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SIMULTANEOUS MEASUREMENTS OF SPIRACULAR AND CUTICULAR WATER LOSSES IN *PERIPLANETA AMERICANA*: IMPLICATIONS FOR WHOLE-ANIMAL MASS LOSS STUDIES

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Summary

Spiracular and cutaneous water loss through the cuticle and spiracles of *Periplaneta americana* was measured in animals of differing initial water contents under controlled temperature, humidity and airflow conditions, by continuous weighing (resolution $\pm 10\,\mu g$). Stable water loss rates (2.09×10^{-8} to $3.47\times10^{-8}\,g\,h^{-1}\,cm^{-2}\,Pa^{-1}$) were as much as an order of magnitude less than those reported in earlier studies employing intermittent weighing techniques. We suggest that increased water loss caused by substantial increases in ventilatory water loss during the episodic disturbances required by intermittent weighing is the principal contributor to this discrepancy.

Water losses, as well as the interval between spiracular ventilations, decreased with water content. However, greater variation was primarily due to interruptions of the regular cyclic pattern of ventilation by highly variable, activity-related bouts of increased ventilatory loss. Variations in water loss appear to be too large and too rapid to be convincingly explained in terms of cuticular conductance. Our results suggest that previous experiments using 5% CO_2 anaesthesia, linking changes in total water loss to the conducting properties of the cuticle, were not valid. There seems to be no adaptive value for the proposed voluntary increases in cuticular permeability in an animal where ventilatory water losses can be so high.

Introduction

Deriving the permeability to water of insect integuments by measuring the mass loss of whole animals over hours or days (see Loveridge, 1980) has been widely practiced because it is believed that spiracular water losses are trivial in comparison with those sustained through cuticular fluxes. It has been assumed that spiracular-closing and water-conserving mechanisms are intact in living insects under experimental situations, whereas spiracular water loss in recently killed specimens is reduced to simple diffusion through the open spiracles and represents

Key words: *Periplaneta americana*, cuticular water loss, cuticle permeability, spiracular water loss, ventilatory cycles.

loss from a very small part of the total surface area. Several techniques have been tried to seal the spiracles to reduce this loss from dead animals but doubts about their efficacy have discouraged widespread acceptance (Loveridge, 1980).

Evidence suggesting that the nervous system may be involved in regulating integumental water loss in *Periplaneta americana* has been provided by whole-animal mass loss measurements of Noble-Nesbitt and Al-Shukur (1987, 1988*a*,*b*). Refinements of Treherne and Willmer's (1975) decapitation techniques and injections of homogenates of brain and terminal abdominal ganglion were used. Noble-Nesbitt and Al-Shukur (1987) responded to the possibility of errors from direct handling (Machin *et al.* 1986) by enclosing their experimental animals in perforated containers. The claim that handling does not affect water loss was based on the lack of difference between decapitation-induced losses in two differently handled, dehydrated groups. Changes in rates of water loss induced by decapitation and homogenate injection are attributed to a complex two-stage mechanism involving promoting and reducing factors controlling cuticular water permeability. It is presumed that water loss is adaptively reduced when the cockroach is dehydrated and enhanced when it is over-hydrated (Noble-Nesbitt and Al-Shukur, 1988*a*,*b*).

Interpretation of these events, primarily in terms of changes in cuticular conductance, had its origin in Treherne and Willmer's (1975) experiment using 5 % CO_2 in air. It is argued that, since the increase in water loss following decapitation still occurs in 5 % CO_2, where they assumed that the spiracles remained open, the rise must be due to a change in cuticular permeability. We question this reasoning because it assumes that the amount of water lost from the tracheal system depends only on the length of time that the spiracles are open. This seems possible only when respiratory exchange is entirely diffusive. It has been established that, although insects hold their spiracles open above 3 % CO_2, they ventilate continuously when CO_2 concentration reaches 5 % (Hazelhoff, 1926). We suggest that convective water loss could easily be influenced by changes in ventilatory dynamics with the type of experimental handling, manipulations of the nervous system or CO_2 anaesthesia employed in the earlier studies, invalidating Treherne and Willmer's (1975) conclusion.

