WATER AND CARBON DIOXIDE LOSS FROM THE COCKROACH *PERIPLANETA AMERICANA* (L.) MEASURED USING RADIOACTIVE ISOTOPES

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Summary

Tritiated water (THO) loss from *Periplaneta americana* is used to measure cuticular permeability (P_d). In dry air, following various periods of predesiccation, the values of P_d lie around $0.5 \times 10^{-9}\,\mathrm{m\,s^{-1}}$, with the lowest values below $0.3 \times 10^{-9}\,\mathrm{m\,s^{-1}}$, close to the lowest permeabilities previously reported. There is no obvious relationship between permeability and initial mass of the insect. A significant lowering of P_d was found after 72 h of predesiccation. A marked sudden decrease in permeability occurred when the airstream flowing over an insect was changed from humid air to dry air. Labelling the bicarbonate pool using $^{14}CO_3^{2-}$ enabled the output of CO_2 to be estimated. An average output of about $390\,\mu\mathrm{l\,g^{-1}\,h^{-1}}$ was found. Simultaneous measurement of THO and $^{14}CO_2$ loss showed that tracheal water loss is only a small component of total water loss, with an average value of 3.8 %. The total water loss can therefore be used to make close estimates of cuticular permeability in *Periplaneta americana*. 2 min sampling periods clearly show the pulsatile nature of $^{14}CO_2$ release but, even when the effect is most discernible, the periodic release of CO_2 has only a small effect on water loss.

Key words: *Periplaneta americana*, cockroach, water loss, CO_2 output, water permeability, tritiated water, $^{14}CO_2$.

Introduction

Following the classical study of Ramsay (1935*a*,*b*), there have been numerous further studies of water loss into air from cockroaches and other insects. Interpretation of some of this work has been confused by failure to understand the basic physics of diffusion processes and by the use of inappropriate and sometimes incorrect units. The data have been collated and revised by Noble-Nesbitt (1991). Some of the problems have been considered by Croghan and Noble-Nesbitt (1989) and Noble-Nesbitt (1991), who point out the advantages of using permeability coefficients to describe cuticular water loss.

Isotopic methods have a number of advantages and these are considered in an accompanying paper (Croghan *et al.* 1995), which describes a technique using radioisotopes for the measurement and analysis of water and CO_2 loss into air from terrestrial insects. This technique is used here to analyse water loss from the American cockroach *Periplaneta americana*. In particular, the effects of desiccation on cuticular permeability are considered. In some cases, simultaneous measurements of water and CO_2 loss were made and these are used to analyse the contribution of the cuticular and the tracheal systems to water loss in this species.

Materials and methods

Adult male *Periplaneta americana* (L.) were removed from stock culture (Noble-Nesbitt and Al-Shukur, 1987) and kept without food or water either in humid air for at least 15 h (overnight) or isolated and predesiccated in dry air for 24–96 h before an experiment commenced.

The techniques used to investigate water and carbon dioxide loss are described in Croghan *et al.* (1995). Tritiated water (THO) and $^{14}CO_2$ were absorbed continuously from the airstream flowing past the insect. The apparatus allowed the investigation to be carried out using relatively unrestrained cockroaches. Before passing over the insect, the airstream was either dried by being drawn through a drying column of 6-16 mesh self-indicating silica gel (Fisons) or humidified by bubbling briskly through two gas bottles in series, both containing saturated NaCl solution. Air in equilibrium with this solution has a relative humidity (RH) of 75 % at ambient temperature (O'Brien, 1948; Winston and Bates, 1960). In practice, the airstream was found to be humidified to 68 ± 2 % RH, as measured with a Vaisala temperature–humidity probe. An air flow rate of $10\,\mathrm{ml\,min^{-1}}$ was used in all experiments.

