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SHORT COMMUNICATION

REGULATION OF BODY FLUID COMPARTMENTS DURING DEHYDRATION OF THE TENEBRIONID BEETLE *RHYTINOTA PRAELONGA*

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Tenebrionid beetles are an important element of the fauna on the African dry savanna. In contrast to the majority of savanna insect groups, which spend the dry season as larvae, the tenebrionids pass the dry season as adults. The adult tenebrionids probably feed on dry plant material and detritus (Louw and Seely, 1982), and they may undergo severe dehydration during the dry season (authors' observation), probably because of their limited access to water.

The ability of the tenebrionids to survive the dry season as adults is based on a number of behavioural and physiological adaptations. The adults spend the hot days hidden under stones and logs, where they avoid the high temperatures and low air humidities that prevail on the exposed surface. They seek to reduce the loss of body water by producing extremely dry faeces and by having low rates of transpiratory water loss (Edney, 1977; Zachariassen *et al.* 1987*a*). The low rates of transpiratory water loss appear mainly to be the result of a low cuticular water permeability, leaving the respiratory water loss over the spiracles as the main component (Zachariassen, 1991). They display low metabolic rates, implying that the respiratory water loss is also low (Zachariassen *et al.* 1987*a*). Most tenebrionid species also have fused elytra, which form a closed subelytral chamber, serving to reduce further the respiratory water loss from the abdominal spiracles (Cloudsley-Thompson, 1964; Ahearn and Hadley, 1969; Zachariassen, 1991). The long survival time secured by these mechanisms also allows a substantial metabolic water production, which provides a further increase in the survival time (Edney, 1977; Zachariassen *et al.* 1987*b*).

Another important adaptation for survival with limited access to water is the great tolerance to dehydration displayed by the tenebrionid beetles. While fully hydrated, tenebrionids may have a relative water content as high as 70%, but the water content after several weeks of dehydration may drop below 40% (authors' observation). This represents a loss of almost 75% of the total body water. A water loss of this magnitude

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raises questions about how the reductions are reflected in the volumes of the various body fluid compartments and in the solute concentrations in the body fluids.

The regulation of haemolymph volume and solute concentrations in insects undergoing dehydration has been studied by several investigators. By using two different methods, Richardson *et al.* (1931) found that larvae of the silkworm *Bombyx mori* and the greater wax moth *Galleria mellonella* had haemolymph masses amounting to 30–40% of the total body mass. Edney (1968) used an inulin dilution technique and found that in the desert cockroach *Arenivaga* sp. and *Periplaneta americana* the haemolymph volume dropped with increasing dehydration. He could not draw any conclusions regarding the relative reductions in haemolymph and cellular compartments. He found that a removal of chloride ions from the haemolymph contributed to the regulation of the extracellular solute concentration. By using a filter paper method, Coutchié and Crowe (1979) found that there is a linear relationship between the water content and the haemolymph volume of larvae of the tenebrionid beetle *Onymacris marginipennis* undergoing hydration and dehydration. The haemolymph osmolality was regulated during the dehydration process, and all major haemolymph solutes were found to contribute to the regulation.

The purpose of the present investigation has been to study the relative changes in the volumes of the extracellular and intracellular body fluid compartments of adult *Rhytinota praelonga* tenebrionid beetles undergoing transpiratory water loss in the laboratory. The possible role of sodium as an osmolyte in the volume regulation of the body fluid compartments has also been investigated. *R. praelonga* beetles are common on dry savanna in East Africa, and the adult stage can be found throughout the dry seasons.

Adult *Rhytinota praelonga* beetles with body mass ranging from 120 to 250mg were collected from their natural habitats under stones in the vicinity of the township of Oltepesi, Kenya. The beetles were brought to the laboratory in small plastic bottles. Prior to the experiments they were kept in the laboratory for 1 week and given water and potato. The beetles were dehydrated at room temperature (20–24˚C) inside a desiccator in which the air was kept dry (relative humidity <5%) by means of silica gel. The dehydration experiments took place over a period of 3 weeks. The beetles were neither fed nor given water during the dehydration period.