Kestler (1978*a*, 1985) has shown how continuous gravimetric recordings of mass loss in quiescent and unrestrained cockroaches can be separated into respiratory and cuticular components. Mass recordings show three periods: constriction (C), the lowest loss rate when the spiracles are closed; transitional flutter (F), when air driven by a pressure differential is admitted into the tracheal system in a series of rapid micro-openings; and ventilation (V), when respiratory gases and water vapour are exchanged in a series of coordinated ventilatory movements. The composite CFV cycles have been shown to reflect complex mechanisms for minimizing respiratory water loss, involving combined diffusive–convective exchanges (Miller, 1982; Kestler, 1971, 1978*b*, 1980, 1982, 1985). These refined analyses have resulted in estimates of cuticular permeability as low as $1.5\times10^{-8}\,g\,h^{-1}\,cm^{-2}\,Pa^{-1}$ (Machin and Lampert, 1987). This value is markedly

lower than most of the values formerly believed to represent the water-conducting properties of the cuticle, suggesting that spiracular losses may represent a more significant component of total water loss than was previously thought. In this paper we further exploit Kestler's technique of separating spiracular and cuticular water fluxes to explore the extent to which changes in cuticle permeability can be inferred from whole-animal mass losses in *Periplaneta americana*. We will also attempt to understand the highly variable water losses in this species as well as the conservation mechanisms that appear to be activated by progressive desiccation.

Materials and methods

Adult *Periplaneta americana* (L.) of both sexes were obtained from a laboratory culture, provided with unlimited dry laboratory chow and water and maintained at a regulated temperature of 24–27°C and 43–45 % relative humidity (RH). Their water contents were 69.5±0.5 % (N=12), with no significant difference between the sexes (P>0.7). These were comparable to earlier estimates in similar conditions [Edney (1968) 68.7 %; Wharton *et al.* (1968) 71.3 %; Tucker (1977) 68.6 %; and Hyatt and Marshall (1977) 66.0 %] but lower than the 72 % water contents reported by Noble-Nesbitt and Al-Shukur (1987). It has been our practice to remove cockroaches from the general culture by holding them in individual containers for at least 3 days prior to experimentation (Machin and Lampert, 1987). Although unlimited food and water in the containers produced humidities exceeding 80 % RH, body water content still ranged between only 67 and 71 % (mean=69.3±0.3 %, N=14) of wet mass. To obtain higher water contents, we made use of the well-established observation (Tucker, 1977; Hyatt and Marshall, 1977) that prior dehydration elevates water content when cockroaches regain access to water. Cockroaches were dehydrated in individual containers without food or water for 5–8 days then allowed access to water alone for 24 h before experimentation. This yielded water contents from 72.0 to 77.2 % of wet mass. Some of these values could overestimate hydration levels in the haemolymph and tissues because they may include a quantity of water retained in the crop. Dehydrated animals were obtained by isolating individuals, without food or water, for 5–8 days. Body water contents of this group were 65.1±0.7 % (N=4) of wet mass, matching the values obtained for dehydrated, starved animals by Noble-Nesbitt and Al-Shukur (1987).

Cockroaches were selected randomly for experimentation. To avoid stress they were allowed to explore and spontaneously enter a metal gauze weighing cage from the individual containers (Kestler, 1991). This behaviour was assisted by darkening the cage relative to the container. The cage was then closed and installed in the weighing chamber of a Sartorius 4410 electromagnetic microbalance (Kestler, 1978*a*, 1985; Machin *et al.* 1985; Machin and Lampert, 1987) and the weighing chamber was wrapped in opaque black plastic. Masses were continuously recorded at 20°C with 10 μg resolution (1 mg full scale) in air dried

with silica gel (partial pressure difference between haemolymph and air, 2.2 kPa) flowing at 800 ml min^{-1}.

The cockroaches were grouped, as in Noble-Nesbitt and Al-Shukur's (1987) study, according to three hydration levels; 'hydrated' (water content >72 %), 'normal' and 'dehydrated' (water content<66 %). Mass losses of three individuals were recorded for 8 days, long enough for water contents to have fallen to dehydrated levels. The weighing chamber was then opened and about 1 ml of water was introduced into the weighing cage with a syringe. The wire mesh of the cage retained the water by surface tension. The animal was therefore provided with drinking water with minimal disturbance and without handling, while the remaining water evaporated overnight. Following the rapid mass loss attributable to evaporation of the residual drinking water, net mass change provided a measure of the imbibed water and of the change in water content of the animal. Mass loss recording of the rehydrated animal was then resumed in dry flowing air for a further 2 days.

Shorter mass loss records (1–2 days) were obtained from a larger number of cockroaches (2–9) of various water contents. Losses during the initial period of activity were assessed as the time taken for 1 mg to be lost (one chart width). Stable sections of the recordings were then identified and CFV cycle variables were measured for five consecutive cycles. In some individuals, CFV cycles marked by occasional single spike-like disturbances produced by grooming movements could not be avoided. Very brief disturbances of this kind have no noticeable effect on the CFV cycle or on the rate of mass loss. CFV cycles for active animals were obtained from sections of the trace where the ventilatory period was noticeably disturbed but still retained a definable CF period. A similar series of recordings with 'normally' hydrated individuals, was made in dry flowing air with 5 % CO_2. At the end of each experiment, dry mass was obtained by drying all experimental animals for approximately 24 h at 60 °C. Water content could then be estimated at any point in the record (with a small error due to occasional faecal loss).

