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EFFECTS OF DESICCATION, WATER-STRESS AND DECAPITATION ON INTEGUMENTARY WATER LOSS IN THE COCKROACH, *PERIPLANETA AMERICANA*

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SUMMARY

Cockroaches decapitated in a fully hydrated state at first lose water under desiccation much more rapidly than their intact counterparts. The rate of loss decreases with time of desiccation; this decrease is more marked in decapitated than in intact cockroaches.

The initial rate of water loss is lower and less variable in predesiccated cockroaches and continues to fall gradually during further desiccation. Decapitation of predesiccated cockroaches has little effect on the rate of water loss. Similarly, the rate of water loss is low with low variability in water-stressed cockroaches taken from dry culture conditions, and decapitation has no significant effect.

The lowering of the rate of water loss is not a simple response to lowering of the body water content, but is a two-stage, time-related physiological response of acclimatization to water-stress or desiccation. The initial phase of rapid response requires the presence of the head, whereas the subsequent gradual phase does not.

With the techniques used in this investigation, handling-related effects of the decapitation procedure are not significant.

INTRODUCTION

The major avenues for water loss from the cockroach are the excretory system, the tracheal (respiratory gas exchange) system and the integument. Neuroendocrine regulation of excretion in insects in response to changes in ionic, osmotic and water balance is well established (Edney, 1977; Raabe, 1982). Restriction of tracheal water loss through control of spiracular opening is also well established in insects as a physiological response to desiccation (Mellanby, 1934; Wigglesworth, 1945; Burkett & Schneidermann, 1968; Miller, 1964; Edney, 1977).

Regulation of integumentary water loss is less well established. The long-standing generalization that, once fully formed, the cuticle provides an unchanging, relatively impermeable barrier restricting water loss through the integument, dating from the early work of Ramsay (1935), has been challenged in a number of cases. The most

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widely accepted of these is the intermoult repair of cuticular abrasion (Wigglesworth, 1945). Wall (1967) suggested that antidiuretic responses in *Periplaneta* could involve restriction of integumentary water loss. Penzlin & Stolzner (1971) showed that an accelerated water loss can be induced by removal of the frontal ganglion or severance of the frontal connectives in *Periplaneta*.

Treherne & Willmer (1975) further strengthened the view that endocrine control is exercised over integumentary water loss in a series of experiments that investigated the involvement of the cephalic endocrine system in the regulation of water loss. Using live insects, they demonstrated that decapitated cockroaches lose water by evapotranspiration at a greater rate than intact cockroaches when exposed to desiccating conditions. When they injected decapitated cockroaches with brain homogenates, rates of water loss were similar to those of intact cockroaches. Such injections into intact cockroaches 24 h before decapitation were ineffective: high rates of water loss were subsequently obtained following decapitation. This suggested that the brain factor is inactivated in the intact insect. Since the effect of the factor in decapitated insects appeared to be long-lasting (for 72 h or more), they suggested that inactivation required the presence of the head. Although decapitation was a central technique in their investigation, they also employed a number of additional techniques to investigate possible involvement of other body systems, including the ventral central nervous system, the excretory system and the tracheal system. After eliminating the effects of regulation on other possible avenues for water loss, they concluded that at least part of the water loss is under neuroendocrine control, and that the cephalic neuroendocrine system (the brain and associated corpora cardiaca) is the source of a potentially long-lived hormone which reduces integumentary permeability.

These conclusions of Treherne & Willmer (1975) have recently been questioned. Machin, Kestler & O'Donnell (1986) suggest that non-endocrine factors can equally well account for both their own results and those of Treherne & Willmer. They show possible effects of handling stress and cuticular abrasion, and question the reliability of Treherne & Willmer's results on tracheal water loss.

