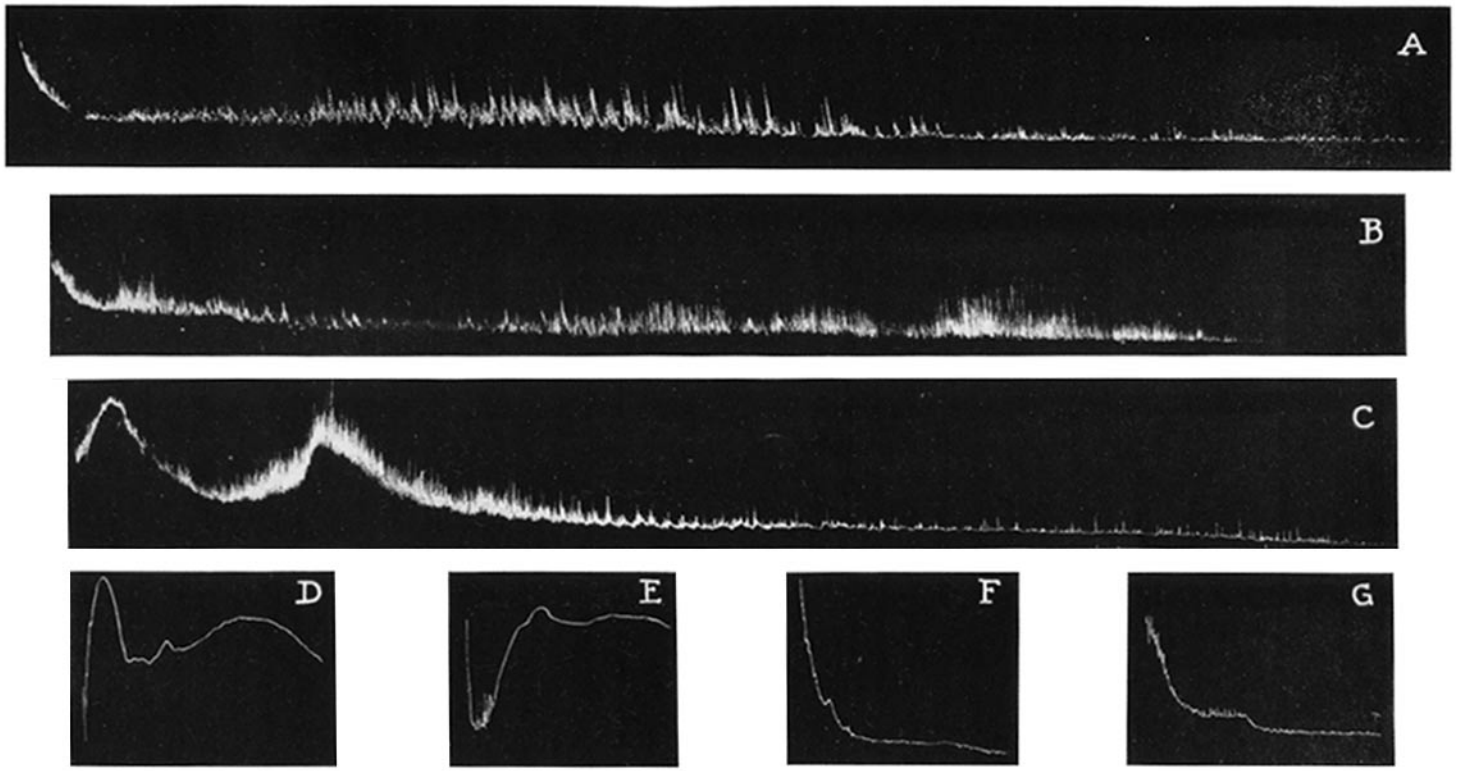

FIG. 2. Typical kymograph records of contractions of isolated American roach foreguts in salt solutions at pH of 7.8.

A, record from male in 1.4 per cent NaCl, 0.02 per cent KCl, 0.04 per cent $CaCl_2$. B, male, 1.4 per cent NaCl, 0.02 per cent KCl, 0.02 per cent $CaCl_2$. C, female, 1.0 per cent NaCl, 0.02 per cent KCl, 0.02 per cent $CaCl_2$. D, female, 1.0 per cent NaCl, 0.02 per cent $CaCl_2$. E, female, 1.0 per cent NaCl, 0.04 per cent $CaCl_2$. F, male, 1.0 per cent NaCl. G, female, 1.0 per cent NaCl, 0.02 per cent KCl.

utes, or at intervals of 1 cm. on the record, a measure of amplitude of contraction was made in centimeters. When these amplitude figures were averaged and corrected for lever amplification, the numerical product was the actual average amplitude of contraction in centimeters. Multiplication of the actual average amplitude and the time for duration of activity yielded an *activity product* in centimeter-hours which was used as a measure of activity of the individual crop and, subsequently, as a criterion of adequacy of a given solution. Because it was necessary to use a very slowly revolving kymograph drum, individual strokes of the recording lever were so close together that it was impossible to measure frequency of contraction in the present work (Fig. 2). Therefore, only amplitude and duration of activity were used to calculate the activity product.