The insects were loaded with THO and in some cases also with $^{14}CO_3^{2-}$, following overnight THO equilibration, as

described in Croghan *et al.* (1995). Most experiments were run for 8 h, with samples being collected every 10 min. At the end of an experiment, an abdominal sample was taken to obtain residual activity. The THO and $^{14}CO_2$ activities were determined by β-scintillation counting. The data were processed as described in Croghan *et al.* (1995) and the efflux of THO and $^{14}CO_2$ are expressed as efflux rate constants. In most cases, four replicate experiments were carried out simultaneously. Experiments were carried out at ambient temperatures between 19 and 24 °C.

The THO efflux rate constant can be converted to a permeability coefficient (Croghan and Noble-Nesbitt, 1989; Croghan *et al.* 1995) if the volume of water in the animal (taken as 72 % of mass) and the surface area through which diffusion is occurring are known. The permeability coefficient P_d (m s^{-1}) is defined as:

$$P_d = k_0 \frac{V}{A}, \tag{1}$$

where k_0 is the efflux rate constant (s^{-1}), V is the volume of water (m^3) in the animal and A is the area (m^2) through which diffusion is occurring. The surface area was calculated using the formula for adult *Periplaneta* given by Machin *et al.* (1986):

$$A = 14.5 \times 10^{-4} W^{0.63}, \tag{2}$$

where A is the surface area (m^2) and W the mass of the insect (g) at removal from the culture.

Results

Some representative examples of experiments carried out in a dry airstream using THO alone are given in Fig. 1. In most cases, the running values of the efflux rate constant were relatively steady, with fluctuations that are taken to represent real fluctuations in water loss. However, it was easy to read off a steady value of the efflux rate constant in order to calculate the permeability coefficient. These permeability coefficients,

for individuals that had not been injected, are collated in Fig. 2. The permeability data are plotted against initial mass at removal from the culture, hours of predesiccation and proportion of water lost between removal from the culture and the end of experiment. The mean permeability coefficient for unpredesiccated individuals is $0.54 \times 10^{-9} \pm 0.062 \times 10^{-9}$ m s^{-1} (S.E.M., $N=12$) and for individuals predesiccated for 72 h the mean permeability coefficient is $0.35 \times 10^{-9} \pm 0.050 \times 10^{-9}$ m s^{-1} (S.E.M., $N=4$). This difference is significant ($0.02 < P < 0.05$, t-test, variance not assumed to be equal).
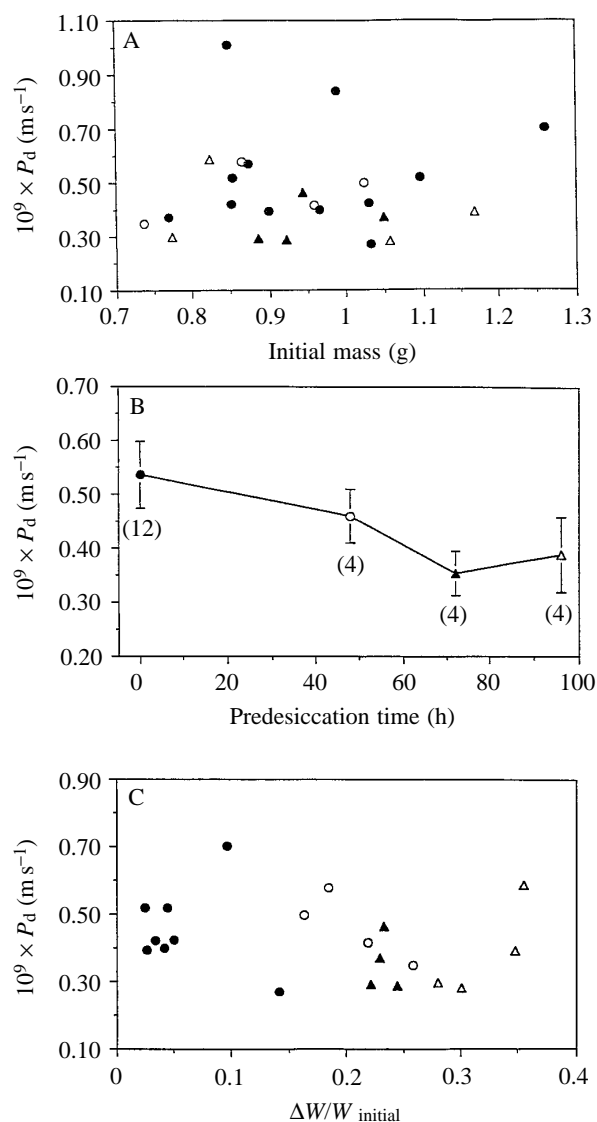
Fig. 2. (A) Cuticular permeability data (P_d) plotted against initial mass (at removal from the culture). (B) Cuticular permeability (mean ± S.E.M., N in parentheses) plotted against time of predesiccation. (C) Cuticular permeability data plotted against proportion of water lost, $\Delta W/W_{initial}$ (where ΔW is the loss of mass between removal from the culture and the end of the experiment and $W_{initial}$ is the mass of water in the insect at removal from the culture). Filled circles, 0 h of predesiccation; open circles, 48 h of predesiccation; filled triangles, 72 h of predesiccation; open triangles, 96 h of predesiccation.
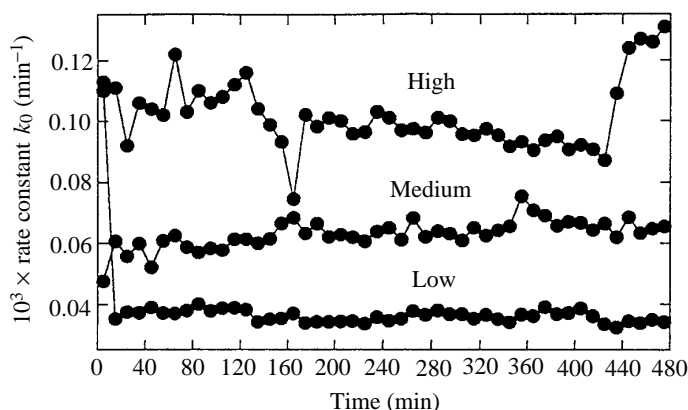

