Comp. Biochem. Physiol. Vol. 93A, No. 4, pp. 691–694, 1989
Printed in Great Britain

0300-9629/89 $3.00 + 0.00

EFFECTS OF DEHYDRATION ON OSMOTIC AND IONIC REGULATION IN *ORCHESELLA CINCTA* (L.) AND *TOMOCERUS MINOR* (LUBBOCK) (COLLEMBOLA) AND THE ROLE OF THE COELOMODUCT KIDNEYS

H. A. VERHOEF and J. E. PRAST

Department of Ecology and Ecotoxicology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands. Telephone: 020-5482909

(*Received* 22 *December* 1988)

Abstract—1. In both collembolan species, *Orchesella cincta* and *Tomocerus minor*, dehydration and rehydration experiments show the absence of osmoregulation.

2. Ionic regulation has been found in *O. cincta*.

3. *T. minor* shows ionic regulation only at slow dehydration (96% RH) and not at fast dehydration (36% RH).

4. Both ion excretion and ion storage play a role in ionic regulation.

5. The urine of fully hydrated animals appears to be hypoosmotic with the haemolymph. After dehydration the urine becomes isoosmotic.

6. In rehydrated *O. cincta* the ionic U/B ratio returns to its original value. In *T. minor* only animals rehydrated after slow and not after fast dehydration show a U/B ratio, which returns to the original value.

7. The ecophysiological significance of these results is discussed.

INTRODUCTION

Aerial dehydration forms a major problem among terrestrial arthropods. As the water activity of their haemolymph ranges from 0.995 to 0.998 (i.e. 300–600 mOsm/kg), unless the water vapour activity of the ambient air is saturated, the activity gradient favours the net loss of water from the animal to the atmosphere by simple diffusion. Although water loss tolerances vary considerably between arthropods (Arlian and Veselica, 1979), many arthropods regulate their body water content between relatively narrow critical limits (Sell and Houlihan, 1985; Cooper, 1985). Besides this maintenance of a critical water content, simultaneous osmotic regulation has been found for locusts, beetles and cockroaches, to ensure that internal water concentration of both haemolymph and tissues remains between tolerable limits (Machin, 1981; Hyatt and Marshall, 1985).

In Collembola, an important group of soil arthropods, clear relations exist between water economy and water conditions of their habitats (Verhoef and Witteveen, 1980). Species living in permanently wet conditions, such as *Onychiurus fimatus*, dehydrate fast (transpiration rate is 813 μg/cm^2/hr/mm Hg), whereas species from dry surroundings, such as *Seira domestica*, lose their water very slowly (transpiration rate is 3 μg/cm^2/hr/mm Hg). Even species which coexist in the same habitat, i.e. coniferous forest soils, show strong differences in transpiration rate: *Tomocerus minor*, which occurs in deeper, humid, fermentation layers, has a transpiration rate five times that of *Orchesella cincta* which can be found in the drier litter layers and on the bark of trees (van der Woude and Verhoef, 1986). Furthermore, lost water can be replenished by the latter species 8–10 times as fast as by the former one (Verhoef, 1981; Eisenbeis, 1982). This apparent relationship between water balance mechanisms and the water conditions of their habitat may also exist with respect to their osmotic and ionic regulatory abilities. Organs which may play a role in osmoregulation are the coelomoduct kidneys (Verhoef *et al.*, 1979, 1983), rudimentary nephridia (Humbert, 1975) and ventral tube vesicles (Eisenbeis, 1982).

The present paper deals with the abilities of *O. cincta* and *T. minor* for osmotic and ionic regulation by means of dehydration and rehydration experiments. The role of the coelomoduct kidneys is studied by determining the osmolality and ion concentration of the urine.

MATERIALS AND METHODS

O. cincta and *T. minor* were sampled in a *Pinus nigra* (Arn.) var. *austriaca* stand near Dronten, the Netherlands. Only adults (1.0–1.2 mg fresh weight) were selected. These animals were kept individually in 4.5 mm diameter plastic boxes with a bottom layer of moistened plaster of Paris, at 19°C with a 12–12 LD photoperiod. The animals were fed a suspension of green algae.

To reduce individual differences due to different physiological stages, only animals 24 hr after ecdysis were selected (see Verhoef, 1981).

Osmotic and ionic regulation in these animals were studied as follows: ten individuals of both *O. cincta* and *T. minor* were dehydrated until 20% of their fresh weight was lost. (Weighing was done with a Sartorius microbalance; ± 1 μg.) Dehydration in *O. cincta* ($n = 5$) was performed at 36% RH (using a glycerol/water mixture of 6.14:1) and 19°C. Dehydration time for 20% weight loss was about 3 hr.