RESULTS AND DISCUSSION

Because of the multiplicity of factors involved in the make-up and testing of the salt solutions, it was found desirable to isolate the individual variables and to study them separately. Therefore, several series of experiments were performed in such a manner as to determine, if possible, the optimum concentration of NaCl, KCl, and $CaCl_2$; the optimum $KCl/CaCl_2$ ratio; and the optimum pH value. Results of these experiments will be discussed in separate sections. Data will appear in the order in which they were collected in the laboratory.

Satisfactory $KCl/CaCl_2$ Ratios with 1.0 Per Cent NaCl

Most insect physiological salt mixtures have contained approximately 1.0 per cent NaCl. It had previously been determined by Griffiths and Tauber (13) that a solution containing 0.02 per cent $NaHCO_3$ afforded a pH value which was apparently satisfactory for prolonged activity of the excised crop of the American roach. In order to ascertain a satisfactory $KCl/CaCl_2$ ratio, these percentages of $NaHCO_3$ (0.02) and of NaCl (1.0) were used as a basic mixture with which KCl and $CaCl_2$ were tested in concentrations of 0.0, 0.2, 0.4, 0.6, and 0.8 gm. per liter of solution. Average activity products, expressed in centimeter-hours, from these 25 solutions (five crops from males and five from females in each mixture) are given in Table I.

In these 25 solutions, 125 crops from females yielded an average activity product of 0.110, while crops from males produced one of 0.280. This sex difference was noted by Griffiths and Tauber (13) in their preliminary work. It will be discussed in later paragraphs.

Seven of the 25 solutions produced activity products greater than 0.375. Previous experience indicated that an activity product below this level was generally a measure of a rather unsatisfactory solution. These seven activity products are shown in bold-face type in the body of Table I. The $KCl/CaCl_2$ ratios (0.2/0.2, 0.4/0.4, 0.6/0.6, 0.8/0.8, 0.2/0.4, 0.4/0.6, and 0.6/0.8) of these seven solutions were selected as the ones to be used for continued experimenta-

tion with increased NaCl concentrations. The seven $KCl/CaCl_2$ ratios were within the range of 0.5 to 1.0.

Examination of activity products in Table I shows that satisfactory activity did not result in absence of either KCl, or $CaCl_2$, or both. In mixtures containing KCl, but no $CaCl_2$, the crops registered an initial loss of tone and stopped activity in a state of relaxation (Fig. 2, G). This phenomenon is in accord with the generally recognized depression effect of potassium upon all types of muscle tissue.

When $CaCl_2$ was present, but KCl was lacking, results were not so uniform. In slightly more than half the cases, the crop exhibited a sharp initial increase in tone and contractions ceased with the foregut in a state of contraction (Fig. 2, D and E). It appears that $CaCl_2$ is responsible for this increase in tonus,

TABLE I

Average Activity Products for Solutions Containing 1.0 per cent Sodium Chloride, but in Which KCl/$CaCl_2$ Ratios Were Varied

		KCl, *gm. per liter*				
		0.0	0.2	0.4	0.6	0.8
		cm.-hrs.	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*
$CaCl_2$, *gm. per liter*	0.8	0.009	0.109	0.162	**0.422**	**0.406**
	0.6	0.013	0.283	**0.523**	**0.398**	0.126
	0.4	0.012	**0.433**	**0.390**	0.080	0.306
	0.2	0.010	**0.549**	0.259	0.293	0.012
	0.0	0.012	0.033	0.012	0.002	0.001

but that the tonic contraction is not always evident. Excess calcium has often been shown to produce tonic contraction in many types of muscle tissue. This same response seems to be at least partially demonstrated by the striated muscle of the insect foregut which was tested in this series of experiments.

When solutions lacked both Ca and K ions, muscle fibers of the crop very often rapidly lost their tonus and remained in a relaxed condition (Fig. 2, F).

