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USE OF A VENTILATED CAPSULE AND TRITIATED WATER TO MEASURE EVAPORATIVE WATER LOSSES IN A TENEBRIONID BEETLE

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Evaporative water losses (EWL) in arthropods have traditionally been measured by gravimetric methods (Loveridge, 1980), although electronic sensing of water has recently been employed (Hadley, Stuart & Quinlan, 1982; Hadley & Quinlan, 1982). Difficulties are encountered in separating the cuticular and respiratory components of total transpiration. Cuticular water loss has frequently been measured in freshly killed specimens with blocked spiracles, and the results subtracted from the total EWL of living animals to obtain values for respiratory transpiration. Unfortunately, however, cuticular transpiration is higher in dead than in living arthropods (e.g. Hadley, 1970) and is further augmented by the appreciable damage to the cuticular waterproofing caused by blocking the spiracles (Beament, 1959). An additional problem arises when surface areas are estimated from standard equations, in order to express cuticular EWL in units independent of body weight. Loveridge (1980) has suggested that such estimates of surface area may be as much as an order of magnitude too low. We describe here an alternative technique using tritiated water (THO) and ventilated capsules to determine transpiration rates across small, known areas of cuticle in living insects. Tenebrionid beetles were selected as experimental animals and, because of the anatomy of their respiratory system, our method has also proved suitable for direct measurement of water losses from the subelytral cavity.

The beetles (*Onymacris plana* Péringuey, mean weight 0·852 g) were collected from sand dunes near Gobabeb, Namibia, and maintained on a diet of oats and lettuce. Before each experiment a weighed beetle was completely immobilized by covering the legs with adhesive tape, and injected with 20 μl THO (specific activity 630 000 c.p.m. μl^{-1}), using a Hamilton syringe and 26 gauge needle. The injection site between the abdominal sternites was sealed immediately with a wax-resin mixture.

An initial experiment established the time needed for equilibration of injected THO with total body water. Seven *O. plana* were injected with THO and 1-μl haemolymph samples were taken after 15 min and then every hour for 7 h. Fig. 1 shows that equilibration was complete 3 h after injection.

Water loss experiments were therefore started after this equilibration period, by connecting the beetle to the apparatus shown in Fig. 2. Room air was pumped (Beckman Gas Sampling System Model Y102) at a constant rate of 150 ml min^{-1} through the ventilated capsule attached to the cuticle of the insect. Flow rates were measured

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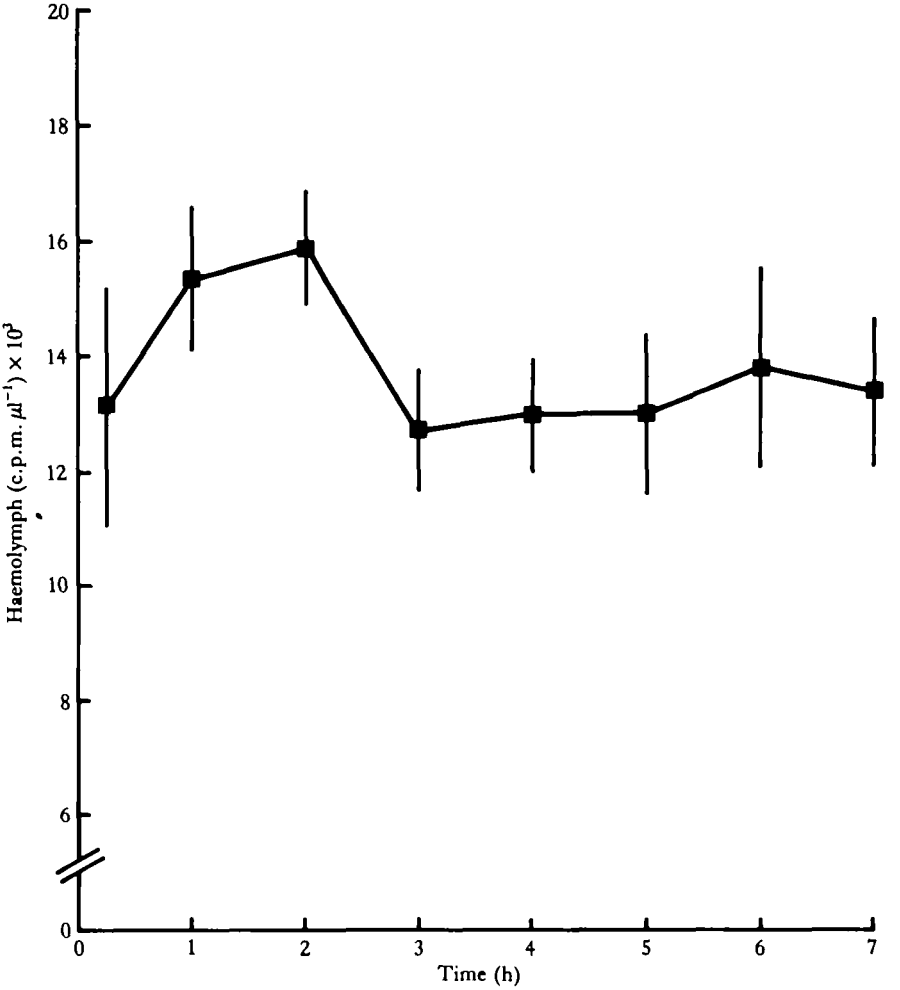