Fig. 1. Examples of rates of water loss for *Periplaneta americana*, illustrating high, medium and low THO rate constants. None of these specimens had been predesiccated.
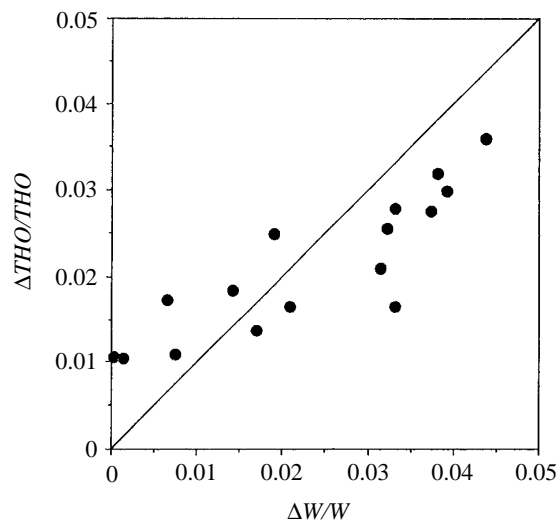
Fig. 3. Relationship between tritiated and gravimetric water loss measurements. Proportion of THO lost is $\Delta THO/THO$ (where *THO* is the amount of THO in the insect at the start of the measurement). Proportion of water lost is $\Delta W/W$ (where *W* is the mass of water in the insect at the start of the measurement).

As most studies of insect water loss have used gravimetric methods, it was considered useful to compare the gravimetric and isotopic methods directly. In some experiments, using unpredesiccated insects, insects were weighed immediately before being placed in the chamber and then immediately after the end of the experiment. Although some THO loss would occur during the weighing or transfer processes, those times were short compared with the total efflux measurement time. The isotopic loss ratio $\Delta THO/THO$ (where *THO* is the amount of THO in the insect at the start of the measurement) can be compared directly with mass loss ratio $\Delta W/W$ (where *W* is the mass of water in the insect at the start of the measurement) over the same time period (Fig. 3).