Effect of Changes in NaCl Concentration

When NaCl concentrations were increased, higher osmotic pressure, with possible hypertonicity, became a factor to be considered. Several investigators have reported osmotic pressures of insect hemolymphs. Most of the work involved a freezing point technique with resulting values expressed as per cent NaCl necessary to produce the same freezing point depression and, consequently, the same osmotic pressure. Maluf (19), in 1939, summarized certain of the information concerning osmotic pressure in insect body fluids. He quoted values ranging between 0.8 per cent and 1.5 per cent NaCl, with a

mean of about 0.9 per cent. Backman (1) reported values between 0.8 per cent and 1.9 per cent NaCl for certain water beetles. For honey bee larvae, Bishop (3) stated that the osmotic pressure was equivalent to 1.5 per cent NaCl. Krogh (18) questioned the correctness of these high osmotic values obtained for insect hemolymph. No osmotic values for cockroach hemolymph have been recorded. However, in 1939, Yeager (24) used as high as 1.19 per cent NaCl in his work with the isolated dorsal vessel of the American roach; and Griffiths and Tauber (13) recommended 1.46 per cent NaCl as optimum for the excised foregut of the same insect. In view of these facts, it appears likely that osmotic pressure values above those equivalent to 1.0 per cent NaCl may very well be isotonic with certain insect hemolymphs.

It has been demonstrated with different muscle tissues that NaCl concentrations can be varied considerably with little or no effect upon activity of contracting tissue. Cole (8*a*) found that decreases and increases in osmotic pressure, up to 15 per cent, caused no significant changes in the rate and character of lobster heart beat, for several hours. Concerning the use of NaCl with the frog heart, Burridge (4, p. 25) stated, ".... we find divers workers obtaining satisfactory percentages varying from 0.6 to 0.75 per cent...." Clark (8), in 1913, varied NaCl from 0.5 per cent to 0.7 per cent with no significant effect upon the contracting frog heart. However, NaCl increases which produced definitely hypertonic solutions caused decreased muscular activity, according to Carlson (6, 7), Howell (17), Steggerda (22), and Cardot (5). It appears that slightly hypertonic solutions may produce little or no effect, but with increased hypertonicity, there is subsequent depression of activity.

To find how roach foregut muscles react to varying NaCl concentrations, the seven $KCl/CaCl_2$ ratios which gave the better activity products when combined with 1.0 per cent NaCl (in Table I) were further tested with 1.2 per cent, 1.3 per cent, 1.4 per cent, 1.5 per cent, 1.6 per cent, 1.7 per cent, and 1.8 per cent NaCl. All these solutions contained 0.02 per cent $NaHCO_3$. The average activity product for these 56 mixtures is given in Table II. Each solution mean is an average for five crops from male and five crops from female roaches. In addition, the combined means for the eight NaCl levels, and similar means for the seven $KCl/CaCl_2$ ratios, are presented.

Information summarized in Table II raises the question as to what NaCl level represents an isotonic condition for the foregut of *Periplaneta americana*. Comparatively satisfactory results were obtained between 1.0 per cent and 1.8 per cent NaCl. This is an extremely wide range. However, activity products obtained with 1.4 per cent, 1.5 per cent, and 1.6 per cent NaCl were definitely superior to other sodium levels. This finding is in agreement with preliminary results (13). If high average activity products, derived from actual measurement of muscle responses, may be interpreted as indicative of isotonicity, solutions which contain 1.4 per cent to 1.6 per cent NaCl seem to approach iso-

tonicity for muscle in the foregut of the American roach. However, the apparent abnormal lowering of activity at the 1.5 per cent level complicates the problem. This average activity product dropped about 20 per cent below the average of the other two. In 1940, Griffiths and Tauber (13), with more tests per solution, found decreased activity in solutions which contained 1.62 per cent NaCl, as compared to those which contained 1.46 per cent. Thus, it seems possible that more extensive experimentation would show that the average activity product at the 1.6 per cent level of the present series of trials is too high. There is considerable evidence which points to this view. Four of the seven

TABLE II

Average Activity Products for Solutions with Seven KCl/$CaCl_2$ Ratios and Eight NaCl Concentrations

		KCl/$CaCl_2$ concentrations, *gm. per liter*							Means
		0.2/0.4	0.4/0.6	0.6/0.8	0.2/0.2	0.4/0.4	0.6/0.6	0.8/0.8	
		Ratio of KCl/$CaCl_2$							
		0.5	0.66	0.75	1.0	1.0	1.0	1.0	
		cm.-hrs.	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*
NaCl, *per cent*	1.0	0.443	0.523	0.422	0.549	0.390	0.398	0.406	0.447
	1.2	0.552	0.296	0.767	0.382	0.475	0.689	0.377	0.505
	1.3	0.657	0.617	0.715	0.505	0.607	0.395	0.309	0.544
	1.4	1.169	0.755	0.637	1.286	0.835	0.550	0.330	0.795
	1.5	1.339	0.671	0.481	0.497	0.504	0.465	0.494	0.636
	1.6	1.546	1.109	0.240	0.686	0.600	0.642	0.459	0.755
	1.7	0.565	0.789	0.466	0.756	0.583	0.662	0.390	0.602
	1.8	0.524	0.910	0.260	0.477	0.407	0.678	0.343	0.514
Means		0.849	0.709	0.499	0.642	0.550	0.560	0.389	