Following different periods in the dry atmosphere, which allowed the beetles to reach different degrees of dehydration, they were taken out from the desiccator and weighed to determine their total body mass (TBM). For analysis of solute concentrations, samples of haemolymph were taken by a method described by Zachariassen *et al.* (1983). A hole was made in the ventral surface between the middle and hind pair of legs, and the attenuated flame-drawn end of a glass capillary was inserted through the hole, allowing the haemolymph to enter the capillary by means of the capillary force. The haemolymph sample was isolated from air by means of liquid paraffin and the distal end of the capillary melted in a flame. The capillary was then centrifuged so that the haemolymph was situated in the melted end, isolated from air by the liquid paraffin. The samples were stored at −25˚C until the analyses were carried out.

The remaining haemolymph was then removed by means of a filter paper method similar to that described by Richardson *et al.* (1931). The ventral cuticle of the beetles was cut open along the median line with a small surgical scissor, and small pieces of filter

paper (Schleicher & Schüll no. 300009) were introduced into the opening so that they came into contact with the haemolymph, which consequently was absorbed by the filter paper. Since beetles have an open circulatory system, the filter paper capillarity is likely to remove practically all extracellular fluid. Assuming that evaporation from the extracellular fluid does not create an osmotic efflux of water from the cells, intracellular fluid is not likely to be removed by this procedure.

The haemolymph compartment was assumed to be exhausted when no more fluid could be drawn into the filter paper. When this stage was reached, the beetles were weighed, and the difference between this mass and the TBM was taken as a measure of the haemolymph mass (HM).

Following the removal of the haemolymph, the beetles were dried for 6–10h at 105˚C and weighed once more to determine their dry body mass (DBM). The difference between the TBM and the DBM was taken as the water mass (WM) of the beetles, and the difference between the DBM and the mass after the haemolymph had been removed was taken as the cell water mass (CWM).

The haemolymph osmolality was determined from the melting points in a Clifton nanolitre osmometer. The temperature at which the last tiny ice crystal disappeared during slow heating of a frozen 20nl haemolymph sample was taken as the melting point.

For determination of the extracellular sodium concentration, 5 μl samples of haemolymph were transferred to 10ml flasks by means of a micro syringe and diluted to 10ml in deionized water. For determination of the total sodium content, beetles dried at 105˚C were transferred to platinum crucibles and ashed overnight at 570˚C. The ashes were dissolved in five droplets of concentrated HNO_3 and diluted to 20ml with deionized water in a glass flask. The concentration of sodium in the diluted samples was measured on a Perkin Elmer 2100 atomic absorption spectrophotometer at 290nm. The intracellular concentration of sodium was calculated by dividing the amount of sodium in the dried beetles by the cellular water mass (CWM).

The statistical significance of the slopes of the regression lines was tested by the use of *t*-test formulae from Zar (1984).

Fig. 1 shows that in the fully hydrated beetles (beetles containing about 200% water in relation to dry mass) the haemolymph made up about two-thirds of the cell water mass. As the beetles lost body water, there was a marked reduction of both the haemolymph and the cell water. The reduction was most pronounced in the haemolymph, in that the haemolymph mass reached zero while the cell water content still made up 60% of the dry mass.

The results obtained in Fig. 1 imply that in fully hydrated beetles the intracellular fluid makes up more than half (about 60%) the total body water. As the beetles become dehydrated, the amounts of intracellular fluid as well as haemolymph are gradually reduced. The reduction is most pronounced in the extracellular fluid, and when the haemolymph mass reaches zero, the cell water content is reduced by only about 50%. The beetles tolerate this tremendous reduction in extracellular fluid volume, and they seem to be in a good shape even when the extracellular fluid makes up as little as 5% of the dry mass. Death seems to occur when the extracellular fluid reservoir is exhausted, with the consequence that further dehydration must take place at the expense of the remaining cellular water.

