TRANSPORT OF WATER VAPOR BY TENEBRIONID BEETLES. II. REGULATION OF THE OSMOLARITY AND COMPOSITION OF THE HEMOLYMPH[1]

PAMELA ANN COUTCHIÉ[2] AND JOHN H. CROWE
Department of Zoology, University of California, Davis, California 95616
(Accepted 8/15/78)

Water content was determined for hydrated and dehydrated larvae of *Onymacris marginipennis*. In addition, the volume and osmolarity of the hemolymph were measured, as were the major components of the hemolymph: sodium, potassium, magnesium, chloride ions, amino acids, and trehalose. There is a positive linear relationship between the water content (mg H_2O/mg dry weight) and the volume of the hemolymph (μl/mg dry weight). The osmolarity of the hemolymph is regulated between 320 and 450 mosmol/liter. The concentrations of the ions, amino acids, and trehalose are relatively constant despite changes in the volume of the hemolymph. The quantity of each of the major components increases as the volume of the hemolymph increases. These increases account for the observed regulation of the osmolarity of the hemolymph. All the major components of the hemolymph participate in the regulation.

INTRODUCTION

Previous work (Coutchié and Crowe 1979) has shown that larvae of the tenebrionid beetles *Onymacris plana* and *O. marginipennis* absorb water vapor from subsaturated atmospheres, increasing their weight to 140% of the original. This could result in severe osmotic stress. Marcuzzi (1955, 1956, 1957–58) and Machin (1975) showed that larvae of *Tenebrio molitor* regulate the osmolarity of the blood under similar conditions. One purpose of the present investigation was to examine the possibility that *O. marginipennis* possesses the capability to regulate the osmolarity of its blood under conditions of different water contents. A second purpose was to attempt to account for the role of major constituents of hemolymph as osmotic effectors during osmoregulation.

The larvae of *O. marginipennis* proved particularly useful for this study for the following reasons. (1) One can easily obtain animals possessing a large range of water contents. (2) The animals are quite large, reaching 6.0 cm in length prior to pupation. (3) Hemolymph samples of 30–40 μl can be obtained from a 200-mg individual. Such a volume is sufficient for multiple analyses. Consequently, we have examined the body water content, volume of the hemolymph, osmotic pressure, and the concentrations of cations, chloride ion, amino acids, and carbohydrates in the hemolymph, with a view toward elucidating their roles in osmoregulation.

MATERIAL AND METHODS

Culture methods for *Onymacris marginipennis* have been described previously

[1] We are grateful to Drs. E. B. Edney and William J. Hamilton III for many invaluable discussions and for their critical reading of the manuscript, and to Dr. Mary Seely who supplied the original adults from which cultures were established. This work was supported by a grant from the Patent Fund of the University of California to Pamela A. Coutchié and by grant BMS73-06897 from the National Science Foundation to John H. Crowe.

[2] Present address: Department of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 1A1.

(Coutchié and Crowe 1979). Larvae weighing 125–300 mg were selected from the colony and weighed and placed in individual glass vials, which were then placed in a humidity chamber at 94.5% relative humidity (RH) or at 0% RH. The respective humidities were maintained by a sulfuric acid and water solution (Solomon 1951) or $CaCl_2$ (Drierite). Individuals were weighed daily to ±0.1 mg, and fecal matter was weighed after drying at 60 C for 24 h. Hemolymph samples were taken on the fifth day in dehydrated animals and on the day after the maximal weight was attained in hydrated animals (fourth to sixth day). The cuticle was punctured with an insect pin and the hemolymph was collected in a 5 μl or 25 μl microcapillary tube (Drummond Scientific Co.). A known volume was evacuated into vials containing solutions appropriate for later analyses.

VOLUME OF HEMOLYMPH

The volume of the hemolymph in each individual was determined by the method of Wall (1970). After all the hemolymph samples were taken from each animal, it was carefully and rapidly dissected and the hemolymph was blotted with fine strips of filter paper. The animal was reweighed immediately and the hemolymph volume determined gravimetrically based on the difference between the final wet weight and the post-dissection wet weight. The animal was dried for 24 h at 60 C. From the final wet weight, the final water content was determined as follows: (final wet weight − final dry weight)/dry weight = mg H_2O/mg dry weight.

OSMOLARITY

The osmotic pressure of the hemolymph was measured immediately upon collection. An 8-μl sample was pipetted onto the paper disc of a Wescor vapor pressure osmometer which had been calibrated with factory-supplied NaCl standards. After determining the osmotic pressure, in those cases where the total hemolymph available was low, this disc was washed into another vial using 2–10 μl washes of glass distilled water for an additional analysis.

INORGANIC IONS

A 10-μl sample of hemolymph was used to measure chloride ions on a Buchler-Cotlove chloridometer. A 5-μl volume of hemolymph was placed in glass distilled water containing 0.2% La, 500 ppm Cs, and 1.0% HNO_3 for the analysis of sodium, potassium, and magnesium, using an atomic absorption spectrophotometer (Varian Techtron Model 1200).

AMINO ACIDS

The 5 μl of hemolymph were placed in 2 ml of 80% ETOH and centrifuged at 4 C at 10,000 g for 30 min to precipitate the protein. Fluorescamine (Roche Diagnostics), which reacts with primary amines, was added to an appropriate volume of the ethanol extract to produce a fluorescent product which was measured on a Turner Model 111 Fluorometer calibrated with glycine standards by the method of Udenfriend et al. (1972). Proline was determined by the isatin reaction (Boctor 1971). The concentration of the blue product formed was measured on a spectrophotometer calibrated with proline standards.