Dehydration in *T. minor* ($n = 5$) at 36% RH and 19°C took 45 min, because of its high transpiration rate. Therefore, this species was dehydrated during a dehydration time comparable with that of *O. cincta*. At 96% RH

(glycerol/water mixture of 0.22:1) and 19°C dehydration time for 20% weight loss was 2.5 hr. Thus, for *T. minor*, distinction has been made between fast (36% RH) and slow (96% RH) dehydration.

For rehydration, 20%-dehydrated animals ($n = 5$) were placed on filter paper wetted with deionized water for 24 hr. Then the animals were fully rehydrated. Five fully hydrated animals of each species were used as control animals.

Haemolymph samples were taken from both dehydrated and rehydrated animals (for each, $n = 10$) by amputation of an antenna under paraffin oil. Small droplets (< 1 nl) were collected in a quartz capillary and enclosed in paraffin oil in a glass capillary tube. The total osmolality of the haemolymph was determined by measuring the melting-point depression (Ramsay and Brown, 1955; Verhoef and Witteveen, 1980) using a nanoliter osmometer with a picture monitor (see van der Woude, 1988).

Large droplets (> 8 nl) were used for determination of the ionic fraction, by measuring the conductivity of the droplet by means of a small conductivity cell, with electrodes, 0.2 mm apart, of 0.15 mm surface diameter. The conductivity cell was connected to a conductivity meter (Philips PW 9505). The measurements were calibrated with NaCl standards of 100, 290 and 500 mOsm/kg. The conductivity of the haemolymph was expressed as an equivalent of NaCl solutions in osmotic units (van der Woude, 1987). The osmotic value of the non-ionic fraction, i.e. the organic fraction, is established by subtracting the ionic fraction from the total osmotic value.

Expected values for the total osmolality and the ionic and organic fraction after dehydration and rehydration were calculated on the assumption that, in the absence of regulation, water loss and gain cause an equivalent change in osmotic value (Verhoef, 1981). The expected value after rehydration is based on the actual value after dehydration.

The urine was collected under paraffin oil from the ventral groove at the head–thorax region with a fine polyethylene tube (see Verhoef *et al.* 1983). Total osmolality and ionic fraction were determined as mentioned above. Based on these data urine-haemolymph ratios (U/B ratios) were calculated.

RESULTS

Changes in total osmolality, ionic and organic fraction of the haemolymph

Values of total osmolality, ionic and organic fraction of the haemolymph after dehydration and rehydration for *O. cincta* are given in Fig. 1. After 20% dehydration total osmolality increases from 350 ± 25 to 494 ± 16 mOsm/kg (mean values ± SD). This is not significantly different from the expected rise without osmoregulation. After rehydration total osmolality returns to the original (pre-dehydration) value (338 ± 15 mOsm/kg). This suggests the absence of any osmoregulation.

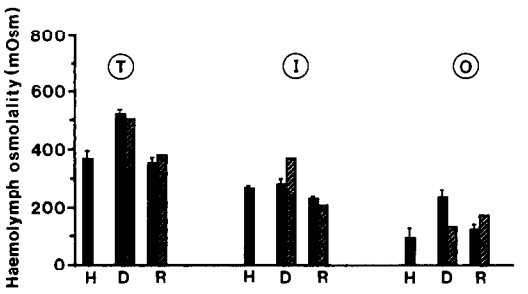

Fig. 1. Haemolymph osmolalities for hydrated (H), dehydrated (D) and rehydrated (R) *Orchesella cincta*. Total osmolality (T), ionic (I) and organic (O) fraction, actual (■) and expected (▨) values are presented.

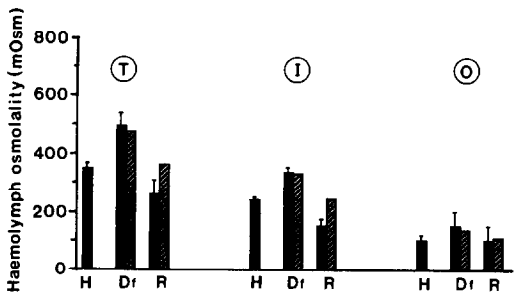

Fig. 2. Haemolymph osmolalities for hydrated (H), fast dehydrated (Df) and rehydrated (R) *Tomocerus minor*. For further explanation see Fig. 1.