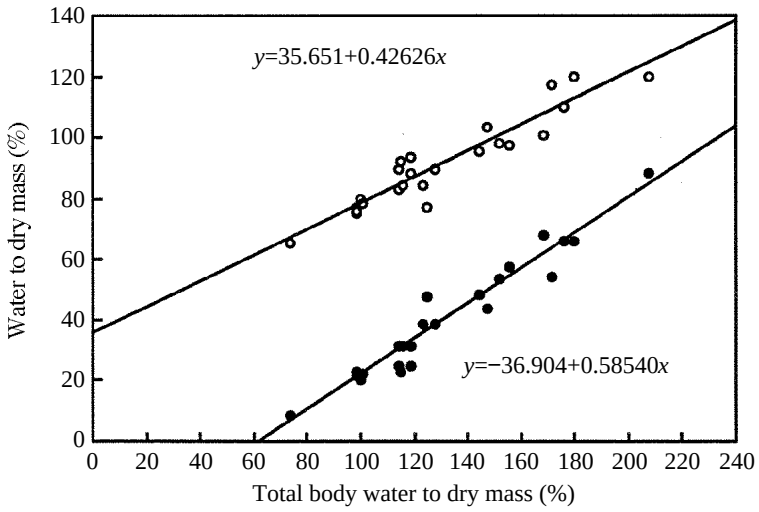

Fig. 1. The percentage of haemolymph mass (●) and cell water mass (○) to dry body mass (100×HM/DBM and 100×CWM/DBM) of *Rhytinota praelonga* beetles which have undergone different degrees of dehydration plotted as a function of total water content relative to dry mass (100×WM/DBM). The haemolymph volumes were determined by means of the filter paper method. All values are related to dry mass of beetles to correct for differences in body size. The lines are linear regressions.

Both vertebrates and invertebrates have developed effective mechanisms to maintain cell volume under situations with changing body fluid osmolality (Lange, 1972; Hoffmann, 1977). This suggests that the maintenance of the cell volume at an optimal level is physiologically important to animals. The present results reveal that *R. praelonga* beetles seek to restrict the reduction in cell volume when they become dehydrated, and that they do this at the expense of the haemolymph volume. They die when this regulation can no longer prevent excessive cellular volume reduction.

These results support the contention of Mellanby (1939), who claimed that the haemolymph of insects acts as a water reservoir during dehydration. This implies that the more water the beetles can store in the extracellular compartment, the longer they can survive under dry conditions without dietary water. Accordingly, by storing a large amount of water in the haemolymph during the rainy season, the beetles have a great chance of surviving the water shortage experienced during the dry season.

The relationship between total body water content and haemolymph mass observed for *R. praelonga* in the present study (Fig. 1) agrees well with the results obtained for *Onymacris* larvae by Coutchié and Crowe (1979). In the present study, the slope of the regression line was 0.58, whereas the slope of the line obtained by Coutchié and Crowe was 0.66. The similarity between the manners in which the haemolymph volume changes in the two experiments suggests that the haemolymph volume is regulated by closely related mechanisms in the two species of tenebrionids. The ranges of the values of relative haemolymph mass and relative water content of the *Onymacris* larvae were

substantially higher than those of the adult *R. praelonga* beetles, but this is likely to be due to the heavy cuticular shell of *R. praelonga.*

The relatively moderate reduction of the cell water content, together with the great reduction in haemolymph volume, requires a comprehensive redistribution of osmolytes. If the relative numbers of osmolytes in the different body fluid compartments were to remain unaltered, evaporative water loss from the haemolymph would result in an osmotic influx of water from the cells and other compartments so that osmotic equilibrium could be maintained, i.e. there would be a proportional reduction in the water content of all compartments. Thus, in order to ensure that the loss of water predominantly takes place at the expense of the haemolymph, there must be a continuous reduction in the amount of osmolytes in the haemolymph relative to the osmolyte content of other fluid compartments. The fact that the haemolymph volume at the end of the dehydration experiment approached zero while there was still a substantial amount of cellular water implies that this regulatory mechanism must involve the removal of nearly all osmolytes from the haemolymph.

The relationship between haemolymph sodium concentration and haemolymph osmolality is shown in Fig. 2. As the beetles become dehydrated (not shown in the figure), there is an increase in haemolymph osmolality as well as in haemolymph sodium concentration. The relative increase in haemolymph sodium concentration is substantially lower than the increase in osmolality. The regression line deviates from a hypothetical line representing proportional increases at the level $P<0.001$ (Student's t-test, $t=7.29$, $N-2=9$).