CARBOHYDRATES

The presence of glucose and trehalose in the hemolymph was determined by chromatographing on paper a volume of the ethanol extract as prepared above. The chromatograph was developed in a solvent of composition 5 butanol:4 ethanol:3 acetone:2 water (vol/vol) ac-

cording to the method of Trevelyan, Proctor, and Harrison (1950). The sugars were detected by spraying the paper with silver nitrate in sodium hydroxide and R_f values were compared with those of standards run simultaneously. Total carbohydrates were measured by the anthrone method of Dimler et al. (1952) on a 5-μl sample of hemolymph in glass-distilled water.

RESULTS

VOLUME OF THE HEMOLYMPH

There is a positive linear relationship between water content and volume of the hemolymph (fig. 1). Water which is not accounted for in the volume of the hemolymph is presumed to be the tissue water content. Most of the water absorbed appears to go directly into the hemocoel. Nicolson et al. (1974) suggested that there may be an overestimation in determining the volume by this method. However, in our procedures any error seems constant. Therefore, the line shown in figure 1 can be used to predict the relative volume of the hemolymph for animals of known water content. We used the equation of this line to calculate such a volume for some of the animals in the results described below.

OSMOLARITY

Osmotic pressure seems to remain relatively constant over a wide range of water contents (fig. 2). The volume 1.77 μl/mg dry weight (μl/mg dw) was chosen as an arbitrary reference point. In animals having a volume of hemolymph greater than 1.77 μl/mg dw, the osmotic pressure remains constant at about 320 milliosmoles/liter (mosmol/liter). Considering the dilution which would result from an increase in the volume of the hemolymph from 1.77 μl/mg dw to 3.5 μl/dw (which we will call hydrated), one would predict a decrease in the osmolarity to 182 mosmol/liter if there were no regulation (fig. 2, predicted line). However, no such decrease was observed (fig. 2, observed line). In animals with the volumes of hemolymph less than 1.77 μl/mg dw, osmotic pressure increases

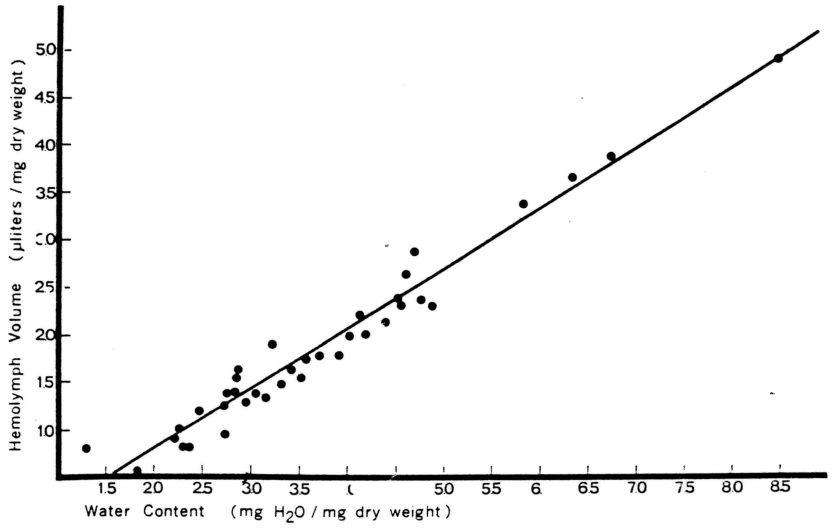


FIG. 1.—Relationship between water content (mg H_2O/mg dry weight) and the volume of the hemolymph (μl/mg dry weight).

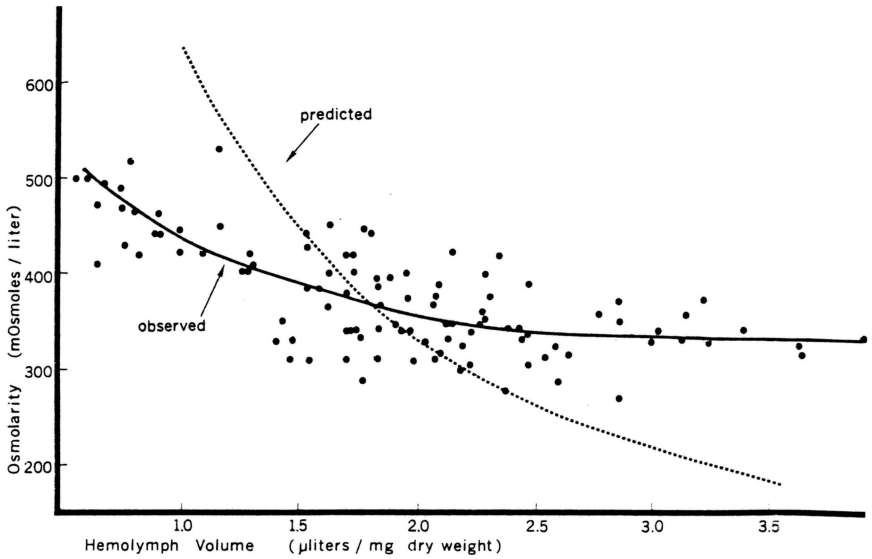


FIG. 2.—Relationship between the hemolymph volume and osmolarity. The predicted line was generated by assuming an arbitrary initial volume of 1.77 μl/mg dry weight with an initial osmolarity of 366 mosmol/liter and changing the volume without changing the number of millimosmoles present. The solid line represents the osmolarity observed in individuals with the indicated volumes of hemolymph. Note the strong regulation of the osmolarity in both dehydrated and hydrated animals.

slightly, but only to 500 mosmol/liter, while 728 mosmol/liter is expected due to solute concentration at a volume of 0.88 μl/mg dw (termed dehydrated), by the same line of reasoning as above. We conclude that osmotic pressure of the hemolymph is strongly regulated by the addition or removal of approximately 200 mosmol of solute.