KCl/$CaCl_2$ ratios tested with 1.4 per cent NaCl yielded average activity products greater than 0.750. In contrast, there was only one solution for the 1.5 per cent level, and only two for the 1.6 per cent level, which had activity products of this magnitude. In addition, and most important, the mixtures with 1.4 per cent NaCl produced the highest average activity product, namely, 0.795. Also, if the means at the right end of Table II are examined it will be seen that the deviation at the 1.5 per cent NaCl level is the only break in an activity curve from 1.0 per cent to 1.8 per cent NaCl. Therefore, it may be tentatively concluded that, of the NaCl concentrations tested, the one containing 14.0 gm. per liter of solution offered the optimum environment for the isolated crop of *Periplaneta americana*. Additional evidence for this interpretation will be found in later paragraphs where other experimental data are presented.

Records from excised crops in 1.4 per cent NaCl are pictured in Fig. 2, A and B. Activity at this sodium level was usually characterized by a prompt initiation of contractions which were maintained over considerable periods of time. Some loss of tonus was usually manifested in the beginning. After small irregularities at first, the baseline is continued in a regular manner. Often, in mixtures in which ionic concentrations were apparently unbalanced, the amplitude, frequency, and baseline were extremely irregular; and activity time was of short duration. In test solution 33 (NaCl = 1.4 per cent, KCl = 0.02 per cent, $CaCl_2$ = 0.04 per cent), where the activity time averaged more than 17 hours, one crop, No. 324, maintained contractions for almost 38 hours.

Optimum KCl/$CaCl_2$ Concentrations

The KCl/$CaCl_2$ ratio which allowed maximum activity was composed of 0.02 per cent KCl and 0.04 per cent $CaCl_2$ (see Table II). Three of the five solutions, in which were obtained activity products of more than 1.000, contained KCl and $CaCl_2$ in this ratio. The lowest average activity product recorded at this ratio was 0.443 for the solution which contained 1.0 per cent NaCl.

In general, Table II indicates that solutions with a KCl/$CaCl_2$ ratio of less than 1.0 produced superior results when compared with solutions with a ratio of 1.0. It appears that, with a particular KCl/$CaCl_2$ ratio, the activity of the excised foregut is decreased as the total amount of these two salts is increased. Thus, for the mixtures with a ratio of 1.0, the average activity product drops from 0.635 in the solutions containing 0.02 per cent KCl and 0.02 per cent $CaCl_2$, to 0.389 in those containing 0.08 per cent of each. In a similar manner, for the solutions with more calcium than potassium, the activity products shift from an average of 0.849 for mixtures with a KCl/$CaCl_2$ ratio of 0.02 per cent/0.04 per cent to an average of 0.499 for the ratio of 0.06 per cent/0.08 per cent.

Another Approach to Determine the Optimum NaCl Concentrations

Since an optimum NaCl level could not be definitely determined by using data presented in Table II, additional experimentation with a different approach, and the use of a statistical analysis seemed expedient. In 1925, Glaser (11) found that the pH of American roach hemolymph lay within the range of 7.5–8.0. In the present experiment, test solutions containing 0.02 per cent $NaHCO_3$ had a pH value which varied between 7.8 and 8.2. It was decided to test an additional series of NaCl levels at a pH of about 7.5. These mixtures were composed of four KCl/$CaCl_2$ ratios (0.2/0.2, 0.4/0.4, 0.2/0.4, 0.4/0.6 gm. per liter) and three NaCl levels (1.4, 1.5, and 1.6 gm. per liter). To each solution was added 0.02 per cent $NaHCO_3$, and the mixture was then titrated with 0.1 normal HCl to a pH value of 7.5. Either while standing, or while flowing through the crop chamber, the pH of these solutions gradually rose to

between 7.8–8.0 within 10 to 14 hours. Thus, although crops were isolated at a pH of 7.5, and tests of activity began at this pH, muscular contractions continued as the OH-ion concentration gradually increased. As before, five crops from each sex were tested in each mixture. Average activity products for each solution at pH 7.5 are given in Table III.