The data in Tables 1–4 (quoted as mean±S.D.) were analysed by two- or three-way analysis of variance (ANOVA) in which variation between animals was separated from that due to physiological state.

Results

Cockroaches introduced into new surroundings initiate periods of searching activity followed by grooming (Kestler, 1971, 1985), distinguished as extracyclic activity (EXCA) in Kestler (1991). As a result, the initial acclimation in the balance was often characterized by mechanically disturbed recordings and rapid mass loss, occasionally punctuated with short sequences of cyclic mass loss. Within 1–3 h, mass loss records all developed the regular cyclical CFV pattern characteristic of resting cockroaches (Kestler, 1971, 1978*b*, 1985). This rhythm was sometimes marked by further bursts of mechanical disturbance, distinguished as

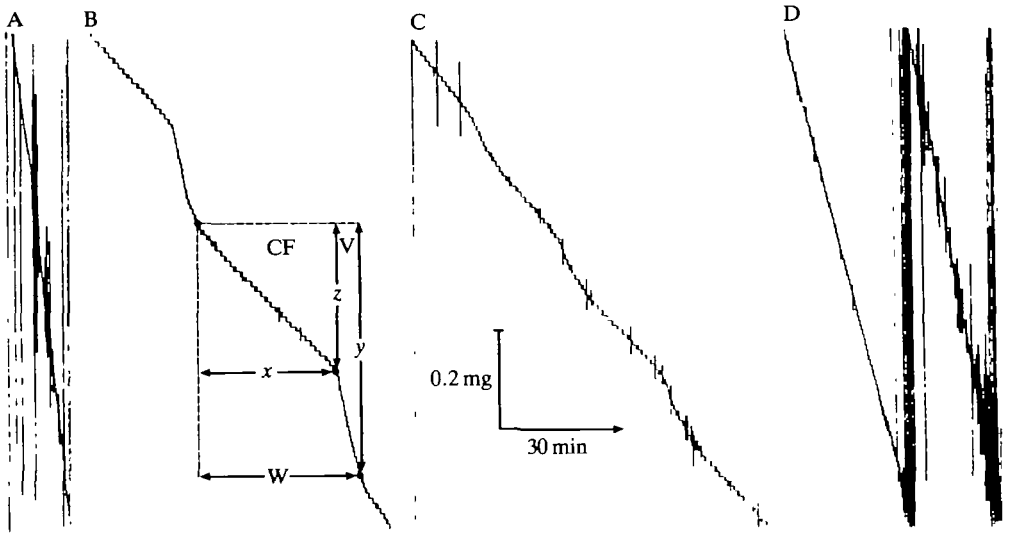

Fig. 1. Typical mass recordings from adult *Periplaneta americana* showing different patterns of loss. A, B and C were produced by the same individual. (A) Losses after introduction into the balance, showing the mechanical disturbances and elevated losses associated with extracyclic activity. (B) Typical cyclic pattern of normally hydrated cockroaches (water content 69.3%), showing constriction plus flutter (CF) and ventilatory periods of the cycle. Arrows indicate measurements used to estimate water losses during different periods of the CFV cycle. (C) CFV cycle 153 h later with a water content of 65.5%. Vertical lines are the result of intracyclic grooming. The scale applies to all four sections. (D) Mass loss in 5% CO_2. The left-hand record was produced during inactivity and the right-hand one during locomotory activity. For further details, see text.

intracyclic activity (INCA), varying between single spikes resulting from grooming to more prolonged periods of greater amplitude due to locomotion (Kestler, 1991). Defecation was marked by a longer (approximately 1 h) period of more rapid, relatively undisturbed, but progressively declining, mass loss. As cockroaches dehydrate, the CFV pattern becomes increasingly regular with little or no disturbance.

Fig. 1 shows typical records from the same cockroach: Fig. 1A active when hydrated, Fig. 1B quiescent when hydrated and Fig. 1C quiescent when dehydrated. The two quiescent traces clearly show the regular CFV cycle characteristic of the respiratory cycle (Kestler, 1978*a*, 1985). It can also be seen by comparing Fig. 1B and Fig. 1C that ventilatory cycles of the dehydrated animal are shorter, with less distinction between constriction plus flutter (CF) and ventilatory slopes. Since the digital output of the balance used in this study could not resolve the rapid events associated with ventilatory pumping or flutter, water flux measurements relate to the ventilatory period as a whole (V) and to CF. Fortunately, the effect of the unresolved flutter on the overall mass loss during constriction is very small (Kestler, 1985). In greatly disturbed recordings at the beginning of experiments, total water flux was obtained simply from the mass trace slope (mass loss/time).