INORGANIC IONS

In general, as many hemolymph components as possible were measured for each individual depending on the volume of hemolymph available. For the purposes of calculations, the results were grouped by hemolymph volume in 0.5 μl/mg dw increments. Both the volumes and the amount of each component were then averaged, and the standard error was determined for both parameters.

The concentration of the cations varied somewhat over the range of hemolymph volumes examined (fig. 3). Sodium concentration was 117 mmol/liter (mM) at 1.77 μl/mg dw. In animals with half this volume the concentration observed was 133 mM, while in the absence of regulation the expected concentration would be 234 mM. At twice the volume, the observed concentration was 75 mM and the predicted was 60 mM. Potassium and magnesium were of approximately equal concentration: 20 mM. They were both strongly regulated at 21 mM in dehydrated animals when the predicted value was 39 mM. In hydrated animals the observed concentration was 14 mM when the predicted value was 1 mM. All the cations seem to be more strongly regulated in dehydrated animals than in the hydrated ones.

Chloride concentration, like that of sodium, varied somewhat over the range of hemolymph volumes (fig. 4). In dehydrated animals 232 mM was the expected value, based on 116 mM for a

volume of 1.77 μl/mg dw; 182 mM was measured in these animals. In hydrated animals 58 mM was predicted, but 104 mM was observed. Thus we conclude that chloride concentration is also regulated.

AMINO ACIDS

The concentration of both the primary amino acids and of proline was very constant despite changes in the volume of the hemolymph (fig. 5). The concentration ranged from 48 mM to 33 mM for proline and from 57 mM to 45 mM for the primary amino acids. However, the predicted concentrations were 82 mM for proline and 105 mM for the primary amino acids in dehydrated animals, and 20 mM and 25 mM, respectively, in hydrated animals. We conclude that the concentration of amino acids, like that of the other components, is strongly regulated.

CARBOHYDRATES

Trehalose was the only low–molecular weight carbohydrate detected in the

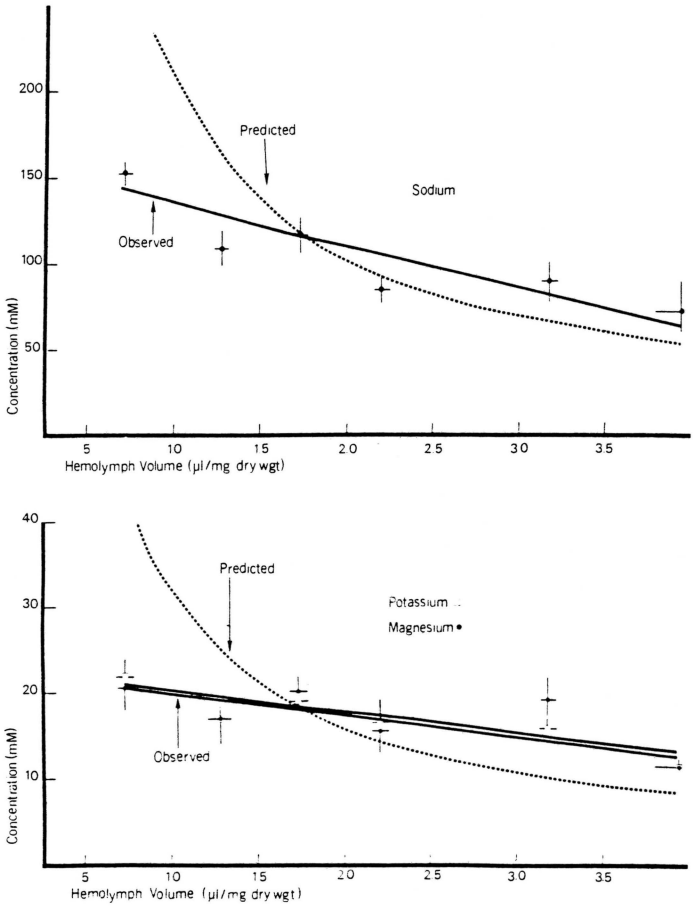


FIG. 3.—Relationship between the concentrations of sodium, potassium, and magnesium and the volume of the hemolymph. For each curve the predicted line is generated by assuming the concentration present at 1.77 μl/mg dry weight and varying the volume without any of the components entering or leaving the hemolymph. Note that the concentration remains relatively constant despite the changes in the volume of the hemolymph when compared to the predicted values which assume no regulation.