In other experiments, using unpredesiccated insects, the efflux of THO was followed for 2 h in humid air, nominally 75 % (measured 68 %) RH. Then the airstream was changed to

dry air and the loss followed for a further 2 h (Fig. 4). A rapid decrease in water loss was seen immediately following the change in relative humidity.

In other experiments, losses of THO and $^{14}CO_2$ were studied simultaneously. Fig. 5 is an example of experiments carried out over 3 h in dry air with samples collected at 10 min intervals. The $^{14}CO_2$ data of Fig. 5 are the same as those used by Croghan *et al.* (1995) in developing a multicompartment model for $^{14}CO_2$ efflux. A running value of the corrected efflux rate constant can be calculated (Croghan *et al.* 1995) and this is given in Fig. 5B. The efflux rate constant for $^{14}CO_2$ is always much greater than that for THO (Fig. 5C). In order to improve time resolution, a series of experiments was carried out using the 2 min collection procedure. An example is given in Fig. 6. Again, the efflux rate constant for $^{14}CO_2$ is much greater than that for THO, but now the loss of CO_2 can be seen to be pulsatile. During these pulses, the loss of THO seems to be relatively unaffected. Another example using the 2 min collection procedure is given in Fig. 7. In this example, an increase of water loss during the ventilation bursts is clearly apparent.
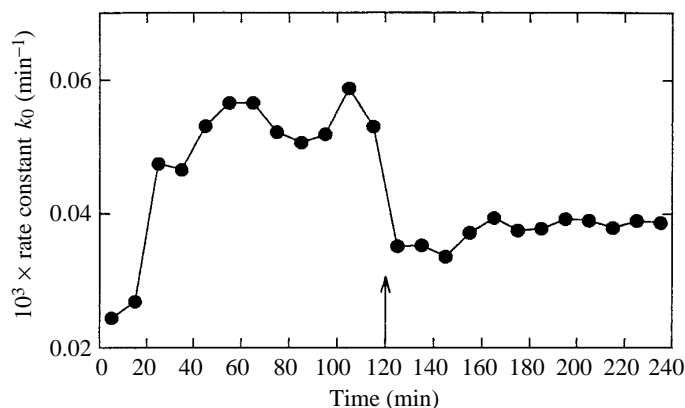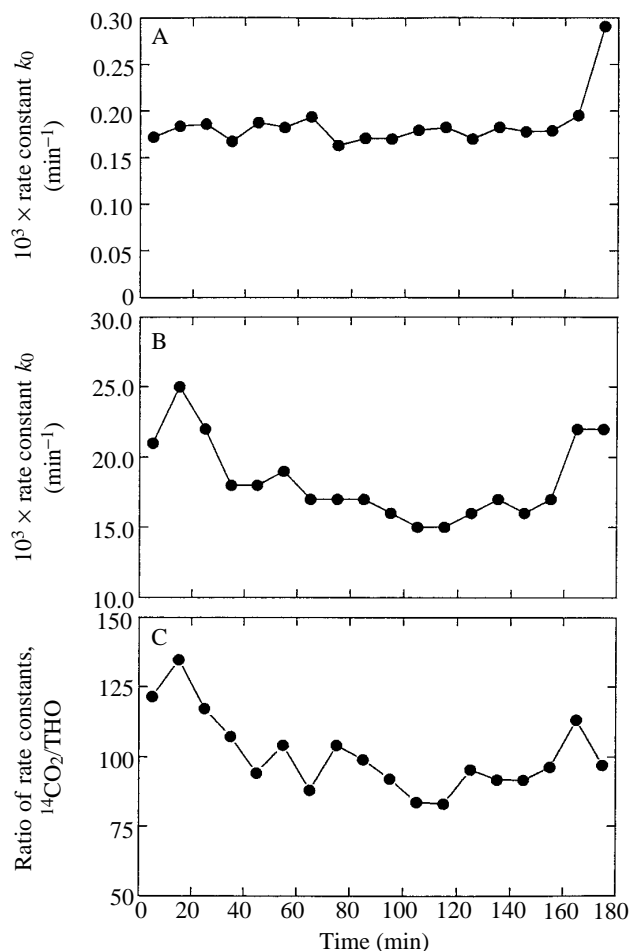

