OSMOREGULATION IN *BLABERUS CRANIIFER* AFTER PARENTERAL INJECTIONS OF NaCl SOLUTIONS

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Abstract—1. After injection of NaCl into the abdominal hemocoel of *Blaberus craniifer*, the osmotic concentration of antennal hemolymph peaked at 1.25 hr and returned to pre-injection concentration by 21 hr. The mean plasma clearance was 2.67 μl/hr.

2. Force-feeding of water 15 min after injection of NaCl prevented the early rise in hemolymph osmotic concentration, but a later gradual increase in osmotic concentration occurred.

3. The onset of spontaneous drinking occurred 0.8 hr after NaCl injection; this correlated with an elevation in hemolymph osmotic concentration.

4. Cumulative spontaneous water intake over 4 days was proportional to the daily injected dose of NaCl.

INTRODUCTION

As indicated by Stobbart & Shaw (1974), the capability of terrestrial insects to regulate the osmotic concentration of their hemolymph is a subject which has received very little investigation. In the reported studies, the investigative approaches generally involved the determination of hemolymph osmotic pressure under the conditions of total water deprivation or the ingestion of saline solutions, which were hypertonic to the hemolymph.

Little change in osmotic pressure was observed after 24 hr of dehydration in the locust *Chortoicetes terminifera* (Djajakusumah & Miles, 1966) or after 9 days in the cockroach *Periplaneta americana* (Wall, 1970), but hemolymph volume decreased in both species. Upon rehydration after a period of dehydration, hemolymph volume increased rapidly, but there was only a slight decrease in osmolality (Wall, 1970). When locusts (*Schistocerca gregaria*) were given hyperosmotic saline to drink instead of tap water, the hemolymph volume decreased and the osmotic pressure increased (Stobbart, 1968). Although a normal hemolymph osmotic concentration was maintained, when cockroaches (*Periplaneta americana*) were given only physiological saline (400 mOsm) to drink for 7 days, drinking a hypertonic saline solution of 700 mOsm caused an increased hemolymph osmotic pressure and an increased blood volume (Heit *et al.*, 1973).

The possible relationship of hemolymph osmotic concentration and spontaneous drinking has been studied in only two species of insects. Spontaneous drinking in the blowfly *Lucilia cuprina* is triggered primarily by increased hemolymph Cl$^-$ concentration, produced either by the injection of salt solu-

tions or by dehydration (Barton Browne, 1968). On the other hand, spontaneous drinking in the blowfly *Phormia regina* depends upon hemolymph volume; the drinking response is abolished by the injection of concentrated sugar or salt solutions into the hemocoel (Dethier & Evans, 1961).

The present work is the first reported study of osmoregulation in the giant cockroach *Blaberus craniifer* Burm. In addition to the acquisition of general information on osmoregulation in this species, the concept of elimination kinetics was applied for the first time to osmoregulation in insects; one very useful and potentially important parameter, which was derived by this analysis, is the mean plasma clearance of osmotically active particles. Finally, spontaneous drinking, as a potential osmoregulatory mechanism, was investigated by an approach which has not been used previously in studies on insects.

MATERIALS AND METHODS

Adult male giant cockroaches of the species, *Blaberus craniifer* Burm., were obtained from our cultures. Their diet prior to experiments consisted of Wayne Lab-blox meal, apples and tap water *ad libitum*.

The osmotic concentration of antennal hemolymph was determined by a previously described comparative melting point method (Gross, 1954). All determinations of osmotic concentration are expressed as milliosmolal (mOsm).

During the experimental period, roaches were suspended by their anterior pair of wings on a rack, and they received no food. For the injection of NaCl solutions, roaches first were anesthetized with CO_2. Then, a 27 gauge hypodermic needle was inserted into the hemocoel between the sternites of the fifth and sixth abdominal segments; the tip of the needle was advanced forward about 1 cm before delivery to prevent leakage.

For the determination of significant differences between the means of two experimental groups, the *t*-test was applied. The 0.05 level of probability was used for the establishment of significance. To determine the level of significance of the closeness of fit of observed points to an estimated line of regression, the *t* statistic was calculated from the correlation coefficient (see Wyatt & Bridges,

1967). Both the correlation coefficient, r, and the probability, P, are reported for each estimation of a line of regression.