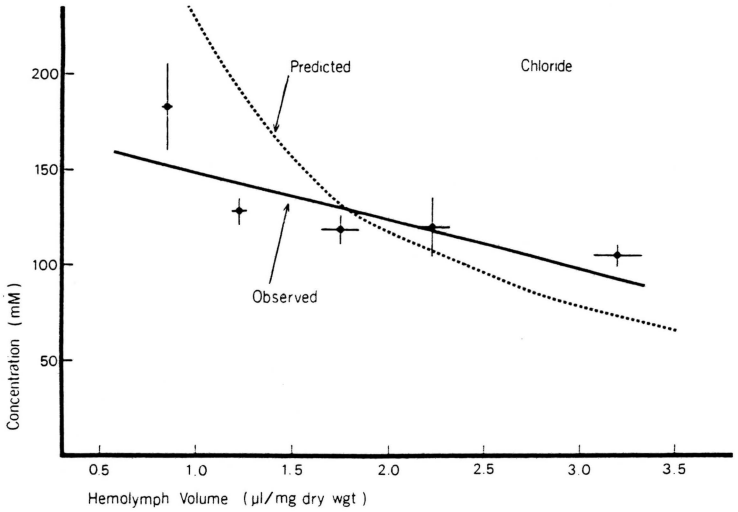
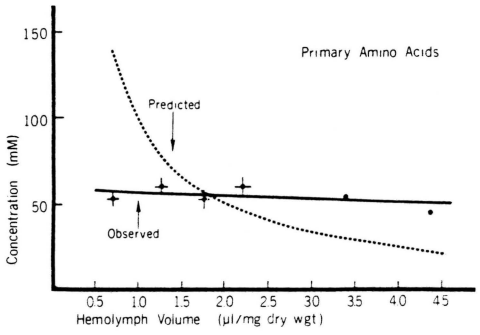


FIG. 4.—Relationship between the concentration of chloride ions and the volume of the hemolymph. See legend to figure 3 for further explanation.

hemolymph. It ranged from 55 mM in dehydrated animals to 45 mM in hydrated ones (fig. 6). Predicted concentrations of 130 mM and 32 mM, respectively, lead to the conclusion that the concentration of trehalose is also regulated.

RELATIVE CONTRIBUTIONS OF MAJOR CONSTITUENTS TO OSMOREGULATION

To assess the relative contribution of the major constituents to the observed osmotic pressure, the measured concen-



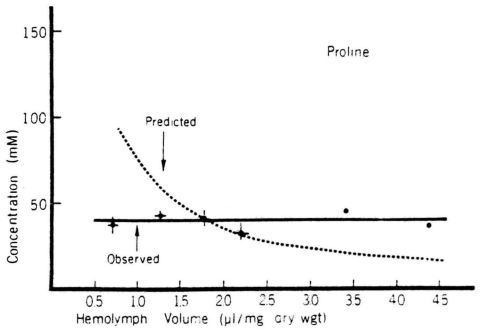


FIG. 5.—Relationship between the concentration of primary amino acids and the volume of the hemolymph. See legend to figure 3 for further explanation.

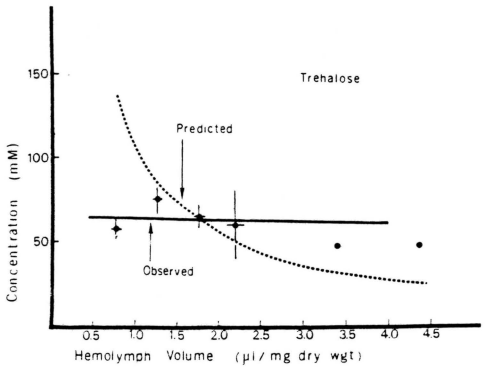


FIG. 6.—Relationship between the concentration of trehalose and the volume of the hemolymph. See legend to figure 3 for futher explanation.

tration was converted to an expression for the total millimoles of any component in the hemolymph/mg dw in the following fashion: mmol/liter × μl hemolymph/mg dw = mM/mg dw, and mosmol/liter × μl hemolymph/mg dw = mosmol/mg dw. The values obtained were then grouped by blood volume at 0.5-μl increments. A regression line was determined for the average values at each of the averaged blood volumes. The results are expressed in figures 7–10.

The quantity of each of the inorganic ions increases with increasing volume of the hemolymph, with sodium and chloride increasing more sharply than potassium and magnesium (fig. 7). For sodium, 0.18×10^{-6} mM/mg dw in-

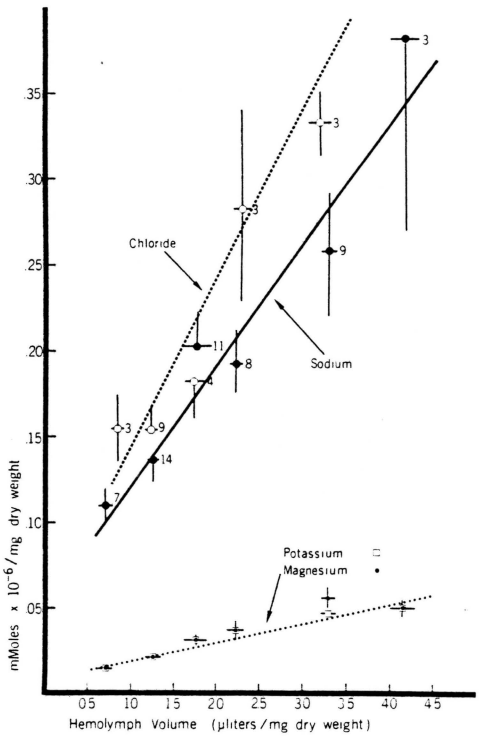


FIG. 7.—Total mmol/mg dry weight of ions plotted against the volume of the hemolymph. The total quantity of each component increases as the volume increases, which is the result expected if the concentration remains constant as seen in figures 3–6.