Fig. 1. Time required for equilibration of injected THO (20 μl; 630 000 c.p.m. μl^{-1}) with total body water. Vertical bars represent S.E. values and $N = 7$. Temperature during equilibration period was 22·5 °C.

with a rotameter-type flowmeter and the relative humidity and temperature of the air were monitored throughout the experiment by means of the humidity sensor and associated thermistor of a Vaisala Model HMI 14 humidity meter. Measured ranges for relative humidity and temperature were 27·5–46·0 % and 22·0–25·3 °C, respectively. The temperature of the cuticle surface inside the capsule was also measured, by means of a fine thermocouple, and did not differ by more than 0·1 °C from the temperature at the humidity sensor. The capsule for attachment to the insect was made by shortening a disposable polypropylene tip of an automatic pipetter (Gilson Pipetman P1000) and glueing the inlet and outlet tubing (polyethylene, i.d. 0·86 mm) into the upper end so that the inlet tubing extended to within 3 mm of the cuticle. Calipers were used to measure the internal diameter of the lower end of the capsule, and the calculated areas ranged from 0·033 to 0·052 cm^2. The capsule was held against the beetle's elytra with a retort clamp and sealed in place with a wax-resin mixture.

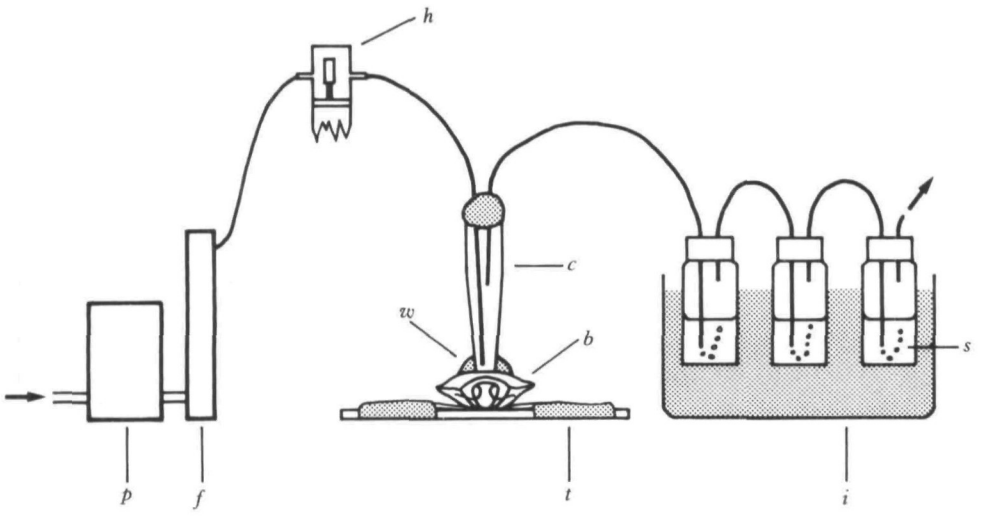

Fig. 2. Diagram of apparatus used to measure evaporative water losses in beetles (not to scale). Capsule has been attached to the elytra of a male *Onymacris plana*. Abbreviations: *p*, pump; *f*, flowmeter; *h*, humidity sensor and thermistor; *c*, capsule; *w*, wax-resin mixture; *b*, beetle; *t*, tape covering legs; *s*, scintillation fluid; *i*, ice.

Male *O. plana*, because of their flattened elytra, are especially suitable for this procedure, but both sexes were used. The wax seal was tested for air leaks by pumping air through the apparatus at twice the normal flow rate and applying a soap solution to the seal. No air leaks were observed.

Air was passed over the cuticle for at least 2 h and the labelled water transpired was trapped in three vials of scintillation cocktail (10 ml Packard Dimilume-30) arranged in series in a bed of ice. Radiation in the third vial never exceeded background levels. At the end of the experiment, duplicate 1-μl haemolymph samples were withdrawn for counting. All samples were counted for 30 min in a Packard Tri-Carb 460C liquid scintillation counter.