When CFV cycles had developed, total losses could be separated into constriction–flutter (CF) and ventilatory (V) components. Total loss, averaged over time, was given by y/w, whereas CF loss was z/x and ventilatory loss $[(y-z)/(w-x)]-(z/x)$ (Fig. 1B). Total loss (CF plus V) during ventilation, used in comparisons with animals in 5 % CO_2, was $(y-z)/(w-x)$. Total CFV duration and the durations of CF and V are inversely proportional to the metabolic rate of the cockroach (Kestler, 1971; Miller, 1982).

To compare data from animals of different size, we followed the convention of earlier studies of dividing by total body surface area, calculated according to Machin *et al.* (1986). To facilitate comparison with other studies the values given in Table 5 were divided by partial pressure difference to obtain standardized cuticular flux or 'mass conductance density' (MCD), in $g\,h^{-1}\,cm^{-2}\,Pa^{-1}$. This new term is compatible with related concepts described in Piiper *et al.* (1971). Although cuticle permeabilities should be expressed in units of $m\,s^{-1}$ (Croghan and Noble-Nesbitt, 1989), arguments in favour of using explicit units in physiology have been made by Piiper *et al.* (1971). More explicit physiological terms to describe the conducting properties of the spiracles will not be used because spiracular area is unknown.

In all experiments before CFV cycles had developed, total water losses were highly variable, ranging between $23.42\times10^{-5}\pm20.12\times10^{-5}$ (N=2), $13.09\times10^{-5}\pm$

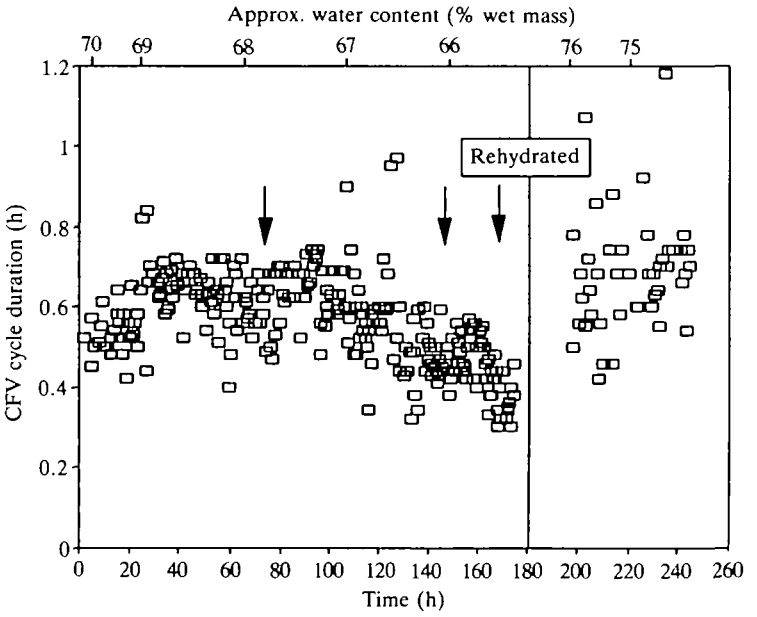

Fig. 2. Changes of ventilatory cycle (CFV) duration in the same cockroach as in Fig. 1A, B and C with time after introduction into the balance. After 8 days of dehydration, the record continues for 2 days following rehydration. The approximate water content of the animal is also indicated. Arrows indicate periods of elevated loss in Fig. 3.

Table 1. *Summary of analysis of representative CFV cycle timing in animals grouped according to water content*

	Water content (%)	*N*	Mean duration (h) CFV	V	CF	V/CFV
Hydration state						
Hydrated	76.61±0.60	10	0.772±0.172	0.183±0.041	0.589±0.142	0.239±0.036
Normal	69.46±0.22	45	0.679±0.180	0.162±0.036	0.517±0.165	0.251±0.069
Dehydrated	65.07±0.69	20	0.534±0.159	0.130±0.044	0.404±0.141	0.253±0.088
P values						
Hydrated–normal			<0.01	<0.05	<0.05	>0.8
Hydrated–dehydrated			<0.001	>0.1	<0.001	>0.3
Normal–dehydrated			<0.001	<0.001	<0.001	>0.7

Probabilities of differences between hydration states are also given.
N refers to the total number of replicates, five per animal.
Values are mean±S.D.