In a re-examination and extension of Treherne & Willmer's work (Al-Shukur, 1984), outlined in a preliminary report (Noble-Nesbitt & Al-Shukur, 1984), it became clear that a fuller reappraisal of their results and interpretations was necessary. Early preliminary experiments using cockroaches drawn from normal culture confirmed the results obtained by Treherne & Willmer. In this culture, moist food (carrots, cabbage) and water were always available along with dry food pellets. The ambient humidity within the culture was kept relatively high with this available moisture. However, some later experiments did not conform to the Treherne & Willmer pattern. Low water loss rates were obtained in decapitated cockroaches. Examination of possible causes for these results suggested that dry culture conditions may have affected the results, indicating that predesiccation could perhaps precondition the insect, and so affect the water loss rates obtained under the experimental regime.

We therefore decided to investigate possible effects of prior water-stress and predesiccation under experimentally controlled conditions on subsequent water loss. This communication sets out the results of that investigation. The results indicate that the intact, living cockroach responds rapidly to environmental changes, sensitively regulating water loss. At the same time, as shown in parallel investigations which will be reported in full separately, the nature of the cuticular surface lipids changes. The two effects may be causally connected.

MATERIALS AND METHODS

Prior to experimentation, cockroaches (*Periplaneta americana* L.) were kept in culture in a room at 24–26 °C and with a relative humidity (RH) of 50–55 %. They were provided with ample water and dry, pelletted food, augmented with wet food (cabbage, carrot). Under these normal culture conditions, the available moisture maintained a high RH (60–70 %) within the microhabitat of the culture tank, which contained corrugated cardboard sheets. For experiments using cockroaches drawn from dry culture conditions, only dry food pellets were provided in the culture, and a water supply was not continuously available. The culture RH was then the same as room RH (50–55 %). Adult male cockroaches were used in all the experiments described here, although other experiments using nymphs and adult females gave similar results (Al-Shukur, 1984).

In view of the known sensitivity of cockroaches to handling and restraint (Beament, 1954, 1958, 1961*a*; Richards, 1951; Loveridge, 1980), recently further indicated by Machin *et al.* (1986), care was taken to adopt handling procedures designed to minimize stress or damage to the cockroach during both routine weighing and decapitation, with minimal differences among different sets of insects to reduce variation. Preliminary tests were carried out using a variety of handling procedures before a standard procedure was adopted.

Cockroaches were removed from culture by carefully seizing a leg with long-handled forceps and gently transferring the insect to isolation, first in a stoppered glass specimen tube and then to a stoppered, perforated polyethylene weighing tube of the same diameter. Subsequent handling only of the tube resulted in minimal specimen disturbance, abrasion or other injury.

Decapitation was carried out using a technique similar to that described by Treherne & Willmer (1975). The cockroach was first lightly anaesthetized with carbon dioxide. A ligature was then put round its neck, tightened and knotted. The neck was then severed anterior to the ligature. Finally, the cut end of the neck was sealed with Newskin, a proprietary product which was found to be effective in sealing spiracles and wounds in *Periplaneta* (Beament, 1961*b*; Treherne & Willmer, 1975). The mass of the insect was recorded both before and after decapitation. Control insects were handled similarly up to the point were the ligature was applied.

Experimental insects, both intact and decapitated, were kept individually in closed, perforated polyethylene polypots of known mass. This greatly facilitated handling and weighing, with minimal effect on the insect, which remained enclosed

in the polypot throughout the experimental desiccation and weighing period. Trials with empty perforated polypots showed that insignificant mass changes (<0·1 mg) occurred during 72 h of exposure to the desiccating conditions used in the experiments and that mass changes from this source could therefore be ignored.

Sets of 10 or 12 intact males and 10 or 12 decapitated males were exposed to a low-humidity, desiccating atmosphere of 10–15 % RH (in a large glass desiccator, over self-indicating silica gel) at room temperature (20–22°C) for 3 days. To follow the time course of water loss, they were weighed after 1, 2, 3, 4, 5, 24, 48 and 72 h exposure to the desiccating atmosphere. To do this, they were briefly removed from the desiccator for weighing on an electrobalance accurate to 0·1 mg. Before resealing the desiccator, it was flushed out with dry air from a gas cylinder, to minimize humidity fluctuation. At the end of the experiment, water content was measured by drying the insects to constant mass at 60°C.