TABLE III

Average Activity Products for Solutions at pH 7.5

NaCl	Per cent $KCl/CaCl_2$				Means at pH 7.5
	0.02/0.02	0.04/0.04	0.02/0.04	0.04/0.06	
per cent	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*
1.4	0.656	0.980	0.725	0.462	0.705
1.5	0.503	0.285	0.751	0.555	0.524
1.6	0.606	0.575	0.175	0.534	0.473
Means at pH 7.5....	0.588	0.613	0.550	0.517	0.568

TABLE IV

Average Activity Products for Solutions at pH 8.0

NaCl	Per cent $KCl/CaCl_2$				Means
	0.02/0.02	0.04/0.04	0.02/0.04	0.04/0.06	
per cent	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*	*cm.-hrs.*
1.4	0.923	0.738	1.165	0.891	1.011
1.5	0.683	0.564	0.991	0.855	0.753
1.6	0.664	0.633	0.897	0.789	0.589
Means.....	0.790	0.645	1.351	0.845	0.784

As a basis for comparison, another series of five crops from each sex was tested in a set of mixtures with an initial pH of 8.0. These results are given in Table IV.

It is evident from Tables III and IV that there is a real difference between mixtures tested at pH 7.5 and pH 8.0, with the latter seemingly better. However, at both levels of alkalinity, optimum NaCl concentration for muscle contractions is 1.4 per cent and there is a reduction in crop activity with each increase of NaCl. These results are in accord with the previous suggestion that 1.4 per cent represents the optimum NaCl concentration. At pH 8.0, the $KCl/CaCl_2$ ratio with the highest average activity product was again the 0.02 per cent/0.04 per cent ratio.

To obtain a better understanding of the interactions which played a part in

the experiments, data from the four $KCl/CaCl_2$ ratios (0.2/0.2, 0.4/0.4, 0.2/0.4, and 0.4/0.6 gm. per liter) tested in combination with 1.4 per cent, 1.5 per cent, and 1.6 per cent NaCl at pH values of 7.5 and 8.0 were statistically analyzed by the method of analysis of variance (20). The summary of this analysis is presented in Table V. Due to difficulties involved in interpretation, other subclass interactions than those listed were not tested. Subclasses in this analysis involved crops from a particular sex, tested on a given day, in any one solution. Test solutions had been used for 2 days of experimentation, and

TABLE V

Analysis of Variance for Solutions at pH 7.5 and pH 8.0

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	239	125.66	
Within subclass (sampling error)	144	56.01	0.39
pH	1	3.81	3.81*
Sex	1	0.77	0.77
K/Ca	3	3.72	1.24*
NaCl	2	1.88	0.94‡
Days	1	10.14	10.14*
pH and sex	1	3.34	3.34*
pH and K/Ca	3	7.93	2.64*
Sex and K/Ca	3	2.01	0.67*
pH and NaCl	2	4.11	2.06*
Sex and NaCl	2	2.91	1.46*
K/Ca and NaCl	6	3.38	0.56
Days and pH	1	3.55	3.55*
Sex and days	1	1.70	1.70‡
K/Ca and days	3	1.32	0.44
NaCl and days	2	1.47	0.74
Discrepance (error)	63	17.61	0.28

* Highly significant.

‡ Significant.

a preliminary survey of data had indicated a possible difference between crop activity on the 1st and 2nd day of the solution's use, in spite of the fact that the pH was adjusted at the beginning of each day's run.

Although the average activity product for crops from males (0.790) was greater than that from females (0.700), there was no significant difference. Crops tested on the 1st day yielded an activity product of 0.950 in contrast to 0.530 on the 2nd day. However, when the interaction between these two variables (sex, and day-tested) was analyzed, it was found that while the crops from males were considerably less active in day old solution (activity product 1.100 to 0.800), the females' crop activity was greater on the 2nd day (0.490 to 0.560). At present no adequate explanation can be offered for this response.

Since there is a significant difference between NaCl levels, and between KCl/$CaCl_2$ ratios, and since 1.4 per cent NaCl and 0.02 per cent KCl/0.04 per cent $CaCl_2$ produced the highest average activity products, it may be concluded that, under the conditions prevailing, these percentages form the medium which affords an optimum environment for activity of the excised crop of the American roach.

With these factors of NaCl concentration and KCl/$CaCl_2$ ratio rather well circumscribed, further experimentation upon the effect of pH appeared to be desirable. Therefore, four KCl/$CaCl_2$ ratios (0.2/0.2, 0.4/0.4, 0.2/0.4, and 0.4/0.6 gm. per liter) were tested with 1.4 per cent NaCl at initial pH values of 6.8 and 8.9 and the results were compared with the comparable solutions at pH 7.5 and 8.0.

An Optimum pH Value

In order to study the effect of changes in pH, solutions to be tested were made up with either phosphate or bicarbonate. Standard HCl or NaOH was added and solutions were titrated by means of a glass electrode to the desired pH. It was found that the pH of acid solutions (pH 6.0–6.5) which were buffered with both phosphate and bicarbonate, or bicarbonate alone, tended to rise rapidly to a pH above 7.0. Since this shift was undesirable, only phosphate was used in acid solutions, at the rate of 0.5 cc. of 0.25 normal NaH_2PO_4 per liter. After titration, it was possible to maintain a pH between 6.5 and 6.9. Occasionally, however, the acidity increased and it was necessary to check the pH regularly and remedy any change.