10.71×10^{-5} (N=9) and $10.12\times10^{-5}\pm4.07\times10^{-5}$ (N=4) $g\,h^{-1}\,cm^{-2}$ in hydrated, normal and dehydrated groups, respectively. Figs 2–4 are derived from the CFV cycles recorded over a 10-day period using a single animal. The other two individuals recorded in the same way produced essentially the same results. It can be seen from the water content axis that the animal began the experiment in a hydrated state and became dehydrated (water content <66%) before being returned to full hydration. Fig. 2 shows that CFV cycle durations were initially short (approximately 30 min) during an acclimation period of about 20 h. Following this there was a decreasing trend in CFV duration as dehydration progressed. Cycle duration was elevated and more variable following the second hydration, where human interference was less, showing little of the initial shortening noted above. In the shorter experiments with larger numbers of individuals, there was considerable individual variability in all CFV variables, resulting in significant differences (P<0.001) between all experimental groups. Nonetheless, mean CFV, CF and V duration of stable CFV cycles (Table 1) did show significant hydration-related differences, confirming the cycle shortening characterized in Fig. 2. No significant trends were observed in the proportion of the ventilatory cycle occupied by CF or V.

CF losses calculated once CFV cycles had developed were initially elevated then rapidly declined over the first 16 h (Fig. 3). Rehydration resulted in an increase in CF loss and greater variability. Below water contents of about 69%, CF losses were much less variable with a slight tendency to increase with time. Imposed on this pattern were short periods of elevated losses associated with moderate, probably extracyclic, locomotor activity involving several consecutive CFV cycles (marked with arrows in Fig. 3). Comparison with Figs 2 and 4 suggests some correlation with exaggerated ventilatory loss and perhaps with depressed CFV

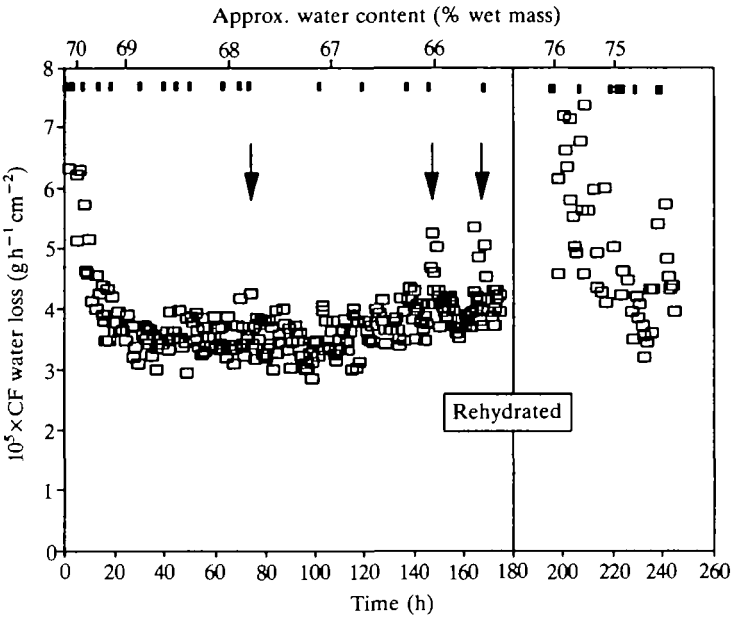

Fig. 3. Changes in CF water loss in the same cockroach as in Fig. 1A, B and C with time after introduction into the balance. After 8 days of dehydration, the record continues for 2 days following rehydration. The approximate water content of the animal is also indicated. Vertical bars indicate periods of disturbed readings due to animal activity. Arrows indicate periods of elevated CF loss associated with animal activity.

duration, both indicating increased metabolic rate (Miller, 1982; Kestler, 1971). Analysis of typical CFV cycles (Table 2) confirms that CF losses are higher in hydrated animals, lower during normal hydration, and slightly but significantly higher in dehydrated individuals. Ventilatory losses (Fig. 4) in hydrated animals were elevated and variable. Below water contents of 69 % they were somewhat more variable than CF losses but, unlike them, showed a steady decline during dehydration. Table 2 confirms that ventilatory losses decline between hydrated and normally hydrated states. However, differences between normal and dehydrated states are not significant.