RESULTS

1. Hemolymph osmotic concentration after the injection of NaCl solution

A. *The time-course of the hemolymph osmotic concentration and the effect of water ingestion.* The time-course of the hemolymph osmotic concentration after the injection of 0.018 ml of 9569 mOsm NaCl solution into the abdominal hemocoel of roaches is presented in Fig. 1. There were the following two experimental groups of eight roaches each: one group (I) received no water and the other group (II) was force-fed distilled water 15 min after the injection of the NaCl solution. The latter procedure was accomplished by cannulating the esophagus with a short length of polyethylene tubing, which was connected to a syringe. Although approximately 0.35 ml of distilled water was delivered into the crop of each roach in group II, only a volume of 0.267 ± 0.052 ml (mean ± S.D.) was retained.

In group I, which received no water, the mean osmotic concentration of antennal hemolymph began to rise sharply by 30 min after injection, peaked at 1.25 hr, fell sharply at first, then more gradually. Mean hemolymph osmotic concentrations were significantly different from the control value, obtained before injection, at 1.0 hr $(P < 0.002)$, at 1.25 hr $(P < 0.001)$ and 1.5 hr $(P < 0.003)$. None of the other mean osmotic concentrations were significantly different from the control value.

The results from group II, which was force-fed water, contrasted sharply with the data from group I. The only mean osmotic concentrations which were significantly different from the control value were at 1.25 hr $(P < 0.05)$ and at 14.5 hr $(P < 0.01)$. At 1.25 hr there was also a significant difference between the mean concentrations of groups I and II $(P < 0.001)$.
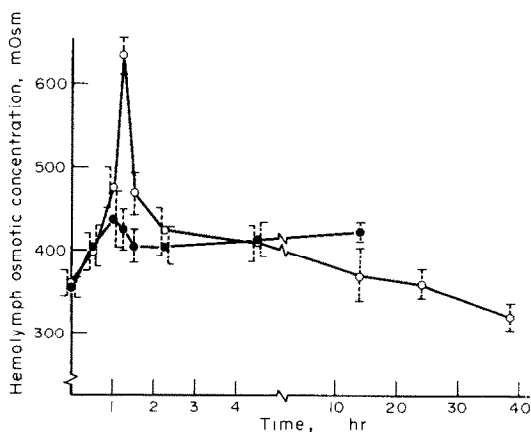

Fig. 1. The time-course of hemolymph osmotic concentration after the injection of 0.018 ml of 9569 mOsm NaCl solution. Roaches in group I (O) received no water; whereas roaches in group II (●) were force-fed distilled water 15 min after the injection of the NaCl solution. Each point represents eight roaches. The vertical broken lines represent the extent of twice the standard error of the means.

From these findings, it was concluded that the drinking of water was a potentially effective mechanism in the depression of an elevated hemolymph osmotic concentration in *Blaberus*. However, before drinking could be accepted as a useful mechanism of osmoregulation in *Blaberus*, it was necessary to demonstrate that an elevated hemolymph osmotic concentration actually triggered spontaneous drinking. Another characteristic of group II, which contrasts sharply with group I, is that after 1.5 hr the hemolymph osmotic concentration rises slowly and continuously until 14.5 hr; the experiment was discontinued at this point, so that the further time-course of the osmotic concentration of group II is unknown.

B. *Elimination kinetics and hemolymph volume determination.* Hemolymph volume was determined from the data of group I by two methods. The first method was the commonly used dilution technique (see Heit *et al.*, 1973), which is described by equation (1).

$$V_h = \frac{V_i(C_i - C_2)}{C_2 - C_1},$$ (1)

where
C_1 is initial osmotic concentration of hemolymph (361 mOsm), C_2 is peak osmotic concentration of hemolymph after the injection of NaCl (632 mOsm), C_i is osmotic concentration of the injected NaCl solution (9569 mOsm), V_h is volume of hemolymph; V_i is volume of injected NaCl solution (0.018 ml). The hemolymph volume calculated by this method was 0.594 ml. The body weight of roaches in group I was 2.71 ± 0.31 g (mean ± S.D., $N = 8$); consequently the hemolymph volume, as determined by this method, constitutes 21.9% of the body weight.