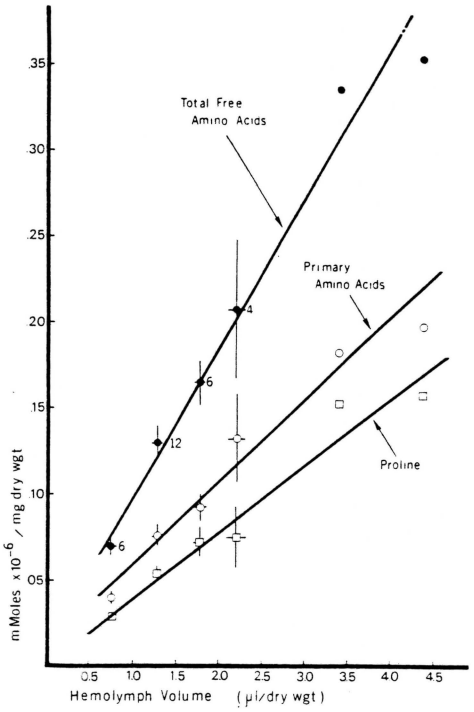


FIG. 8.—Total mmol/mg dry weight of amino acids plotted against the volume of the hemolymph. See legend to figure 7 for further explanation.

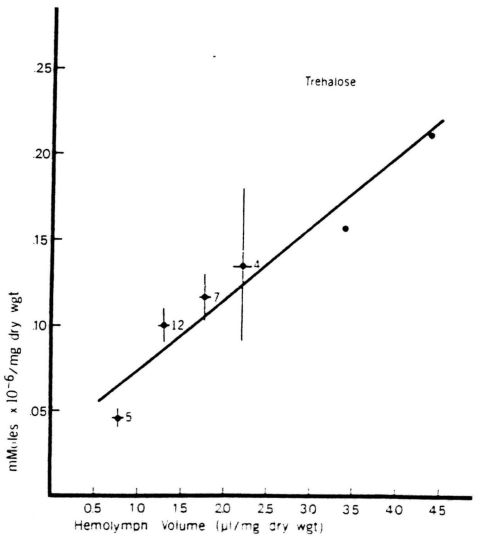


FIG. 9.—Total mmol/mg dry weight of trehalose plotted against the volume of the hemolymph. See legend to figure 7 for further explanation.

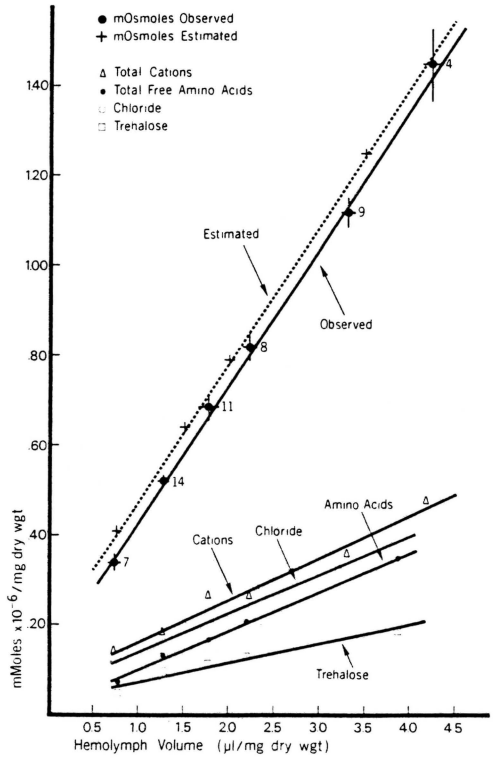


FIG. 10.—Summary graph expressing the relationship between the measured components and the volume of the hemolymph. From the measurement of the osmotic pressure, the total milliosmoles observed was calculated. The values for each of the measured components were summed to obtain a predicted value for the osmotic pressure (labeled "estimated"), assuming all components have an independent osmotic effect. Note that the slopes of the observed and estimated lines for the osmotic pressure are indistinguishable.

creases to 0.30×10^{-6} mM/mg dw at twice the volume, less than the 0.36×10^{-6} mM/mg dw which would be expected if the sodium concentration were perfectly regulated. A similar pattern is seen in chloride ion where the quantity increases from $.21 \times 10^{-6}$ mM/mg dw to only 0.35×10^{-6} mM/mg dw at twice the volume. While magnesium and potassium also increase with blood volume, the contributions of these ions as osmotic effectors are relatively minor. The total mMoles of free amino acids also show sharp increases while the hemolymph volume increases (fig. 8). The primary amino acids contribute slightly more to the total than does proline. The total quantity of amino acids increases from $.165 \times 10^{-6}$ mM/mg dw to $.31 \times 10^{-6}$ mM/mg dw. Trehalose does not increase as sharply as the other constituents do when the volume of hemolymph increases (fig. 9), rising from 0.1×10^{-6} mM/mg dw to 0.15×10^{-6} mM/mg dw.

The total mosmol/mg dw increased with the increasing blood volume (fig. 10). This result is expected if the osmotic pressure is to remain constant in the face of an increased volume of hemolymph. From the blood volume of 1.77 µl/mg dw the number of milliosmoles nearly doubles from 0.68×10^{-6} mosmol/mg dw to 1.28×10^{-6} mosmol/mg dw, a value which is close to the 1.36×10^{-6} mosmol/mg dw needed to maintain a constant total solute concentration at twice the blood volume.

Figure 10 is a summary of the relative contributions of these major components. The values obtained from the regression lines for each component have been summed at appropriate hemolymph volumes in order to obtain the dotted line. Assuming complete ionization of the ions and independent and equal osmotic activity of each molecule of amino acid or carbohydrate, this dotted line represents an estimation of the osmotic pressure that would be observed. Any molecular interactions would decrease the actual osmotic pressure produced by the concentrations measured. Therefore, it is not surprising that the sum of the amounts of solutes detected in the hemolymph is actually greater than that calculated from the measured osmolarity. However, the slope of the dotted line is identical to that

obtained for the regression line for the osmolarity.

For cations, amino acids, and chloride, the slopes of the regression lines are about equal, as are the amounts of each of the solutes at any given volume of hemolymph. Each of these groups of components contributes approximately 25% to the total observed osmolarity, given the assumptions above regarding their activity in solution.