The sets of insects were drawn as batches either from normal culture, or from dry culture, or from normal culture but then predesiccated in the intact state for 3 days over silica gel at room temperature without access to food or water.

Expression of results

Results are expressed conventionally, providing ease of comparison with results already in the literature. Transpiratory water loss through the cuticle is assumed to equal total mass loss (Beament, 1961*a*; Edney, 1977; Loveridge, 1980). Since water loss through the cuticle is a function of surface area, rates of water loss are expressed in units of $mg\,cm^{-2}\,h^{-1}$, using the relationship of Edney (1977) as modified by Machin *et al.* (1986) for surface area, A (in cm^2) = k (initial mass, in g)$^{0·63}$, where k = 14·5. From this is subtracted 0·4 cm^2 for headless individuals. Estimates of cuticular permeability in units of $mg\,cm^{-2}\,h^{-1}\,mmHg^{-1}$ are derived by dividing the water loss rate by the saturation deficit (in mmHg, 1 mmHg = 133·3 Pa) of the air within the experimental desiccators. Cuticle thickness is assumed to be constant and is not taken further into account. Although results expressed in this way are only first approximations, in the absence of reliable direct measurements of cuticular water loss they provide probably the best estimates currently available for cuticular water loss and permeability.

RESULTS

Intact cockroaches from normal culture quickly settle to a low transpiratory water loss rate (Fig. 1). Linear regression analysis of the data over the first 5 h of desiccation shows that water loss rate remains fairly constant. Extrapolation back to time zero shows a very small positive intercept, indicating that any initial adjustment of water loss rate is rapid – within minutes rather than hours – and within a span of very slight water loss. Tracking of data for individual cockroaches showed that over this initial 5 h, water loss rate fluctuated. This is a major contributory factor to the variability of the data during this initial period, although inter-individual differences

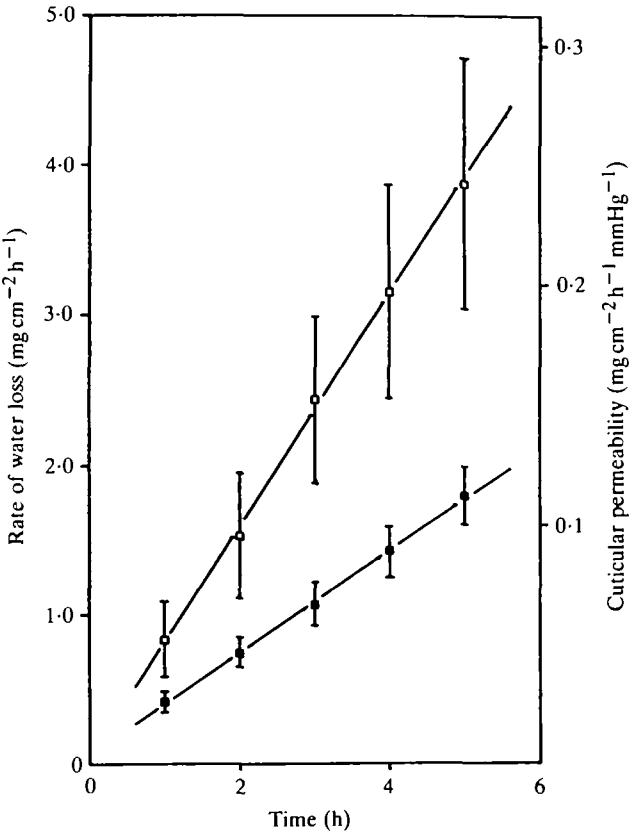

Fig. 1. Early water loss under desiccating conditions from fully hydrated intact (■) and decapitated (□) cockroaches taken direct from normal culture conditions. In this and subsequent figures, vertical bars show standard errors and continuous lines are regression lines. N = 10 or 12 cockroaches for each point.

also occur. By 24 h, the rate has settled still further at a lower and more constant level, with less variability. This rate is maintained over days 2 and 3 (Fig. 2).