The osmoregulatory capability of *Blaberus* was analyzed in terms of elimination kinetics. For a complete description of the method of analysis, see Wagner (1975). Based on anatomical considerations, it was assumed that the two-compartment open model was most applicable (Fig. 2) where compartment No. 1 represents the functional extracellular space (hemocoel) and compartment No. 2 represents the functional intracellular space.

With application of the back-projection or stripping technique to the osmotic concentration, time data of group I for the time interval of 1.25–38.5 hr, two exponentials were obtained (Fig. 3); this technique involved the application of the method of least squares to the natural logarithms of the original osmotic concentrations (or residuals) and the corresponding time values. The correlation coefficients and the levels of significance for the curve fitting process were as follows: for the slower (terminal) exponential curve, $r = 0.497$ and $P < 0.002$; for the faster exponential, $r = 0.836$ and $P < 0.003$. From the stripping procedure, the equation for these data may be expressed as follows:

$$C_1 = Ae^{-\alpha t} + Be^{-\beta t} = 17,100e^{-3.44t} + 418e^{-0.0069t}, (2)$$

where C_1 is the osmotic concentration in compartment No. 1 at any time t between 1.25 and 38.5 hr; the intercept and elimination rate constant for the slower exponential are B and β; for the faster exponential, A and α. The elimination half-lives for the slower and faster exponentials are 100 hr and 12.1 min, respectively. The line generated by the
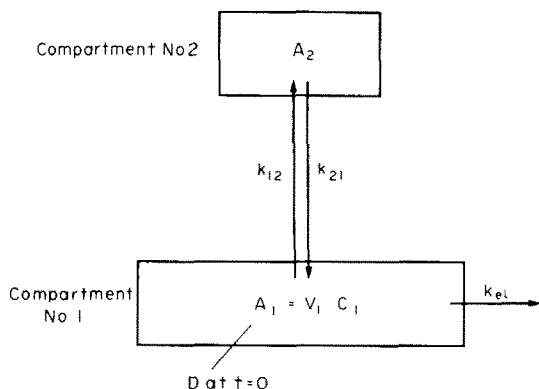
Fig. 2. Scheme of the two compartment open model. A single dose (D) is injected into compartment No. 1 at time $t = 0$, and elimination occurs only from compartment No. 1. The meaning of the symbolism is as follows: k_{12}, first order rate constant for transfer of solute from compartment No. 1 to compartment No. 2; k_{21}, first order rate constant for transfer of solute from compartment No. 2 to compartment No. 1; k_{el}, first order rate constant for elimination of solute by all mechanisms from compartment No. 1; A_1, amount of solute in compartment No. 1 at time t; A_2, amount of solute in compartment No. 2 at time t; C_1, osmotic concentration in compartment No. 1 at time t; V_1, volume of compartment No. 1.

slower exponential intersects the mean initial osmotic concentration (361 mOsm) at 21 hr after injection.

With reference to the symbolism in Fig. 2, the following parameters of the model were obtained: k_{el}, $0.267\,hr^{-1}$; k_{12}, $3.09\,hr^{-1}$; k_{21}, $0.0888\,hr^{-1}$; V_1, $0.010\,ml$; the extrapolated volume of distribution, V_{dext}, $0.419\,ml$; the volume of distribution calculated from the area under the concentration–time curve, V_{darea}, $0.387\,ml$; the volume of distribution at steady state, V_{dss}, $0.358\,ml$; the mean plasma clearance, $2.67\,\mu l/hr$.

2. Onset of spontaneous drinking after the injection of NaCl solution

In this section, all roaches were suspended by their anterior wings as described previously; but only those of the experimental groups were injected with 0.025 ml of 1.0 M NaCl solution. Distilled water was presented with a 0.1 ml Hamilton syringe, and the roaches were permitted to take drops from the needle tip for a period of 15 min. In a control group of 30 roaches, the amount of spontaneous water ingestion under basal conditions (i.e. immediately after removal from culture) was 0.151 ± 0.074 ml (mean ± S.D.).