The ionic fraction, however, does not change significantly during dehydration, remaining lower than the expected value without regulation. This points to a form of ionic regulation.

So, the observed increased total osmolality can only be explained by a rise in the organic fraction. The calculated value for the organic fraction after dehydration (Fig. 1) is higher than the expected value without regulation.

After rehydration the ionic fraction decreases to a value (221 ± 7 mOsm/kg), which is slightly lower than the original value and higher than the expected value. The organic fraction decreases after rehydration to a value slightly higher than the original value, and lower than the expected value.

Fast dehydration (at 36% RH) causes in *T. minor* an increase in total osmolality from 354 ± 17 to 500 ± 44 mOsm/kg, which is not significantly different from the expected rise without osmoregulation (Fig. 2).

In this species fast dehydration causes an increase in the ionic fraction, which rises from 248 ± 9 to 342 ± 17 mOsm/kg, which is not significantly different from the expected rise, suggesting the absence of ionic regulation. The rise in total osmolality is completed by a rise in the organic fraction.

After rehydration total osmolality decreases to a value (265 ± 48 mOsm/kg) significantly lower ($P < 0.05$; t-test) than the original (and expected) value. This is caused by a similar decrease in the ionic fraction to 159 ± 19 mOsm/kg ($P < 0.001$); the organic fraction returns to the original value.

Slow dehydration (at 96% RH) (Fig. 3) causes in *T. minor* a high increase in total osmolality compared with fast dehydration (560 ± 54 and 500 ± 44 mOsm/kg, respectively). This value is significantly higher ($P < 0.05$) than the osmolality expected without osmoregulation.

Slow dehydration causes a small, but significant, increase in the ionic fraction (248 ± 9 to 293 ± 1 mOsm/kg) to a value which is lower than the expected value. This suggests ionic regulation, which implies that the increase in total osmolality is mainly caused by an increase in the organic fraction (see Fig. 3). This means an increase of more than 150% (from 106 to 267 mOsm/kg).

After rehydration total osmolality decreases to a

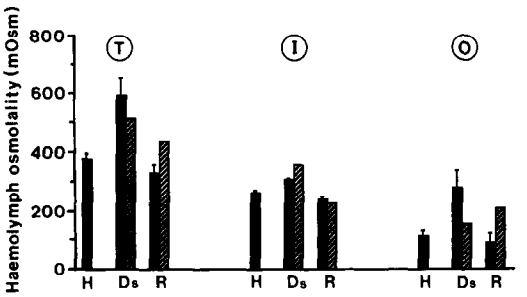

Fig. 3. Haemolymph osmolalities for hydrated (H), slowly dehydrated (Ds) and rehydrated (R) *Tomocerus minor*. For further explanation see Fig. 1.

value significantly lower ($P < 0.01$) than the expected value. This is caused by a similar decrease in the organic fraction to 106 mOsm/kg; the ionic fraction returns to a value slightly below the original value ($P < 0.01$).

Changes in U/B ratio

Fully hydrated *O. cincta* and *T. minor* show similar U/B ratios of 0.35 and 0.39, respectively, which indicates that the urine is hypoosmotic with the haemolymph (Table 1). The ionic U/B ratios of the species are even lower (0.27 and 0.21, respectively). This suggests for both species a higher reabsorption rate for ions than for the organic fraction.

After dehydration urine becomes isoosmotic with the haemolymph, with a U/B ratio of 1.01. Since urine production decreases strongly during dehydration, quantities necessary for the determination of the ionic fraction could not be collected, even when large numbers of animals were used.

After rehydration the U/B ratios for *O. cincta* return to the original values, suggesting a normal functioning of the kidneys, which produce hypoosmotic urine.

In slowly dehydrated *T. minor* only the ionic U/B ratio returns after rehydration to the original level; the organic U/B ratio increases to a value >1, suggesting an increased excretion of organic substances. This is also found for fast dehydrated *T. minor*. For these animals the U/B ratio stays at the same level (0.93) after rehydration, which indicates that the urine is isoosmotic with the haemolymph. After 24 hr rehydration, the kidneys have not returned to their normal functioning.

DISCUSSION

This study suggests a relationship between ionic regulatory ability and water conditions of the habitat for the two collembolan species studied: at 36% RH the xeric *Orchesella cincta* shows ionic regulation, whereas the hygric *Tomocerus minor* fails to regulate.

The stable ion concentration of the blood during dehydration in the former species is performed by two processes: ion excretion and ion storage. Ion excretion can be deduced from the fact that after rehydration the ionic fraction remains lower than the original value. Ion storage appears from the fact that ionic fraction after rehydration with deionized water is higher than the expected value.