Fig. 4. THO efflux rate constant in a humid (68 % relative humidity) airstream initially, followed by a dry airstream. The vertical arrow indicates the time when the airstreams were switched.

Fig. 5. Simultaneously determined efflux rate constants for (A) THO and (B) $^{14}CO_2$, and (C) the ratio of $^{14}CO_2$ and THO efflux rate constants. Data were obtained with 10 min sampling periods.

Fig. 6. Simultaneously determined efflux rate constants for (A) THO and (B) $^{14}CO_2$, and (C) the ratio of $^{14}CO_2$ and THO efflux rate constants. Data were obtained with 2 min sampling periods. $k_w=0.11\times10^{-3}\,min^{-1}$; $k_{CO_2}=4.2\times10^{-3}\,min^{-1}$.
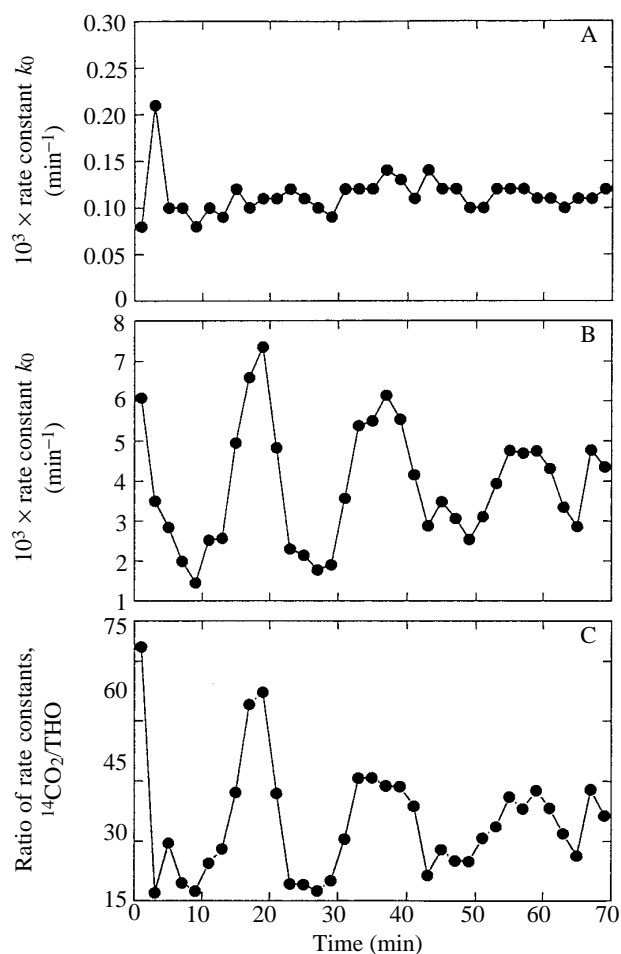

Fig. 7. Simultaneously determined efflux rate constants for (A) THO and (B) $^{14}CO_2$, and (C) the ratio of $^{14}CO_2$ and THO efflux rate constants. Data obtained with 2 min sampling periods. $k_w=0.085\times10^{-3}\,min^{-1}$; $k_{CO_2}=29.9\times10^{-3}\,min^{-1}$.