Of the 40 roaches in the experimental groups, two of them were offered distilled water at 0.5, 1.0 and 3.5 hr; four roaches at 1.5 hr; and 10 roaches at 2.0, 2.5 and 3.0 hr after injection. To determine the onset of drinking due to the injected NaCl, it was first necessary to determine statistically the groups in which the drinking of water was significantly increased over basal conditions. To achieve this end, the confidence interval for the mean fluid intake of the control group was calculated at the 0.95 level. If the fluid intake of any roaches in the experimental groups was greater than the upper boundary of the confidence interval, it was interpreted as significantly elevated. No significant elevations occurred in the

0.5 hr group, one elevation was present in each of the 1.0 and 1.5 hr groups, four out of 10 were elevated at 2 hr, and all were significantly elevated in the remaining experimental groups. It appeared that under these experimental conditions the onset of drinking occurred between 0.5 and 1.0 hr.

To test the above theory, fluid intake in those experimental groups containing a member with a significantly elevated intake was plotted versus time after injection (see Fig. 4); the estimated line of regression fits the observed points with a high level of significance $(P < 0.001, r = 0.627)$. The intercept of the x axis, which indicates the time of onset of drinking after injection, is 0.8 hr. Between 0.5 and 1.0 hr, the mean osmotic concentration of antennal hemolymph becomes significantly elevated over the mean initial osmotic concentration (see the osmotic concentration–time curve in Fig. 1). Therefore, there is a good correlation between the onset of drinking and the significant elevation of the mean hemolymph osmotic concentration after the injection of NaCl.

3. The relationship of spontaneous drinking and the magnitude of the daily injected dose

On the first day of the experiment, roaches were suspended and offered distilled water in the manner described in the previous section. As indicated in the previous section, the amount of spontaneous water ingestion on the first day (i.e. under basal conditions) was 0.151 ± 0.074 ml (mean ± S.D., $N = 30$). After a preliminary study, a selection was made of a daily dosage range of injected NaCl solution, which resulted in a progressively increased daily water con-
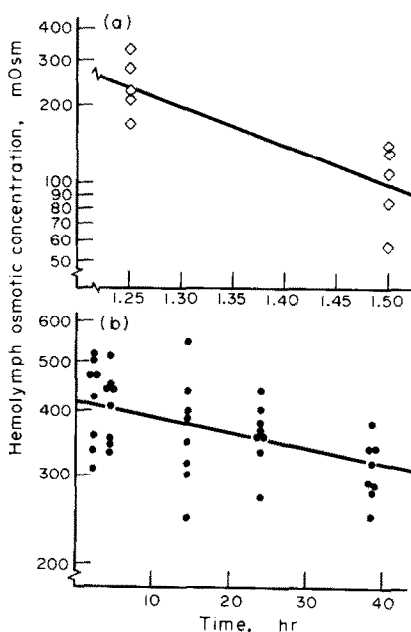

Fig. 3. Semilogarithmic plot of the two exponentials obtained by the back-projection or stripping technique from the elimination phase of the osmotic concentration, time data of group I (i.e. 1.25–38.5 hr after injection). The faster exponential curve (◇) appears in (a) and the slower (terminal) exponential curve (●) appears in (b). The correlation coefficients, levels of significance, the equation for these curves and the parameters of the model appear in the text.
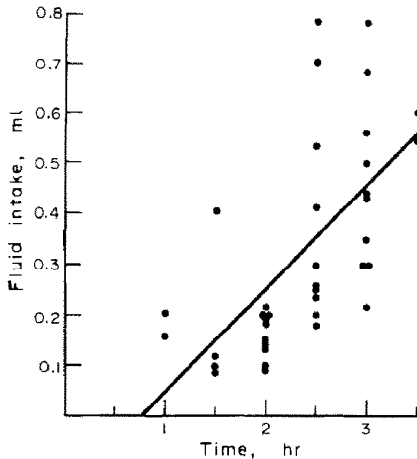
Fig. 4. Scatter diagram and regression line for the relationship between the spontaneous drinking of water and the elapsed time after the injection of 0.025 ml of 1.0 M NaCl solution. Each point represents one roach. The intercept on the x axis (0.8 hr) indicates the time of onset of spontaneous drinking after the injection of the NaCl solution.