DISCUSSION

The linear relationship which exists between water content and the volume of the hemolymph suggests that water absorbed from the air is stored in the hemocoel. We tentatively suggest that water is transferred to body tissues at the expense of the hemolymph volume when the animal is under stress due to dehydration. We have also found in the course of these studies that animals with decreased water content appear flat and flaccid, while animals of high water content were rounded in appearance. In fact, when the hydrated animals were punctured in order to obtain the hemolymph sample, the hemolymph literally spurted several centimeters into the air. Okasha (1971, 1972) has previously suggested that the uptake of water vapor from subsaturated air by *Thermobia* may constitute a mechanism for the regulation of the volume of the hemolymph. Coutchié and Crowe (1979) showed that in *Onymacris* water uptake ceases after a point, when an animal has reached an apparent maximal water content (or hemolymph volume). It seems likely that the control may be determined by the animal's volume and, from what is known concerning the role of abdominal stretch receptors in the volume regulation in insects like *Rhodnius* (Wigglesworth 1964), we suspect that this control could be mediated by stretch receptors.

Data have been accumulated over the past decade by a number of workers which demonstrate that terrestrial insects are capable of strongly regulating the osmolarity of their hemolymph. For example, Broza, Borut, and Pener (1976) noted that the adult tenebrionid *Trachyderma philistina* controlled the osmolarity of its hemolymph between 385 and 528 mosmol/liter as do adult *Eleodes hispilabris* (Riddle, Crawford, and Zeitone 1976). Machin (1975) noted that *Tenebrio* which had increased their water content by 25%–30% showed a decrease in the concentration of their hemolymph from 575 to 433 mosmol/liter. Marcuzzi (1955) reported an osmotic pressure for "normal" *Tenebrio* larvae of 356 mosmol/liter. Although Marcuzzi saw a relatively constant osmotic pressure, there were no data on the volume changes, so the extent of the osmoregulation cannot be fully determined. In the present study, we found that osmoregulation in *Onymacris* is between 320 and 480 mosmol/liter. Other insects, such as lepidopterans, are known to osmoregulate between 200 and 300 mosmol/liter (Fyhn and Saether 1970). The orthopterans which have been studied also osmoregulate in similar ranges: *Arenivaga* and *Periplaneta*, 407–470 mosmol/liter (Edney 1966, 1968; Wall 1970); *Leucophaea*, 326–353 mosmol/liter (Laird and Winston 1975); and *Chortoicetes*, 200–250 mosmol/liter (Djajakusumah and Miles 1966). In general the terrestrial insects studied seem to be able to regulate the osmolarity of their hemolymph within a range of 100–150 mosmol/liter.

Even though the regulation is becoming well documented, the mechanism by which it is accomplished is not yet clear. Edney (1966) suggested that the

removal of chloride ions might contribute to the osmoregulation he observed in *Arenivaga* and Okasha (1973) reported relatively constant concentration of chloride in *Thermobia* of different water contents, as did Tucker (1977*a*, 1977*c*, 1977*d*) for *Periplaneta*. Broza et al. (1976) stated that although chloride ion contributed to the osmoregulation they observed in *Trachyderma*, the inorganic ions were not as important as the organic molecule as osmotic effectors. However, when we examined their data closely, it became apparent that chloride ion contributed nearly 50% of the osmotically active substances they reported. In addition, the total osmolarity that would be generated by the substances they measured (chloride ion and free amino acids), represented only about 50% of the osmotically active substances in the hemolymph.

Cations have been suggested as osmotic effectors by a few workers. In millipedes, the combination of sodium, chloride, potassium, and amino acids together accounted for two-thirds of the observed osmotic pressure (Woodring 1974). Wall (1970) observed a strong regulation of the concentrations of potassium and sodium in dehydrated *Periplaneta*, and Okasha (1973) described rather constant concentrations of sodium, potassium, and chloride in *Thermobia* of varied water contents. Results of the present study suggest that sodium, potassium, and magnesium constitute about 25% of the osmotically active substances in the hemolymph, and chloride a further 25%. This contribution is constant with variations in the volume of the hemolymph. Thus the inorganic ions account for 50% of the osmotic effectors.

The potential involvement of free amino acids as osmotic effectors in the hemolymph has been suggested by a number of workers including Sutcliffe (1963), Florkin and Jeuniaux (1974), and Laird and Winston (1975), based on the observation that coleopterans possess high levels of free amino acids in the hemolymph, and on the work of Djajakusumah and Miles (1966) who proposed that in *Chortoicetes* (a locust) an interplay of nonprotein nitrogenous substances and protein might account for the observed osmoregulation. The amino acids in *Chortoicetes* are 15% of the total osmoles present, but the amino acids represent 30% of the milliosmoles added back to the hemolymph when the dehydrated animal was allowed to drink water and replenish the hemolymph volume (Djajakusumah and Miles 1966). From our results in the present study and those of Broza et al. (1976), which clearly demonstrate the involvement of free amino acids as osmotic effectors in the hemolymph of insects, it appears that free amino acids comprise about 25% of the total osmotically active substances. Thus the inorganic ions and free amino acids constitute about 75% of the osmotically active substances.