In normally hydrated cockroaches in 5 % CO_2, mass declined comparatively smoothly with no evident CFV cycles (Fig. 1D, left-hand trace). As with animals in normal P_{CO_2}, records of mass loss were disturbed by activity at the start of the experiment and intermittently thereafter (Fig. 1D, right-hand trace), resulting in local variations in the rate of mass loss. Comparisons of total water loss during ventilation between active and inactive animals in air (showing CFV cycling) and in 5 % CO_2 are made in Table 3. Activity increases mean losses by 30.0 and 53.7 % in the air and CO_2 groups, respectively. Water losses in quiescent and active cockroaches in 5 % CO_2 were 25.1 and 48.0 % greater, respectively, than for corresponding animals breathing air. The increases in water loss with activity in

Table 2. *Summary of CF and ventilatory (V) water loss of representative ventilatory cycles in animals grouped according to water content*

	Water content (%)	*N*	10^5×mean water loss ($g\,h^{-1}\,cm^{-2}$) CF	V
Hydration state				
Hydrated	76.61±0.60	10	5.487±0.967	9.055±2.047
Normal	69.46±0.22	45	3.201±0.541	8.363±2.440
Dehydrated	65.07±0.69	20	3.259±0.576	6.101±4.013
P values				
Hydrated–normal			<0.001	<0.001
Hydrated–dehydrated			<0.001	<0.001
Normal–dehydrated			<0.05	>0.5

Probabilities of differences between hydration states are also given.

Note that ventilatory losses are corrected for animal size differences by dividing by animal, not spiracular, surface area.

N refers to the total number of replicates, five per animal.

Values are mean±S.D.

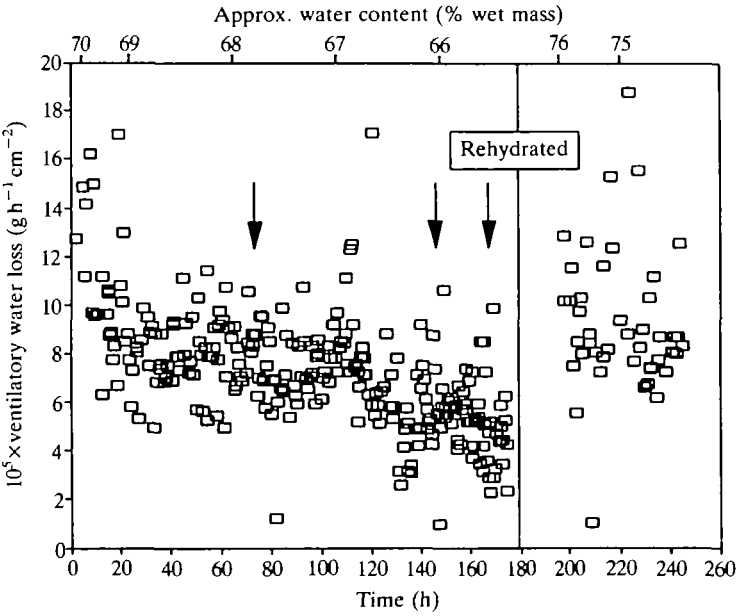

Fig. 4. Changes in ventilatory water loss in the same cockroach as in Fig. 1A, B and C with time after introduction into the balance. After 8 days of dehydration, the record continues for 2 days following rehydration. The approximate water content of the animal is also indicated. Arrows indicate periods of elevated loss in Fig. 3.

5 % CO_2 clearly show that spiracular opening is not the only factor affecting water loss.

Total losses averaged over time are shown in Table 4. The term 'averaged total loss' distinguishes these values from total loss during the ventilatory period only in Table 3. Comparison of CF to averaged total losses (Table 4) shows that CF loss amounts to about two-thirds of the total in quiescent groups, showing no trend

Table 3. *Summary of total water loss during ventilation (V) from normally hydrated, active and inactive cockroaches in dry air and in 5 % CO_2 at 20°C*

	$10^5 \times$mean water loss ($g\,h^{-1}\,cm^{-2}$)				*P* values
	N	Air	*N*	5 % CO_2	Air–CO_2
Activity state					
Inactive	45	11.564±2.767	20	14.470±1.466	<0.05
Active	41	15.030±4.510	20	22.244±5.081	<0.001
P values					
Active–inactive		<0.001		<0.001	

Probabilities of differences according to activity and gaseous environment are also given.
N refers to the total number of replicates, 4–5 per animal.
Values are mean±S.D.