Alkaline solutions were adjusted by adding 0.1 N NaOH to a mixture which contained 0.2 gm. of $NaHCO_3$ as a buffer. If phosphate was used, the pH dropped toward neutrality. If it was omitted, there was only a gradual lowering toward 8.0. Thus, it was possible to maintain a pH above 8.5 for some 12 to 15 hours without a change of solution. The pH values were adjusted at the beginning of each day's run, and at any other time when necessary.

The peculiar shifts in pH noted above are apparently related to the CO_2 exchange between the atmosphere and the test solutions. Although fluctuations occurred both while standing in glass containers and during the course of experimentations, the change was more rapid when the solutions were being aerated and were flowing through the crop chamber. Changes were essentially the same whether insect tissues were present or absent in the experimental set up. Apparently, agitation by bubbling and movement of the fluid afforded a better opportunity for the loss or uptake of the CO_2, depending on the pH of the medium. In acid solutions, part of the $NaHCO_3$ was changed to unstable H_2CO_3. It appeared that this compound decomposed to form water and CO_2. The latter was lost, with the resulting rise in the pH value. However, in a medium with a pH above 8.0 there was no loss, but, instead, an uptake of

CO_2. Uptake of CO_2 was slow and the resultant pH change was, therefore, gradual. The greater rapidity of change in the presence of phosphates may be partially explained. The buffer effect of phosphates is at a minimum at a pH value of about 8.5. This is due to the fact that the tri-sodium form is changing to the di-sodium form at this value. In addition, if $CaCl_2$ is present, the following reaction will take place when the mixture is alkaline:

$$3\ CaCl_2 + 2\ Na_2HPO_4 \rightarrow Ca_3(PO_4)_2 + 4\ NaCl + 2\ HCl$$

However, since the quantities of both reactants were small, the amount of HCl would be very limited. Other factors may be contributing to the shift of pH, but no further explanation can be offered at this time.

Various workers (4, 5, 7, 8, 9, 17) have demonstrated that changes in acidity or alkalinity affect the activity of muscle tissues. These investigators have used different kinds of muscle from several widely separated animal groups. With experiments performed upon different animals and with techniques not the same, results may often be seemingly contradictory. Nevertheless, certain tendencies have made themselves evident.

A review of the literature (12) indicates that moderate and sometimes rather large changes in pH do not materially affect the normal activity of contracting muscle. However, within certain limits, there is a tendency for increase in acidity to depress, and increase in alkalinity, also within a definite range, to stimulate activity. It is probable that the stimulatory effect is a temporary one and that this concentration of hydroxyl ions would prove detrimental if maintained over longer periods, or if the OH-ions were further increased. These same tendencies were noted in the present experiments with roach foregut muscle.

Results from the crops tested in 1.4 per cent NaCl at pH values of 6.8 and 8.9 are compared in Table VI with those at 7.5 and 8.0. It should be remembered that crops in solutions which were initially tested at pH 7.5 and 8.9 continued their activity even though the solutions gradually approached pH 8.0. This change must have affected the activity in these solutions and should be considered when studying data in Table V.

Data of Table VI were statistically analyzed. The summary of this procedure is in Table VII.

Several significant differences may be noticed in Table VII. There was a significant sex difference, and the activity for crops from males (0.850) was better than that for crops from females (0.640). Once again the difference between day's run was highly significant. The interaction between sex and day's run was highly significant and, as noted previously, the activity for males was markedly reduced on the 2nd day (1.160 to 0.540) while the females changed very little (0.630 to 0.640).

Until further evidence may be produced, it has been assumed that the change

in concentration of the phosphate and bicarbonate anions was not responsible for the different results found at the four pH levels, but rather that the differences were caused by a shift in the H-ion concentration. Average activity products produced at the four pH values differ significantly. That at a pH of 8.0 is greatest. Therefore, it may be concluded that this value represents