Discussion

As will be shown below, integumentary water loss in *Periplaneta* is predominantly cuticular and thus the loss can be expressed as a cuticular permeability coefficient (P_d) using equation 1. Although the permeability values for a given individual are consistent (Fig. 1), there is considerable variation between individuals (Fig. 2). The values lie around $0.5\times10^{-9}\,m\,s^{-1}$, with the lowest values below $0.3\times10^{-9}\,m\,s^{-1}$. These isotopic results are consistent with published values for *Periplaneta* obtained using gravimetric methods (Noble-Nesbitt, 1991). A lower permeability for *Periplaneta* ($0.049\times10^{-9}\,m\,s^{-1}$) has been reported by Machin *et al.* (1992), using a ventilated capsule attached to the pronotum. As has been previously pointed out (Croghan and Noble-Nesbitt, 1989; Noble-Nesbitt, 1991), values as low as these are difficult to explain in terms of the water diffusion properties of liquid oil or solid wax.

There is no obvious relationship between water permeability and initial mass of the insect (Fig. 2A). However, there is an interesting relationship between P_d and time of predesiccation
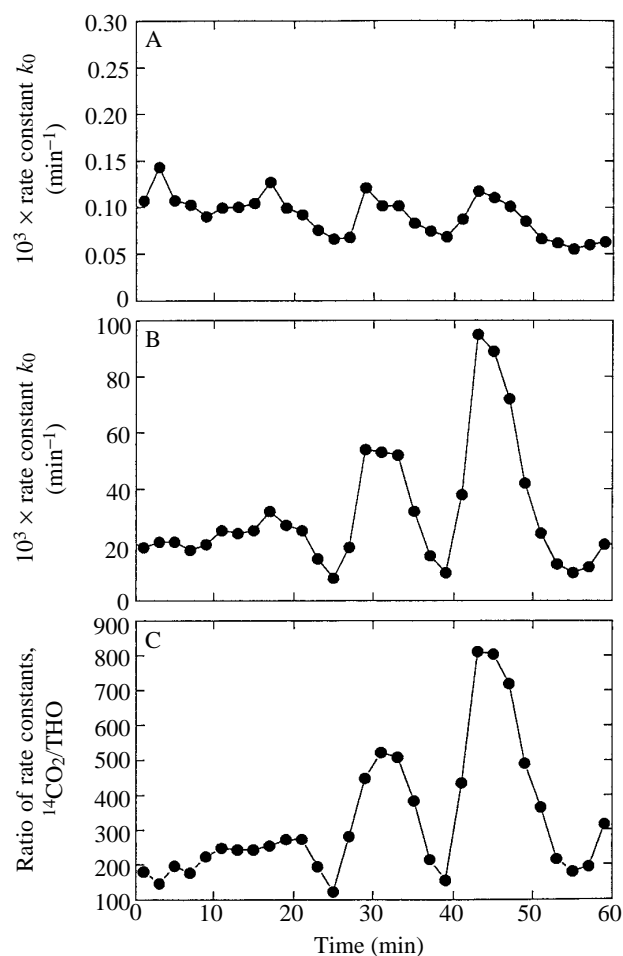
(Fig. 2B). Permeability is reduced after predesiccation for 72 h. This reduced permeability may be related to the reduction of body water during the predesiccation period (Fig. 2C). A reduction in water loss during desiccation has been found previously in *Periplaneta* (Noble-Nesbitt and Al-Shukur, 1987, 1988*a,b*). Permeability is not further reduced after predesiccation for 96 h and the values at this time are more variable. This could be interpreted as the onset of physiological deterioration, since these individuals had lost more than 30 % of their body water (Fig. 2C). This is likely to be close to the lethal limit for water loss and may adversely affect the mechanisms controlling cuticular permeability. Owing to the considerable individual variation, it is clear that further investigations of long-term permeability adaptation should be carried out on the same individual over a long period.