The remaining 25% of the total must be accounted for by still other solutes in the hemolymph. While there are a variety of other inorganic ions, such as calcium ions, phosphate ions, sulfate ions *etc.*, their contributions are relatively minor. The carbohydrate trehalose is probably the most important to consider with respect to potential osmotic contribution. There have been many measurements of the carbohydrate levels in insect hemolymph (see review by Florkin and Jeuniaux 1974; Jungreis 1976). However, there have been few attempts to estimate the osmotic contribution or to make any correlation with osmoregulation. Trehalose is at a

high concentration in the hemolymph and cannot be without some effect on the osmotic pressure. In fact, according to this study, the level of trehalose in the hemolymph represents the final 25% of the osmotic contribution by solutes. Thus, all the major constituents of the hemolymph—cations, free amino acids, chloride ions, and trehalose—contribute to the observed osmotic pressure in about equal proportions. Moreover, the relative contributions do not appear to be altered significantly, despite drastic changes in the volume of the hemolymph. All these components remain at approximately constant concentrations, meaning, of course, that the total amount of each constituent in the hemolymph must increase with the volume of the hemolymph.

Although the quantity of solute which must be added to the hemolymph at increased hemolymph volumes during osmoregulation is supplied by these major components, little is known concerning the mechanism of storage of these solutes during dehydration or how they might be mobilized during hydration. Only a few suggestions concerning how this might be accomplished exist in the literature. Evidence for a storage site for inorganic ions is found in the work of Jungreis and Tojo (1973) who showed that potassium, although of integumental origin, accumulated in the fat body, and Tucker (1977*d*) showed that chloride may be stored in the hindgut. Mullins and Cochran (1974) suggested that sodium and potassium could be stored as urate salts in the fat body, a proposition that is supported by the recent work of Tucker (1977*a*, 1977*b*, 1977*c*, 1977*d*). Given the current study indicating the importance of the inorganic ions in the regulation of the osmolarity of the hemolymph, more work is needed to identify the source of the ions. We should emphasize that the immediate source for these ions is not dietary; the animals were starved during the course of our experiments. Just as the only source of water was from the water vapor of the air, the only source of solutes was within the body of each individual.

The possible sources of free amino acids and trehalose are less difficult to discern. Djajakusumah and Miles (1966) have suggested that the amino acids may be polymerized into proteins or polypetides during dehydration and broken into free amino acids during subsequent rehydration. Collett (1976) has suggested that a peptidase can mediate the storage of amino acids in *Calliphora*. An alternative suggestion might be that amino acids may be metabolized during dehydration and resynthesized during rehydration. Such a possibility is being considered by several workers involved in the area of osmoregulation in euryhaline organisms (Baginski and Pierce 1975). The problem incurred in applying this mechanism to insects is the necessity for a source of NH_3 for the resynthesis pathway. In preliminary studies we have found that the level of ammonia is low in the hemolymph. Work on bacterial symbionts in the fat body of cockroaches has suggested that these symbionts may be involved in the nitrogen metabolism of the host, providing various metabolites as well as ammonia from the uric acid of the fat body (Donnellan and Kilby 1967; Henry and Block 1962). It is not known if there are similar symbionts in *Onymacris*. However, such a pathway would prove of interest, particularly in view of the possible association of the inorganic ions with urate in dehydrated animals. Currently, we are attempting

to determine the fate of the free amino acids in dehydrated animals and their source in hydrated ones.

Trehalose, a disaccharide of glucose, can be converted into glycogen or metabolized by normal oxidative pathways. If trehalose is broken down during dehydration, it could be resynthesized from the subunits of glycogen when the animal became rehydrated. Or, if the glyoxalate pathway exists in insects (Skye and Van Handel 1974), trehalose could be synthesized from fatty acid precursors.

LITERATURE CITED

BAGINSKI, R. M., and S. K. PIERCE. 1975. Anaerobiosis: a possible source of osmotic solute for high-salinity acclimation in marine molluscs. J. Exp. Biol. **62**:589–598.

BOCTOR, F. N. 1971. An improved method for colorimetric determination of proline with isatin. Anal. Biochem. **43**:66–70.

BROZA, M., A. BORUT, and M. P. PENER. 1976. Osmoregulation in the desert tenebrionid beetle *Trachyderma philistina* (Reiche) during dehydration and subsequent rehydration. Israel J. Med. Sci. **12**:868–871.

COLLETT, J. I. 1976. Peptidase-mediated storage of amino acids in small peptides. Insect Biochem. **6**:176–185.

COUTCHIÉ, P. A., and J. H. CROWE. 1979. Transport of water vapor by tenebrionid beetles. I. Kinetics. Physiol. Zool. **52**:67–87.

DIMLER, R. J., W. C. SCHAEFFER, C. S. WISE, and C. E. RISE. 1952. Quantitative paper chromatography of D-glucose and its oligosaccharides. Anal. Chem. **24**:1411–1414.

DJAJAKUSUMAH, T., and P. W. MILES. 1966. Changes in the relative amounts of soluble protein and amino acid in the haemolymph of the locust *Chortoicetes terminifera* Walker (Orthoptera: Acrididae), in relation to dehydration and subsequent hydration. Australian J. Biol. Sci. **19**:1081–1094.

DONNELLAN, J. F., and B. A. KILBY. 1967. Uric acid metabolism by symbiotic bacteria from the fat body of *Periplaneta americana*. Comp. Biochem. Physiol. **22**:235–252.

EDNEY, E. B. 1966. Absorption of water vapour from unsaturated air by *Arenivaga* sp. (Polyphagidae, Dictyoptera). Comp. Biochem. Physiol. **19**:387–408.

———. 1968. The effect of water loss on the haemolymph of *Arenivaga* sp. and *Periplaneta americana*. Comp. Biochem. Physiol. **25**:149–158.