TABLE VI

A Comparison of Records from Solutions with pH Values of 8.9, 8.0, 7.5, and 6.8

pH	Solution No.	$KCl/CaCl_2$	Amplitude	Activity	Activity product
		gm. per liter	*cm.*	*hrs.*	*cm.-hrs.*
8.9	79	0.2/0.2	0.030	10.3	0.324
	80	0.4/0.4	0.066	12.1	0.896
	81	0.2/0.4	0.032	19.7	0.691
	82	0.4/0.6	0.060	18.9	1.075
	Mean.....		0.047	15.3	0.797
8.0	33	0.2/0.2	0.071	17.4	1.286
	34	0.4/0.4	0.067	11.5	0.835
	37	0.2/0.4	0.066	15.2	1.169
	38	0.4/0.6	0.059	9.8	0.755
	Mean.....		0.066	13.5	1.011
7.5	41	0.2/0.2	0.042	18.1	0.656
	42	0.4/0.4	0.058	15.4	0.980
	45	0.2/0.4	0.044	12.7	0.725
	46	0.4/0.6	0.027	9.8	0.462
	Mean.....		0.043	14.0	0.706
6.8	75	0.2/0.2	0.077	10.8	0.879
	76	0.4/0.4	0.066	9.2	0.669
	77	0.2/0.4	0.028	7.1	0.245
	78	0.4/0.6	0.053	6.3	0.351
	Mean.....		0.056	8.4	0.536

the optimum one, of those tested, for the activity of the excised crop of the American roach.

Crop 814, in solution 82, with an initial pH of 8.9, produced an unusual record. At the end of 24 hours of activity, its amplitude had dropped almost to zero. At that time the pH was 8.0. The solution was changed; the pH was again 8.9. Instead of showing an inhibitory effect, contractions increased in magnitude and the best part of the record was made during the next 15 hours.

Altogether, activity had continued over a period of almost 47 hours. No adequate explanation can be offered, but, apparently, the muscle had undergone some change whereby the increased alkalinity served as a stimulus for greater activity. This phenomenon may be allied with the stimulatory effect of the hydroxyl ion which was noted by Carlson (6, 7) and de Burgh Daly (9). A similar but less spectacular result was obtained from crop 820 in the same kind of solution.

TABLE VII

Statistical Analysis for Solutions Tested at pH Values of 8.9, 8.0, 7.5, and 6.8

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	159	81.34	
Within subclass (sampling error)	96	40.38	0.43
Sex	1	2.00	2.00*
pH	3	4.52	1.51*
K/Ca	3	0.78	0.26
Days	1	3.69	3.69‡
pH and sex	3	2.12	0.71
Sex and days	1	3.83	3.83‡
pH and K/Ca	9	7.46	0.83
pH and days	3	1.31	0.44
Days and K/Ca	3	2.43	0.81
Discrepance (error)	33	11.52	0.35

* Significant.

‡ Highly significant.

The Sex Difference

In their preliminary study of the motility of the isolated roach foregut, Griffiths and Tauber (13) stated, "The crops from male roaches produced significantly higher activity products than those from females. Food was present in the foregut of the females more often than in that of the males." In addition, they observed that the quantity of food contained in the crops did not seem to be correlated with the activity of an individual foregut; that is, a large amount of food did not depress activity. It was suggested that reduced activity of crops from female roaches was responsible for reduced motility. The same correlations were noted in the present investigation.

For solutions with pH values of about 8.0, average activity products of crops from males were greater than those from females in 52 of the 74 mixtures. Thus, in slightly more than 70 per cent of the cases, crops from males produced the higher average activity products. If, for the same 74 solutions, the average of all of the activity products for males and females is determined, it is found that the foreguts from males averaged 0.525 and those from females averaged

0.399. The product for males was almost 25 per cent greater than that for females. In the two sets of data which were treated statistically, the average activity product for crops from males was greater than that for crops from females and in one instance the difference was significant. It becomes obvious, therefore, that there is some fundamental difference between the two sexes as regards the motility of the excised foregut.

During the course of the present experimentation, certain modifications in technique were made in the hope that some adequate interpretation of this difference could be advanced. To determine whether there was a significant sex difference in the quantity of food ingested, groups of five males and five

TABLE VIII

Average Amounts of Food Contained in the Crops of 445 Roaches

Time since fed	Male		Female	
	No. of tests	Average amount food*	No. of tests	Average amount food*
hrs.				
16	12	1.92	15	2.07
17	30	1.33	24	2.25
18	35	0.94	44	2.18
19	27	1.37	35	2.00
20	32	0.91	28	2.21
21	22	0.64	33	1.76
22	24	1.13	29	2.38
23	20	0.70	11	1.36
24	20	0.30	4	0.25

* 4.00 represents a full crop and 0.00 represents an empty one.

females were isolated for 3 days and were then allowed access to food for 1 hour. Banana paste, whole wheat bread which had been soaked in water, and moistened starch were used as test foods. 50 males and 50 females in groups of five were fed on each diet. There was no significant difference in the amount of food ingested by the two sexes.