A careful simultaneous comparison was also made of loss of tritiated water and loss of mass (Fig. 3). If the two techniques are measuring the same processes, $\Delta THO/THO$ should be identical to $\Delta W/W$. There are discrepancies, especially at the extremities of the ranges. The nature of these

is uncertain. However, the accuracy of the gravimetric method with active insects is much reduced at low loss rates and unrecorded THO losses occur during the setting up and weighing procedures. Compartmentalisation can also be a source of error in the isotopic method (Croghan *et al.* 1995) and this error could be greater at high rates of water loss. The differences found, using living intact insects, are much less than those given by Machin *et al.* (1992) for excised pronotal discs. However, the differences found in the present paper are comparable with those between isotopic and balance methods in other animals listed by Nagy and Costa (1980).

The isotopic method allows water loss to be studied in airstreams of different relative humidities. The influence of RH on water loss was studied (Fig. 4). A rapid decrease in isotopic water loss was seen on switching from an airstream of 68 % RH to dry air. This decrease is particularly significant since water absorption on surfaces (insect, chamber or tubing downstream from chamber) during the period of high humidity would be expected to result in a transient increase in THO collection after the switch to dry air. The sudden fall in the rate of water loss obtained, when a dry airstream was substituted for a humid airstream, reinforces earlier conclusions that cuticular water loss is under physiological control and responds rapidly when the insect is suddenly exposed to dry air (Noble-Nesbitt and Al-Shukhur, 1987, 1988*a,b*). This rapid reduction of permeability must have occurred before measurements commenced in all the experiments in which a dry airstream was used. It must be a different physiological process from the much slower gradual reduction in permeability seen during prolonged desiccation (Fig. 2B) and water stress (Noble-Nesbitt and Al-Shukur, 1987). A suggested model of cuticular permeability control has been developed by Croghan and Noble-Nesbitt (1990) and future application of this isotopic technique should provide further information on the nature of the control of cuticular permeability.

In a number of cases, simultaneous measurements of the loss of THO and ¹⁴CO₂ were made (Figs 5, 6, 7). Expressing the losses as the same physical quantity (rate constant) enables direct comparison of the losses to be made, particularly when the ratio is plotted. The efflux rate constant for ¹⁴CO₂ (k_{CO_2}) can be used to calculate the CO₂ output from the insect and, if it is assumed that CO₂ is lost from the insect only by ventilation of the tracheal system, the ventilation rate can also be calculated (Croghan *et al.* 1995). For the eight animals studied, the estimated CO₂ output ranged from 87 to 623 $\mu l\,g^{-1}\,h^{-1}$ with an average value of 391 $\mu l\,g^{-1}\,h^{-1}$. This estimated CO₂ output is comparable with the value for *Periplaneta* determined by Kestler (1971). The calculated ventilation rate ranged from 66 to 197 $\mu l\,g^{-1}\,min^{-1}$, with an average value of 124 $\mu l\,g^{-1}\,min^{-1}$.

Assuming that the ventilated volume is saturated with water vapour, the rate constant for water loss by tracheal ventilation ($k_{w,v}$) is given by:

$$k_{w,v} = k_{CO_2}(B/p_{CO_2})p_w M, \qquad (3)$$

where p_w is the water vapour pressure (18.7 mmHg at 21 ˚C;

2.49 kPa), M is the molar mass of water ($18 \times 10^{-3}\,kg\,mol^{-1}$) and B/p_{CO_2} is the ratio of HCO_3^- concentration in the body fluids to pressure of CO₂ in the tracheal system. This ratio is defined by the Henderson–Hasselbalch equation and, assuming pH is 7.1, was taken as $0.5 \times 10^{-3}\,mol\,kg^{-1}\,mmHg^{-1}$ (at 20 ˚C). Dividing $k_{w,v}$ by the total efflux rate constant for THO gives the proportion of total water loss due to tracheal ventilation. Using the data of Fig. 5, for which a multicompartment model has been fitted (Croghan *et al.* 1995), the calculated contribution of tracheal ventilation to total water loss was only 1.7 %. For the eight insects studied, where k_w and k_{CO_2} were determined simultaneously, the tracheal loss ranged from 0.64 % to 8.5 % of total water loss, with an average value of 3.8 %. If some of the CO₂ loss is also by cuticular diffusion, the calculated water loss by tracheal ventilation will be even lower. The values are much lower than those estimated in *Periplaneta* by Machin *et al.* (1991) using a gravimetric method.