sumption. The following three groups of roaches were selected for this experiment (groups are identified by the amount of daily injected NaCl): 0.025 ml of 0.3 M NaCl ($N = 5$); 0.025 ml of 0.48 M NaCl ($N = 4$); and 0.025 ml of 1.0 M NaCl ($N = 4$). Distilled water was offered each day before the roaches received their daily injections. The variation in daily spontaneous water ingestion was considerable, and the cumulative water intake over several days gave a much more uniform result at a given injected dose. Therefore, cumulative fluid intake over four days was plotted against the daily dose of NaCl per body weight (Fig. 5). The observed points were fitted with a line of regression at a high level of significance ($P < 0.001$, $r = 0.832$). Thus, the cumulative fluid intake is proportional to the daily injected dose (mg/g body weight). Theoretically, the y intercept in this case should be the product of the daily basal water intake
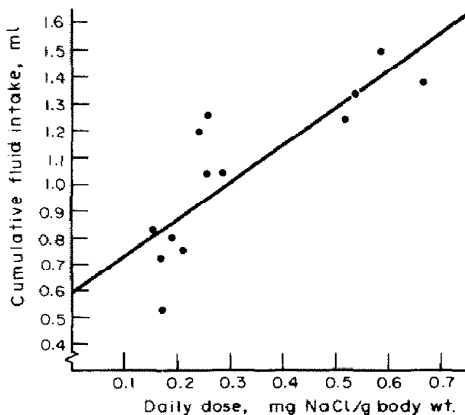

Fig. 5. Scatter diagram and regression line for the relationship between cumulative spontaneous water intake (over 4 days) and the daily injected dose of NaCl. Each point represents one roach. The intercept on the y axis (0.595 ml) is the predicted cumulative basal water intake over four days.
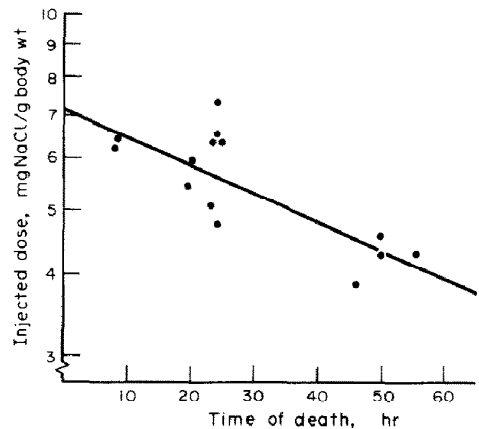

Fig. 6. Semilogarithmic plot of the relationship between the injected dose of NaCl and the subsequent duration of life (i.e. the duration from the time of injection until death). Each point represents one roach.

of 0.151 ml, multiplied by four (days); this calculation yields 0.604 ml. The observed intercept was 0.595 ml, which agrees well with the predicted intercept.

4. The relationship of injected dose and mortality

To delineate the degree of tolerance to marked changes in osmotic concentration of the hemolymph, large amounts of NaCl were injected into the hemocoel, and the subsequent duration of life was determined. Fifteen roaches were used in this experiment. The injected dose of NaCl ranged from 3.89 to 7.31 mg/g body weight, and the injected volume in all cases was 0.06 ml. After consideration of multiple possibilities, it was determined that the relationship of injected dose and subsequent duration of life (Fig. 6) is an exponential one, described by th3 following equation:

$$D_t = Be^{-\beta t} = 7.17e^{-0.0099t}, \qquad (3)$$

where D_t is the dose in mg NaCl/g body weight at time t; B, the intercept, 7.17; β, the elimination rate constant, 0.0099 hr^{-1}; t, the duration from the time of injection until death. The half-time of death was 70 hr. An obtained level of significance ($P < 0.001$, $r = 0.775$) indicates that the estimated line of regression fits the observed points very well. Obviously, the relationship in equation (3) is only valid for a limited range of doses, and it will not hold at very high doses because of the time requirement for the distribution of injected NaCl to vital organs. Furthermore, equation (3) will not hold at very low doses because the duration after injection will be adequate in length to permit clearing of the NaCl load by osmoregulatory mechanisms, and thus prevent the fatal toxic effects of NaCl.