The results in Fig. 2 reveal that sodium makes up about 25% of the total extracellular osmotically active particles, i.e. sodium is one of the most important extracellular

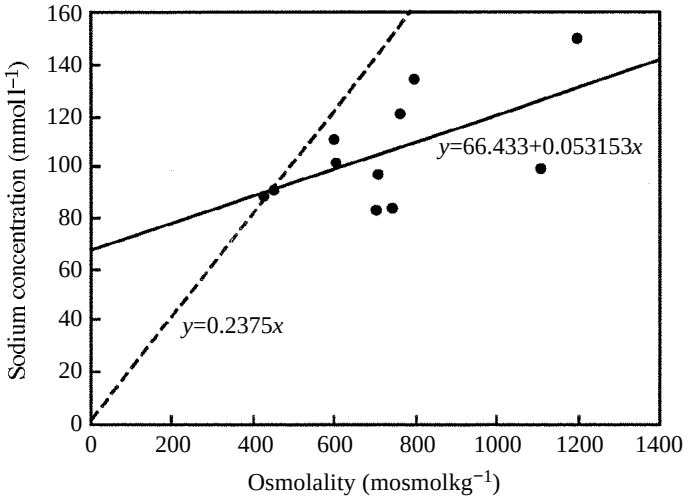

Fig. 2. Haemolymph sodium concentration in *Rhytinota praelonga* beetles undergoing evaporative dehydration plotted as a function of haemolymph osmolality. The solid line is the linear regression line of the data points, whereas the broken line represents proportionality between the two variables.

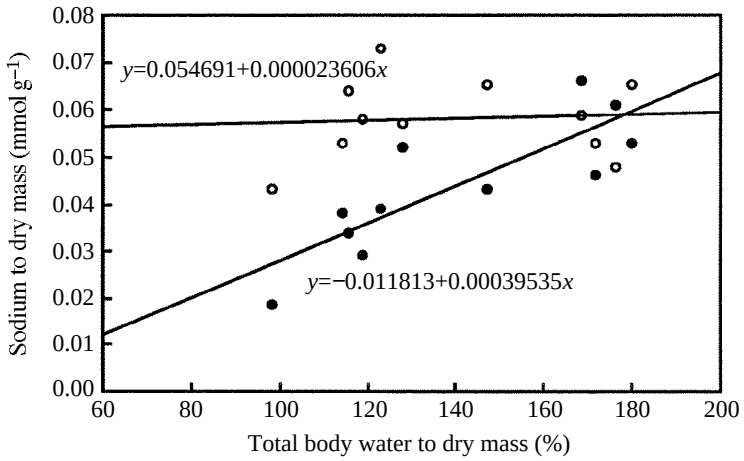

Fig. 3. Sodium content of haemolymph (●) and cells (○) of adult *Rhytinota praelonga* beetles undergoing evaporative dehydration plotted as a function of total water content. The values are related to dry mass to correct for differences in body size. The lines are linear regression lines.

osmolytes in *R. praelonga*. The results also show that sodium is one of the solutes contributing to the redistribution of water from haemolymph to cellular compartments during dehydration. Dehydration is accompanied by an increase in haemolymph sodium concentration, but the increase is considerably smaller than would be expected if there were just a passive increase in the sodium concentration following the reduction in haemolymph volume. This implies that sodium is one of the solutes that are removed from the haemolymph when the beetles become dehydrated.

The results in Fig. 2 show that the total concentration of haemolymph osmolytes increases more than the haemolymph sodium concentration when the beetles become dehydrated. This implies that the relative increase of one or more other haemolymph solutes must be greater than the increase in osmolality. The fact that sodium concentration increases less than the concentration of other solutes also suggests that excretion of sodium from the haemolymph is one of the primary processes that cause the extracellular water volume to drop.

Fig. 3 shows that during dehydration there was a marked decrease in the amount of extracellular sodium, whereas the intracellular amount was constant. The slope of the linear regression line for the extracellular sodium concentration deviates from a horizontal line at a statistical significance level of $P<0.002$ ($t=4.85$, $N-2=9$).

The observation that the haemolymph sodium content dropped substantially while the cellular sodium content was constant implies that the total sodium content of the beetles is substantially reduced when the beetles undergo dehydration. Thus, when *R. praelonga* beetles undergo evaporative dehydration, the haemolymph sodium concentration appears to be regulated by excretion of the ions from the organism. This regulation differs from that observed in other species undergoing dehydration, where sodium and other ions are removed from the body fluids by sequestration in the fat body (Jungreis and Tojo, 1973)

or hindgut (Tucker, 1977), i.e. the ions remain inside the organism. Accordingly, when the beetles become rehydrated after a substantial dehydration, they will probably have to replace sodium from a dietary source.

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