Table 4. *Mean CF losses compared with total water losses (CF plus V) averaged over time in inactive cockroaches at various levels of hydration at 20°C*

			$10^5 \times$mean water loss ($g\,h^{-1}\,cm^{-2}$)		
	Activity state	*N*	CF	Averaged total	CF/ averaged total
Hydration state					
Hydrated	Inactive	10	5.487±0.967	7.676±1.543	0.719±0.046
Normal	Inactive	45	3.201±0.541	5.274±1.111	0.617±0.076
Normal	Active	41	4.085±1.473	7.663±2.908	0.553±0.148
Dehydrated	Inactive	20	3.259±0.576	4.617±0.939	0.711±0.069
P values					
For hydration					
Hydrated–normal			<0.001	<0.001	>0.2
Hydrated–dehydrated			<0.001	<0.1	<0.05
Normal–dehydrated			<0.05	>0.2	>0.1
For activity					
Active–inactive			<0.001	<0.001	<0.001

Comparisons between active and inactive periods are also made in the normally hydrated group.
Mean CF/averaged total ratios were determined from the ratios for each animal.
Probabilities of differences between hydration and activity states are also given.
N refers to the total number of replicates, four to five per animal.
Values are mean±S.D.

with water content. CF losses declined to about half the total in active cockroaches.

Discussion

Rates of total water loss following introduction to the balance show a great deal of variability, with the highest values some five times greater than typical more stable values. Salivation (Quinlan and Hadley, 1982) and increased metabolism (Machin *et al.* 1986), resulting from recent human intervention, and possibly surface desorption (Loveridge, 1980) as the animal is exposed to the dry air environment of the weighing chamber undoubtedly make some early CF and ventilatory fluxes unreliable. However, there are genuine effects on the CFV cycle as well. Elevated CF and ventilatory losses are associated with high hydration levels, since they also recur following rehydration. In contrast, increases in metabolic rate were only associated with human interference since CFV shortening did not recur upon rehydration. Following the initial acclimation period, comparatively stable water losses were sustained in our long studies for seven consecutive days. Ventilatory losses decreased adaptively in response to dehydration in two of the three comparisons. CF losses have also been shown to change with hydration level, surprisingly showing a slight increase in dehydrated animals. Greater control over ventilatory water losses in desiccated insects is well established in the literature (e.g. Miller, 1964, 1974). CFV cycles as well as component CF and V periods were shorter in dehydrated than in normally hydrated animals, perhaps reflecting the decreased CO_2 storage capacitance of declining blood volume.

In addition to the effects of hydration, CF losses are also sometimes subject to rapid change in association with activity. In active individuals the flutter period increases, sometimes with the loss of constriction (Kestler, 1971). Effective spiracular sealing and perfect synchrony have only been demonstrated in quiescent, normally hydrated cockroaches (Kestler, 1985). Although hormonal influences on the cuticle cannot be ruled out, changes in flutter relative to constriction, possibly combined with less effective spiracular sealing, seem to be the likely explanation of higher CF losses in hydrated and active cockroaches. Determination of cuticular MCD, except perhaps in quiescent, normally hydrated or dehydrated animals, would thus be subject to error. However, since the technique used here to separate CF and ventilatory fluxes has produced the lowest cuticular MCDs in the literature for normally hydrated quiescent animals, $1.45\times10^{-8}\pm0.037\times10^{-8}\,g\,h^{-1}\,cm^{-2}\,Pa^{-1}$ (Table 5), these values are probably the best that are currently available. The definitive answer to the question of whether cuticular MCD is under hormonal control must therefore wait until an unambiguous method of cuticle permeability measurement is found.

Table 5 compares standardized water losses derived from data in the Results section (for air-breathing animals only) with the highest and lowest mean losses of intact cockroaches from Noble-Nesbitt and Al-Shukur (1987). Mean values plus

Table 5. *Comparisons between* Periplaneta americana *water losses obtained by intermittent weighing and those derived from continuous mass recordings (present study)*

	$10^8 \times$water losses ($g\,h^{-1}\,cm^{-2}\,Pa^{-1}$)			
	CF	CF +2 S.D.	Total	Total +2 S.D.
Noble-Nesbitt and Al-Shukur (1987)				
Hydrated, weighed hourly	–	–	16.35	–
Dry culture, weighed daily	–	–	5.74	–
Present study				
Following introduction to balance				
Hydrated, handled	–	–	10.58	28.75
Dehydrated, handled	–	–	4.57	8.25
Losses during ventilation only (from Tables 3 and 4)				
Normal, inactive	1.45	1.93	5.22	7.72
Normal, active	1.85	3.18	6.79	10.86
Losses averaged over time (from Table 4)				
Hydrated, inactive	2.48	3.33	3.47	4.86
Dehydrated, inactive	1.47	1.99	2.09	4.32

Values from the Results have been divided by vapour pressure difference to facilitate the comparison between studies.