Decapitated cockroaches from normal culture have a much higher initial loss rate, and higher variability, than their intact counterparts (Fig. 1). Extrapolation back to time zero again shows only a small positive intercept, indicating that large, sudden, immediate losses do not occur. Individual tracking shows both wide inter-individual differences and wide intra-individual fluctuation over this period. This suggests that whilst the state of the individual at the outset is important in determining the starting rate, poor control thereafter causes more 'free running' than is evident in their intact counterparts. By 24 h, the rate has settled to a lower and more constant level, with less variability, but both rate and variability are still greater than that of their intact counterparts (Fig. 2).

The results presented in Figs 1 and 2 were repeated for several batches of cockroaches, similarly drawn direct from normal culture. Although the average loss rate of intact insects varied to some extent between batches, there was a consistent

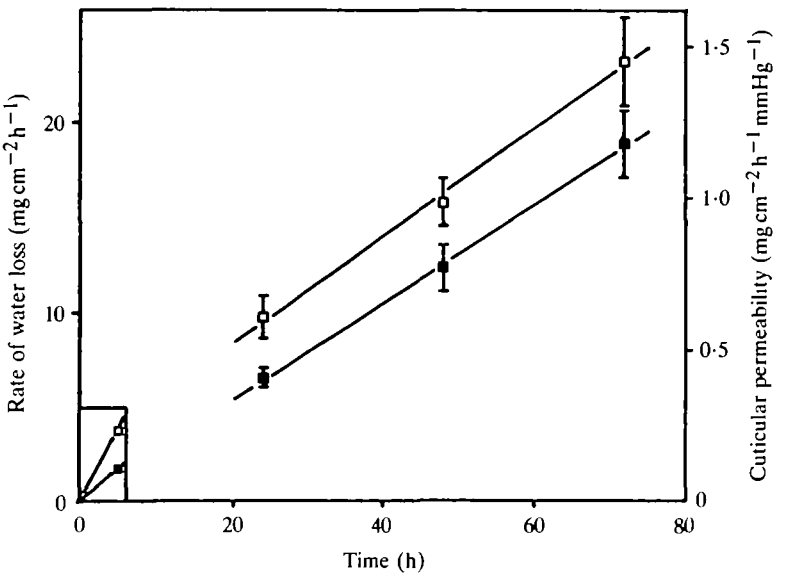

Fig. 2. Later water loss under desiccating conditions from fully hydrated intact (■) and decapitated cockroaches (□) taken direct from normal culture conditions.

difference between intact and decapitated cockroaches. Over the initial 5 h of desiccation, this difference on average amounted to a 46% higher loss rate in decapitated cockroaches than in their intact counterparts. We suggest that the intact results reflect initial differences in the batches of insects, probably due to variation in culture conditions, rather than other sampling variations. The consistently higher loss rates of decapitated cockroaches provide a measure of confidence in comparisons carried out within batches of intact and decapitated cockroaches taken from culture at the same time – an approach we adopted as standard.

Consistently lower loss rates resulted after predesiccation treatment for 3 days, in both intact and decapitated cockroaches. The rates of loss over the first 5 h (Fig. 3) were only a little higher than over days 2 and 3 (Fig. 4). Vartiation was also less.

Similarly, consistently low loss rates followed water-stress treatment in dry culture for 1 week, in both intact and decapitated cockroaches. The rates of loss over the first 5 h (Fig. 3) were little higher than over days 2 and 3 (Fig. 4), and the differences were not significant. Variation was low.

These two sets of results from predesiccated and water-stressed cockroaches demonstrate the overriding nature of the initial physiological state on loss rates subsequently measured under gradient (desiccating) conditions. Differences between intact and decapitated insects are virtually eliminated and variation is much reduced. This latter point emphasizes the tighter physiological control in operation by the end of the predesiccation or water-stress period.

These results also demonstrate that the effects of decapitation on water loss depend more on the physiological state of the insect at the time of decapitation than on factors associated with the decapitation procedure itself (extra handling, cuticular abrasion, etc., see Machin *et al.* 1986) when this is carried out with care.