FLORKIN, M., and C. JEUNIAUX. 1974. Hemolymph: composition. Pages 255–307 *in* M. ROCKSTEIN, ed. The physiology of insecta. Vol. **5**. Academic Press, New York.

FYHN, H. J., and T. SAETHER. 1970. Regulation of the haemolymph osmolarity during metamorphosis in the oak silk moth *Antherae pernyi*. J. Insect Physiol. **16**:263–269.

HENRY, S. M., and R. J. BLOCK. 1962. Amino acid synthesis, a rumen-like effect of the intracellular symbionts of the German cockroach. Fed. Proc. **21**:9.

JUNGREIS, A. M. 1976. Regulation of *Hyalophora cecropia* fat body hexokinase by hexose phosphates common to the pathways of glycolysis, glucogen and trehalose synthesis. Comp. Biochem. Physiol. **53B**:405–413.

JUNGREIS, A. M., and S. TOJO. 1973. Potassium and uric acid content in tissues of the silkmoth *Hyalophora cecropia*. Amer. J. Physiol. **224**:21–26.

LAIRD, T. B., and P. W. WINSTON. 1975. Water and osmotic pressure regulation in the cockroach, *Leucophaea maderae*. J. Insect Physiol. **21**:1055–1060.

MACHIN, J. 1975. Water balance in *Tenebrio molitor*, L. larvae; the effect of atmospheric water absorption. J. Comp. Physiol. **101**:121–132.

MARCUZZI, G. 1955. Osservazioni fisico-chimiche sul sangue dei Coleotteri Tenebrionidi. I. La pressione osmotica nel *Tenebrio molitor* L. Atti Accad. Naz. Lincei. **18**:654–662.

———. 1956. L'osmoregulazione nel *Tenebrio molitor* L. (Col. Tenebrionidae). Atti Accad. Naz. Lincei **20**:492–500.

———. 1957–58. L'osmoregulazione nel *Tenebrio molitor* L. (Coleoptera Tenebrionidae). Boll. Zool. Agr. Bachicultura. Torino **1(SII)**:127–132.

MULLINS, D. E., and D. G. COCHRAN. 1974. Nitrogen metabolism in the American cockroach: an examination of whole body and fat body regulation of cations in response to nitrogen balance. J. Exp. Biol. **61**:557–570.

NICOLSON, S., P. M. HORSFIELD, B. O. C. GARDINER, and S. H. P. MADDRELL. 1974. Effects of starvation and dehydration on osmotic and ionic balance in *Carausius morosus*. J. Insect Physiol. **20**:2061–2069.

OKASHA, A. Y. K. 1971. Water relations in an insect, *Thermobia domestica*. I. Water uptake subsaturated atmospheres as a means of volume regulation. J. Exp. Biol. **55**:435–448.

———. 1972. Water relations in an insect, *Thermobia domestica*. II. Relationships between water content, water uptake from subsaturated atmospheres and water loss. J. Exp. Biol. **57**:285–296.

———. 1973. Water relations in an insect, *Thermobia domestica*. III. Effects of desiccation and rehydration on the haemolymph. J. Exp. Biol. **58**:385–400.

RIDDLE, W. A., C. S. CRAWFORD, and A. M. ZEITONE. 1976. Patterns of hemolymph osmoregulation in three desert arthropods. J. Comp. Physiol. **112**:295–305.

SKYE, G. E., and E. VAN HANDEL. 1974. Malate synthase in insects. Comp. Biochem. Physiol. **49B**:83–86.

SOLOMON, M. E. 1951. The control of humidity with KOH, H_2SO_4, and other solutions. Bull. Entomol. Res. **42**:543–559.

SUTCLIFFE, D. W. 1963. The chemical composition of haemolymph in insects and some other arthropods, in relation to their phylogeny. Comp. Biochem. Physiol. **9**:121–135.

———. 1974. Sodium regulation and adaptation to fresh water in the isopod genus *Asellus*. J. Exp. Biol. **61**:719–736.

TREVELYAN, W. E., D. P. PROCTER, and J. S. HARRISON. 1950. Detection of sugars on paper chromatograms. Nature **166**:444–445.

TUCKER, L. E. 1977*a*. Effect of dehydration and rehydration on the water content and Na^+ and K^+ balance in adult male *Periplaneta americana*. J. Exp. Biol. **71**:49–66.

———. 1977*b*. The influence of diet, age and state of hydration on Na^+, K^+, and urate balance in the fat body of the cockroach *Periplaneta americana*. J. Exp. Biol. **71**:67–80.

———. 1977*c*. The influence of age, diet, and lipid content on survival, water balance and Na^+ and K^+ regulation in dehydrating cockroaches. J. Exp. Biol. **71**:81–94.

———. 1977*d*. Regulation of ions in the haemolymph of the cockroach *Periplaneta americana* during dehydration and rehydration. J. Exp. Biol. **71**:95–110.

UDENFRIEND, S., S. STEIN, P. BOHLEN, W. DAIRMAN, W. LEIMGRUBER, and M. WEIGELE. 1972. Fluorescamine: a reagent for assay of amino acids, peptides, and primary amines in the picomole range. Science **178**:871–872.

WALL, B. J. 1970. Effects of dehydration and rehydration on *Periplaneta americana*. J. Insect Physiol. **16**:1027–1042.

WIGGLESWORTH, V. B. 1964. The hormonal regulation of growth and reproduction in insects. Advance. Insect Physiol. **2**:247–336.

WOODRING, J. P. 1974. Effects of rapid and slow dehydration on the hemolymph osmolarity and Na^+-K^+ concentration in the millipede *Pachydermus crassicutis*. Comp. Biochem. Physiol. **49A**: 115–119.