DISCUSSION

The elimination of osmotically active particles from the hemolymph of Blaberus after the injection of NaCl solution is probably due to excretion by the Malpighian tubule–ileum–rectum system and to the uptake of Na$^+$ and Cl$^-$ by tissues (Stobbart & Shaw, 1974; Wall, 1970). These two mechanisms, working in concert, are probably responsible for the calculated

mean plasma clearance. The osmoregulatory capability of *Blaberus* in clearing a large solute load is presented in Fig. 1 (group I). The peak osmotic concentration of antennal hemolymph occurred 1.25 hr after the injection of solute into the abdominal hemocoel; some variability in the duration from the time of injection until peak concentration may be expected, depending upon the choice of injection and sampling sites.

In the present work, elimination kinetics is described in terms of the two-compartment open model. It must be emphasized that the values of the parameters of the model should be considered a functional description of osmoregulation in *Blaberus* after the introduction of a solute load. The calculated volumes, with one exception (V_{dext}), do not correspond to real anatomical compartments. For example, the volume of compartment No. 1 (V_1) is only a small portion of the real hemolymph volume; but it is the functionally active portion. The obtained values for the parameters of the model are a consequence of the open type of circulatory system in insects. Hemolymph flows very slowly and is not mixed as well as blood in the closed circulatory system of vertebrates. Support for this theory comes from the findings of Pichon (1970) in *Periplaneta americana* that the concentrations of Na^+, K^+, and Ca^{2+} are different in hemolymph samples taken from different regions of the body. One very useful and potentially important parameter is the mean plasma clearance; the latter is a measure of the plasma volume, which is completely cleared of osmotically active particles in a unit of time. It is an ideal parameter to compare the osmoregulatory capability of one insect species with that of another.

The estimation of hemolymph volume in the present study was problematical. The hemolymph volume calculated by the dilution technique [equation (1)] is probably an overestimation because the long mixing time (1.25 hr) permitted some NaCl to be excreted and to be taken up by tissues, and consequently, C_2 was decreased. V_{darea} and V_{dss} are calculated from the elimination rate constant of the faster exponential, and in the calculation of V_1 the intercept of the faster exponential is used. Since both the elimination rate constant and the intercept of the faster exponential depend upon the osmotic concentration values at 1.25 hr, the values of V_{darea}, V_{dss}, and V_1 may be criticized for the same reason as the calculation of the hemolymph volume by the dilution technique. On the other hand, V_{dext} depends only upon the dose and the intercept of the slow exponential; the latter is established over a 36-hr period and after the complete distribution of NaCl had occurred. Therefore, it would appear to be the most reliable indicator of hemolymph volume. However, Wagner (1975) points out that V_{dext} is depressed when plasma protein binding is present. Since Weidler & Sieck (1977) have demonstrated the binding of Na^+ and Cl^- to macromolecules in the hemolymph of another cockroach species (viz. *Periplaneta americana*), the same situation may well obtain in *Blaberus*. Thus, it seems probable that the true hemolymph volume, in terms of body weight, is between 15.5% (V_{dext}) and 21.9% (dilution technique). It is of interest that the V_{dext} of 15.5% in *Blaberus* is very close to the combined plasma

volume and interstitial-lymph volume in man determined with mannitol, 15.9% and with inulin, 15.7% (Schwartz *et al.*, 1950); these combined volumes in man are analogous to the hemolymph volume in *Blaberus*.

The role of spontaneous drinking, as an osmoregulatory mechanism, was investigated in three steps. First, it was demonstrated that the ingestion of water (in this case, forced ingestion) could prevent the massive increase in hemolymph osmotic concentration which occurred from solute loading (Fig. 1). Second, the onset of spontaneous drinking was shown to occur at 0.8 hr after solute loading (Fig. 4); this point coincided with the first significant elevation of the mean osmotic concentration of antennal hemolymph after NaCl loading (Fig. 1). This documented spontaneous drinking as a response to an acute rise in hemolymph osmotic concentration, in Na^+ concentration, or in Cl^- concentration. Third, it was demonstrated that the cumulative fluid intake was proportional to the quantity of the daily injected solute. It may be concluded that with an acute elevation of hemolymph osmotic concentration (or of NaCl concentration), the drinking of water is effective in significantly lowering the elevation, and that the onset of spontaneous drinking occurs at the optimal time to lower the elevated concentration.