Storage of ions has been suggested for cockroaches in the hindgut (Tucker, 1977), the cuticle (Hyatt and Marshall, 1985) and the fat body (Mullins and Cochran, 1974; Tucker, 1977; Hyatt and Marshall, 1985). Upon rehydration these ions are again mobilized and released into the haemolymph (Spring *et al.*, 1986). In *Periplaneta americana*, apart from this storage, small percentages of sodium and potassium are excreted (Hyatt and Marshall, 1985).

In Collembola, ion storage may take place in the ductless cephalic and abdominal nephridia (Humbert, 1975) and in fat tissue. Excretion takes place via the labial nephridia (Verhoef *et al.*, 1979; Verhoef *et al.*, 1988). The urine runs via an external transport system to the ventral tube vesicles, at which further reabsorption can take place (Verhoef *et al.*, 1983; Rusek, 1987). During dehydration, the urine production decreases in both species, as is often found in arthropods (Tyler-Jones and Taylor, 1986); for Collembola it can be explained by their ultrafiltration-reabsorption type kidneys (Verhoef *et al.*, 1979). As haemolymph pressure plays a role in the passage of the blood into the sacculus of the coelomoduct kidney, dehydration may have a negative effect on urine production.

In *O. cincta* the ionic U/B ratio returns to its original value after rehydration, which may point to a restored functioning.

In *T. minor* the ionic U/B ratio remains high after rehydration. In this species the selective ion-reabsorbing capacity of the kidneys does not seem to function optimally (cf. Verhoef *et al.*, 1983).

However, at 96% RH, at which the dehydration time is comparable with that of *O. cincta*, *T. minor* is able to regulate. After rehydration, the ionic U/B

Table 1. Changes in U/B ratios of *Orchesella cincta* and *Tomocerus minor* due to dehydration and rehydration treatment

Species	Treatment	U/B ratio		
		Total osmolality	Ionic fraction	Organic fraction
Orchesella cincta	Hydration	0.35	0.27	0.55
	Dehydration (36% RH)	1.01	*	*
	Rehydration	0.44	0.31	0.69
Tomocerus minor	Hydration	0.39	0.21	0.81
	Dehydration (36% RH)	1.01	*	*
	Rehydration	0.93	0.70	1.27
Tomocerus minor	Hydration	0.39	0.21	0.81
	Dehydration (96% RH)	†	†	†
	Rehydration	0.74	0.34	1.76

*Ionic fraction could not be measured due to a decreased urine production.
†Urine production ceased completely.

ratio returns, like in *O. cincta*, to the original value. This means that under their own specific water conditions both species are able to regulate the ionic fraction of their haemolymph.

A similar result has also been found concerning the water loss tolerance of both species: fast dehydrated (0% RH) *T. minor* tolerate water loss up to 22% of their normal water content (Vegter, 1985). At a more natural dehydration (96% RH), specimens tolerate a loss of 47%, as found for *O. cincta* (Verhoef, unpublished data).

In neither species has osmoregulation been found. Regulation of the ionic component, together with the absence of regulation of the total osmotic value, has also been found in the drought-sensitive isopod *Oniscus asellus*, which is capable of a remarkable degree of sodium control, but incapable of effective osmoregulation (Price and Holdich, 1980).

The rise in the organic fraction in both species might be caused by a failure of the kidney system to excrete the, during dehydration continuing production of, organic excretion products. This has been found especially in slowly dehydrated *T. minor*, where urine production ceased completely. For this species, the organic U/B ratio after rehydration is >1, which points to an increased organic excretion. This can be explained from their metabolic rate during water- and food-shortage. During a 3-week period of starvation and dehydration, *T. minor* stays active and keeps its metabolic rate unchanged (control animals: 341 ± 19.0; starved animals: 337 ± 40.0 μl O_2/g/hr). At the absence of water osmolality increases with 27%. In the presence of water, such an increase does not take place.

In *O. cincta*, food- and water-shortage causes a strong decrease in metabolic (59%) and transpiration rate (31%), resulting in an unchanged osmolality (Verhoef and Li, 1983).

Thus, it can be concluded that *T. minor* is not adapted to fast dehydration and long, dry periods, whereas *O. cincta* is well adapted to such conditions.

Acknowledgements—The authors are grateful to Ms D. Hoonhout for typing the manuscript and to Mr L. Sanna for drawing the figures.

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