The data relating $k_{w,v}/k_w$ and k_w are plotted in Fig. 8. This indicates that in *Periplaneta* the tracheal water loss is a relatively small proportion of total water loss. However, as the loss rate decreases, the proportion of total water loss that is due to ventilation increases. If $k_{w,v}$ were constant, a hyperbolic relationship might be expected and this fits the data of Fig. 8 well. A trivial contribution of tracheal water loss was, of course, the assumption made in calculating cuticular water permeability from the efflux rate constant for THO. The experiments were carried out with relatively quiescent insects. If the insects were more active, hyperventilation and an increase in tracheal water loss would be expected. The assumption would also not necessarily be correct for a more xeric, presumably less permeable, species, where the total water loss might be very low and the contribution of tracheal water loss to total water loss would be expected to be greater.

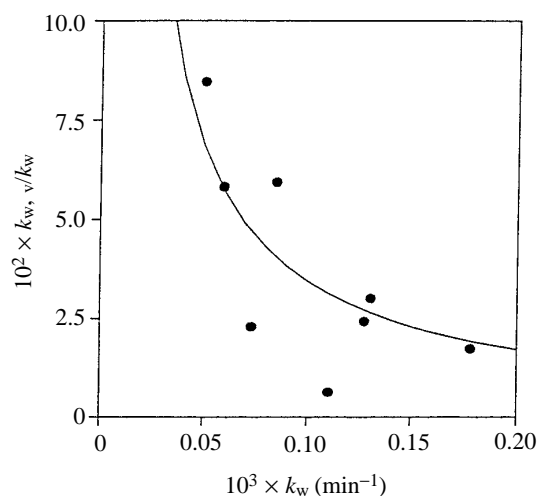In some cases, simultaneous loss of THO and ¹⁴CO₂ was

Fig. 8. Relationship between tracheal and total water loss; k_w is the rate constant for total water loss and $k_{w,v}/k_w$ is the proportion of water loss due to tracheal ventilation. The line is the best fit to the data of the hyperbolic equation $k_{w,v}/k_w = a/k_w$, where $a = 3.44 \times 10^{-6}\,min^{-1}$.

studied using 2 min collection periods (Figs 6, 7). With this time resolution, the CO_2 output can clearly be seen to be periodic. Periodic CO_2 output was initially observed in insects by Punt (1950), although unfortunately he did not illustrate his *Periplaneta* results. However, Wilkins (1960), using an infrared technique, very clearly demonstrated a periodic release of CO_2 in *Periplaneta*. In the example illustrated in Fig. 6, the contribution of tracheal water loss to total water loss was only 0.64 % and increased loss of water during the CO_2 ventilation burst was hardly perceptible. In another example (Fig. 7), where the contribution of tracheal water loss to total water loss was 5.9 %, an increase of water loss during the ventilation burst is clearly seen. The difference between these two particular insects was mainly due to the differences in ventilation. Cuticular water loss, however, remains much the greater component of overall water loss.

A result similar to that illustrated in Fig. 7 was obtained, using a quite different technique, in a mesic grasshopper with similar rates of water loss to those found here for the cockroach, by Hadley and Quinlan (1993). An apparently similar result, suggesting an even higher proportion of water loss in the ventilation burst, has been reported for xeric ants (Lighton, 1992), although in that work the CO_2 output and water loss were measured successively rather than simultaneously as they were in the experiments reported here.

The results reported in this paper provide a more detailed picture of the way water is lost from the mesic insect *Periplaneta americana*. The cuticular permeability has been estimated and some factors that influence the permeability, such as predesiccation and ambient humidity, have been studied. The techniques described in this paper and in the accompanying paper (Croghan *et al.* 1995) provide a basis for obtaining comparable data for a range of mesic and xeric insect types that will be relevant to further studies of the physiological processes involved in adaptations to control water loss.

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