From the present study, it cannot be determined whether the relationship of cumulative fluid intake and daily injected NaCl over 4 days depends upon an elevated hemolymph osmotic concentration or upon another triggering factor. Although by 2.25 hr after injection of NaCl alone (group I) the hemolymph osmotic concentration returned to a level which was not significantly different from the initial concentration, the osmotic concentration at 24 hr after the administration of both NaCl and a large volume of water in *Blaberus* (group II) is unknown. It was shown that the hemolymph osmotic concentration rose continuously from 1.5 hr to 14.5 hr after injection of NaCl in group II, whereas the osmotic concentration decreased continuously over the same period in group I (Fig. 1). It appears that hemolymph osmotic concentration responds differently as a function of time depending on whether NaCl plus a relatively large volume of water is introduced into the hemocoel or whether NaCl alone (i.e. a highly concentrated NaCl solution) is injected into the hemocoel. Based on Fig. 1 (group II), it may be suggested that water is excreted faster than NaCl when both of them are introduced at approximately the same time. Therefore, it may be reasonably postulated that the hemolymph osmotic concentration is elevated 24 hr after the combination of the injection of NaCl and ingestion of a large volume of water, and that the relationship of the cumulative spontaneous water intake and the amount of daily injected NaCl over 4 days is explained by an elevation of hemolymph osmotic concentration at drinking times.

The factor which acutely triggers drinking after the injection of NaCl into *Blaberus* appears to be a rise in osmotic concentration; alternatively, however, it may be a rise in Cl^- concentration, as is the case with the blowfly, *Lucilia cuprina* (Barton Browne, 1964, 1968; Barton Browne & Dudziński, 1968). The increased cumulative spontaneous water ingestion in

Blaberus, which occurred 24 hr after each NaCl injection (Fig. 5), also could be due to an elevated osmotic concentration or Cl⁻ concentration; however, no data were sought or obtained in either the acute or chronic injection studies to support or refute the chloride hypothesis. From the work of Djajakusumah & Miles (1966) and Wall (1970), it appears that solute uptake by tissues occurs under circumstances which tend to elevate hemolymph osmotic pressure; based on these observations, an alternate hypothesis would be that an elevated Na^+ or Cl^- concentration within osmoreceptor cells is responsible for the delayed drinking response. Further investigation is required to elucidate the precise mechanisms responsible for the drinking response in *Blaberus* after NaCl loading.

SUMMARY

In the present study, osmoregulation in the giant cockroach, *Blaberus craniifer* Burm., was investigated. After the injection of a concentrated NaCl solution into the abdominal hemocoel, the osmotic concentration of antennal hemolymph was determined as a function of time by means of the comparative melting point method. Following the injection of NaCl, the osmotic concentration began to rise sharply by 30 min, peaked at 1.25 hr, fell sharply at first, and then more gradually until it reached the mean preinjection level at 21 hr (Fig. 1); however, the mean hemolymph osmotic concentration was not significantly different from the mean initial osmotic concentration by 2.25 hr ($P > 0.05$). When roaches were force-fed water at 15 min after injection, the rise in osmotic concentration was essentially prevented (Fig. 1). This finding established the drinking response as a potentially useful mechanism in osmoregulation. The osmotic concentration, time data were analyzed in terms of elimination kinetics of the two-compartment open model (Fig. 2). Two exponentials were obtained (Fig. 3). The mean plasma clearance was calculated to be 2.67 μl/hr, and the hemolymph volume was determined to be between 15.5 and 21.9% of the body weight, as derived from the extrapolated volume of distribution and the dilution technique, respectively.

After the injection of NaCl, the time of onset of spontaneous drinking was 0.8 hr (Fig. 4); at this point in time, the osmotic concentration of the hemolymph had become significantly elevated over the mean initial osmotic concentration. It was concluded that the onset of spontaneous drinking was triggered by the acute rise in hemolymph osmotic concentration (or a rise in NaCl concentration). The cumulative spontaneous water intake (over 4 days) was found to be proportional to the daily injected dose of NaCl (Fig. 5). In this case, increased spontaneous water intake may be secondary to an elevated hemolymph osmotic concentration or due to a different triggering factor.

Within the dosage range of 3.89–7.31 mg NaCl/g body weight, the duration from the time of injection until death was inversely proportional to the natural logarithm of the dose (Fig. 6).

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