twice their standard deviations were considered to represent the probable upper limits of the various water losses due to random variability in a normal distribution. The greater variability of the total loss data compared with corresponding CF values, indicated by the standard deviations, shows that spiracular losses must account for most of the variation of water loss observed in *Periplaneta*. It is also clear from Table 5 that neither random nor hydration-related differences in CF losses alone could account for the range of values obtained in the other study. Furthermore, the total water losses of our inactive or spontaneously active groups, even during periods of continuous ventilation, are insufficient to account for the losses of Noble-Nesbitt and Al-Shukur's (1987) hydrated group. Only our short-term losses, elevated by recent human contact, can match the sustained values of the other study. Whereas our animals developed relatively stable patterns of reduced water loss within 24 h, the losses of Noble-Nesbitt and Al-Shukur's (1987) cockroaches were still 74 % of initial values after 72 h. We further note that water losses from controls in the injection experiments (5×10^{-8} to $8\times10^{-8}\,g\,h^{-1}\,cm^{-2}\,Pa^{-1}$) (Noble-Nesbitt and Al-Shukur, 1988*a*,*b*) are considerably higher than our stable values, and even higher than those from our animals during ventilation.

We suggest that discrepancies in water losses in the two studies are associated

with differences in the method of weighing. Even though handling was indirect in the previous study, intermittent weighing requires repeated and much greater human interference than continuous mass loss recording. Our technique of allowing the cockroach to enter the weighing cage voluntarily may also be responsible for the more rapid fall in rate of water loss in our study. In addition, mean values calculated from fluctuating accumulative losses in intermittent weighing tend to be skewed towards the highest values. Although Noble-Nesbitt and Al-Shukur (1987) defend their use of intermittent weighing by stating that they have demonstrated that water loss is unaffected by handling, their experiments only show that water loss in differently handled, dehydrated groups is not affected by decapitation. However, their general claim that handling has minimal effect on water loss is incorrect. Predesiccated cockroaches, handled before and after experimental dehydration, lost water more rapidly than those handled only once on their removal from the dry culture. The difference was 1.6-fold in the first 6 h (Noble-Nesbitt and Al-Shukur, 1987; Fig. 3) and 1.4-fold over the next 60 h (their Fig. 4).

The theory of hormonal control of cuticle permeability (Noble-Nesbitt and Al-Shukur 1987, 1988*a*,*b*) rests on two assumptions discussed in the Introduction: that the rate of water loss through spiracles held open by 5 % CO_2 is fixed, and that the majority of water lost by an insect passes through the cuticle. This study has demonstrated that total water losses in the presence of 5 % CO_2 exhibit greater activity-related variability than those of the groups breathing air. Variation in CF losses in Table 5 seems to be too low to offer a plausible explanation for differences in total loss. This study's best estimate of cuticular MCD is $1.45\times10^{-8}\,g\,h^{-1}\,cm^{-2}\,Pa^{-1}$ (Table 5). It is possible that there could be some differences in cuticular MCD between highly inbred cockroach cultures, and indeed differences in the water contents of animals from 'normally' hydrated cultures have already been noted. However, mass loss rates reported in the other studies of intact, hydrated animals (Noble-Nesbitt and Al-Shukur, 1987) and both hydrated and dehydrated control groups (Noble-Nesbitt and Al-Shukur, 1988*a*,*b*) are so much higher than this value (sevenfold and 2.5–4.5 times, respectively) that to explain them largely in terms of differences in cuticle conductance hardly seems plausible. Furthermore, if the low cuticular MCDs obtained by this study are representative, the changes in MCD necessary to account for the effects of experimental decapitation and injection are larger than envisaged by the earlier studies. For example, the increase of $20.6\times10^{-8}\,g\,h^{-1}\,cm^{-2}\,Pa^{-1}$ resulting from the decapitation of hydrated cockroaches (Noble-Nesbitt and Al-Shukur, 1987) would require as much as an 8.3-fold increase in cuticular MCD. More modest changes of over $3\times10^{-8}\,g\,h^{-1}\,cm^{-2}\,Pa^{-1}$ following injection (Noble-Nesbitt and Al-Shukur, 1988*a*) would still require more than a doubling of cuticular MCD. Since ventilation is significantly more variable than CF in active animals (Table 5) with 95 % limits representing an increase of 2.33-fold for CF and 4.07-fold for V, we suggest that changes in total loss are more easily explained in terms of ventilation.

Finally, there is no obvious adaptive value to a cockroach in having variable control over cuticular MCD as suggested by Noble-Nesbitt (1990). Ventilation would be an efficient avenue to augment losses for cooling purposes or overcoming hypo-osmosis, though it should be stressed that no evidence exists for either.

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