During each equilibration period, a control experiment was carried out without a beetle, but with the capsule attached to a microscope slide. The counts obtained from this procedure provided background counts for the three experimental vials. Total counts from all three vials were compared with the haemolymph specific activity in c.p.m. μl^{-1} to give the volume of water transpired in μl.

Cuticular transpiration rates for 11 beetles, expressed in terms of surface area and vapour pressure difference, were $0{\cdot}75 \pm 0{\cdot}12\ \mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$ (mean ± S.E.). This is almost the lowest cuticular permeability ever reported for an insect, and in fact resembles the permeabilities of certain desert scorpions (Edney, 1977). Moreover, our results are comparable to previous measurements of total EWL in *Onymacris plana*: $1{\cdot}53\ \mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$ for beetles in still dry air at 27 °C (Edney, 1971) and $3{\cdot}1\ \mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$ in moving dry air at 30 °C (Hadley & Louw, 1980). In the latter experiments, however, surface areas were derived from a generalized formula.

In flightless tenebrionids the fused elytra enclose an air-filled space known as the subelytral cavity. Because the abdominal spiracles open into this cavity and expired

air leaves through a single aperture above the anus, we wished to measure the abdominal component of respiratory water loss by sealing the ventilated capsule around the tip of the abdomen. Unfortunately, evaporative losses measured in this manner inevitably include water lost from the cuticular lining of the subelytral cavity. Since it is not possible to separate the two components, we refer to them together as subelytral water loss. The procedure used was otherwise identical to that described for cuticular EWL experiments. No faeces were produced during experiments, so it was not necessary to fast the insects beforehand. Subelytral water loss in 11 beetles averaged $0{\cdot}11 \pm 0{\cdot}03\,mg\,g^{-1}\,h^{-1}$ (mean ± S.E.). Table 1 shows that, when expressed in the same units, subelytral and cuticular water losses were approximately equal. In an earlier study of water loss in desert tenebrionids, Ahearn (1970) sealed different parts of the body of *Eleodes armata* and found that the abdominal spiracles contributed most to respiratory EWL, but that at 25 °C respiratory losses were less than 3 % of the total EWL. He did not, however, differentiate between cuticular and respiratory water losses from the subelytral cavity. In spite of the minimal level of activity of *O. plana* in the present experiments, the subelytral component was of major importance.

Total transpiration rates in *O. plana* were determined gravimetrically in 20 beetles immobilized as for the THO experiments, and were found to exceed the sum of cuticular and subelytral losses measured isotopically (Table 1). This finding may reflect differences in the techniques, errors inherent in the use of the formula for surface areas, or a relatively high proportion of expired air leaving the body *via* the thoracic spiracles. Evidence for the latter explanation is seen in a recent re-examination of the components of EWL in *Eleodes* by Cooper (1983). Using spiracular dimensions to calculate water loss from the mesothoracic spiracles, Cooper found that at 30 °C this avenue accounted for half the respiratory EWL.

Tritiated water has been used as a tracer in recent studies of water exchange between arthropods and their environment (reviewed by Arlian & Veselica, 1979). We have now demonstrated its potential for the direct measurement of cuticular water losses. Although it is not possible to measure water loss from all spiracles simultaneously, the ventilated capsule technique can also be applied to single spiracles for detailed investigations of respiratory EWL, particularly in insects where air flow through the tracheal system is known to be unidirectional. In terms of cuticular

Table 1. *Evaporative water losses of* Onymacris plana

	$\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$	$mg\,g^{-1}\,h^{-1}$
1. Cuticular EWL ($N = 11$)	$0{\cdot}75 \pm 0{\cdot}12$	$0{\cdot}13 \pm 0{\cdot}02$*
2. Subelytral EWL ($N = 11$)		$0{\cdot}11 \pm 0{\cdot}03$
3. Total (1 + 2)		0·24
4. Total EWL (gravimetric) ($N = 20$)		$0{\cdot}44 \pm 0{\cdot}02$

All values are means ± S.E.

* Cuticular EWL measured as $\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$ and converted to $mg\,g^{-1}\,h^{-1}$ using Edney's (1971) formula for the relationship between surface area and body mass.

transpiration, our technique has three main advantages. Firstly, we are measuring the cuticular permeability of living arthropods. Secondly, the surface area across which transpiration takes place is accurately known, although surface irregularities remain a problem. In previous studies, only surface areas of isolated preparations of cuticle could be measured with the same accuracy, but such preparations may exhibit water loss rates which greatly exceed those of whole animals (Hadley *et al.* 1982). Finally, little quantitative information is available on permeabilities of different regions of arthropod cuticle (Loveridge, 1980), and our technique is well suited for examining such regional variations.

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