Most roaches used in the gut motility experiments were isolated for 48 hours and were then allowed access to moistened starch for several hours. The starch had been mixed with carmine so that the ingested food could be identified in the gut. Roaches were usually used for experimentation on the day following this feeding. When the foregut was removed, the amount of food in the crop was recorded. If food was present in only one-fourth of the crop, it was recorded as 1; if half of the crop was full, it was listed as 2; if three-fourths full, as 3; and if completely full, as 4. Data for 445 roach crops are presented in Table VIII. This includes information concerning the number of roaches and

the amount of food which remained in the crops from 16 to 24 hours after feeding had ceased. In only one instance (at 24 hours) was the average amount of food contained by the crops greater for males than for females.

Snipes and Tauber (21) reported that for *Periplaneta americana* the average egestion time was 19.6 hours for males and 21.4 hours for females. While this was not a significant difference, the egestion time discrepancy may have been related to the motility difference observed in excised crops from the American roach. Table VIII clearly shows a significant difference in the rate of progression of food in crops from males and females, and, consequently in the amount of food remaining in the foregut. This food progression rate is more rapid in the foreguts from the male roaches.

As stated above, there is a significant sex difference in activity products of crops from males and females, in the amount of food contained in the crops, in the rate of progression of food through the ingluvial region, and in the reaction to the age of the salt solution. However, the amount of food ingested by males and females is not significantly different. Therefore, it appears that variations in motility are produced by inherent activity differences which are normally present in the ingluvial region of the American roach. That the two sexes of the American roach differ in other features has been reported previously by Griffiths and Tauber when they found differences in the duration of the nymphal period (14), the number of molts (14), and the adult longevity (15).

SUMMARY AND CONCLUSIONS

1. Twenty-five solutions which contained KCl (0.0, 0.2, 0.4, 0.6, and 0.8 gm. per liter), in combination with $CaCl_2$ (0.0, 0.2, 0.4, 0.6, and 0.8 gm. per liter), 10.0 gm. of NaCl, and 0.2 gm. of $NaHCO_3$ per liter of solution were tested in order to determine satisfactory $KCl/CaCl_2$ ratios in an insect physiological salt mixture for the maintenance of muscular activity by the isolated crop of the American roach.

Satisfactory activity products (0.390 to 0.549) were obtained in seven mixtures with $KCl/CaCl_2$ ratios of 0.2/0.2, 0.4/0.4, 0.6/0.6, 0.8/0.8, 0.2/0.4, 0.4/0.6, and 0.6/0.8, expressed as gram per liter. These ratios lie between 0.50 and 1.00.

In solutions which contained calcium, but no potassium, approximately 50 per cent of the crops exhibited an initial tone increase and were arrested in rigor. See Fig. 2.

In solutions which contained potassium, but no calcium, all crops showed an initial loss of tone and arrest in relaxation. See Fig. 2.

2. Seven $KCl/CaCl_2$ ratios (see paragraph 1 above) were tested with eight NaCl concentrations (1.0, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, and 1.8 per cent) at a pH of 8.0. In these mixtures, the ones with $KCl/CaCl_2$ ratios of less than 1.0 produced higher activity products than those with ratios equal to 1.00. The

highest average activity product (0.849) was obtained in the solutions with 0.2 gm. of KCl and 0.4 gm. of $CaCl_2$ per liter.

3. Four KCl/$CaCl_2$ ratios (0.2/0.2, 0.4/0.4, 0.2/0.4, and 0.4/0.6 gm. per liter) were tested with 1.4, 1.5, and 1.6 per cent NaCl at a pH of 7.5. When analyzed with data from comparable solutions at a pH of 8.0, it was found that 1.4 per cent NaCl afforded an optimum environment for isolated crop activity.

4. Effects of hydrogen and hydroxyl ion concentrations were studied at pH values of 6.8, 7.5, 8.0, and 8.9. The highest average activity product, 1.011, was produced at a pH of about 8.0.

5. A satisfactory physiological salt solution for the isolated foregut of the American roach, *Periplaneta americana*, would contain 14.0 gm. of NaCl, 0.4 gm. of $CaCl_2$, 0.2 gm. of KCl, and 0.2 gm. of $NaHCO_3$ per liter of solution. This mixture should have a pH value between 7.8 and 8.2.

6. Durations of crop activity extending over periods as long as 25 hours were quite common, and several crops maintained contractions for more than 30 hours. The greatest longevity was for crop 814, from a female, which continued activity for slightly more than 47 hours.

7. A significant difference between the activity products of the crops from males and the crops from females was recorded. Although there was not a significant difference in the amount of food ingested by males and females, 12 hours after feeding there was more food in the females' crops, and the food progressed more rapidly through the males' crops than through the females'. In addition, crops from the two sexes reacted differently to the effects of day old solutions. This sex difference is apparently related to an inherently increased activity of the crop from the male roach.

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