Averaging the rate of water loss over shorter times shows a general tendency towards lower rates as time progresses. All classes of experimental insect, except for decapitated insects drawn from normal culture, have loss rates within a fairly narrow band. Within this band, rates are lower the greater the predesiccation, in a cumulative way. Fig. 5 plots the results in a temporal sequence which takes into account pretreatment periods. Plotted in this way, the results emphasize the cumulative effect of desiccation and water-stress on water loss rate.

This cumulative effect seems to be a temporal one, since dry culture (fed) individuals maintain a body water content (69%) higher than predesiccated (starved) individuals (65%), though lower than individuals from normal culture (72%). This again emphasizes that loss rate is under physiological control, and not simply determined by the degree of depletion of body water, or directly related factors. Long-term physiological adjustment – acclimatization – evidently occurs.

The fall in rate with time in decapitated insects from normal culture suggests that, although initial response to desiccation is impaired, longer term response is not. This suggests a two-phase response, an initial phase requiring the head with its sensory, central nervous and endocrine elements intact, and a second phase which is not dependent on the head.

DISCUSSION

Our results show that the rate of water loss of fully hydrated cockroaches taken from normal culture is affected by the presence or absence of the head. To this extent they confirm the conclusions of Treherne & Willmer (1975). However, our results also point to the crucial importance of the insect's physiological state at the

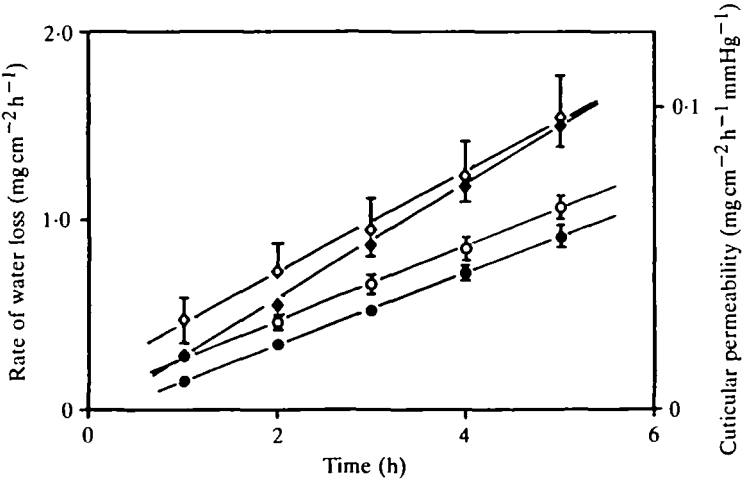

Fig. 3. Early water loss from intact (◆) and decapitated (◇) cockroaches predesiccated in the intact state and from intact (●) and decapitated (○) cockroaches previously water-stressed in dry culture.

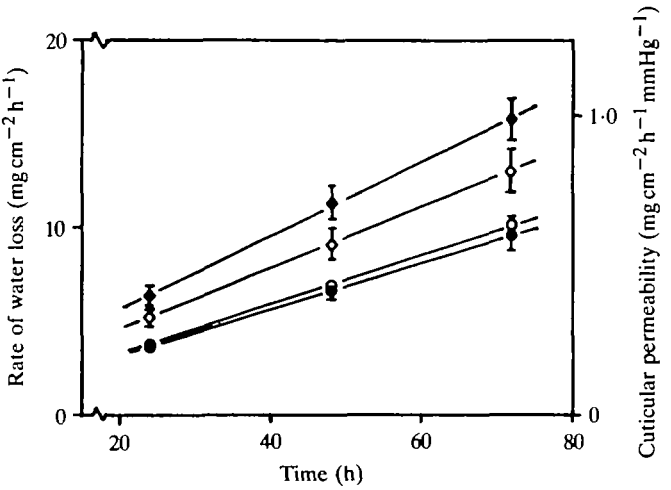

Fig. 4. Later water loss from intact (◆) and decapitated (◇) cockroaches predesiccated in the intact state, and from intact (●) and decapitated (○) cockroaches previously water-stressed in dry culture.

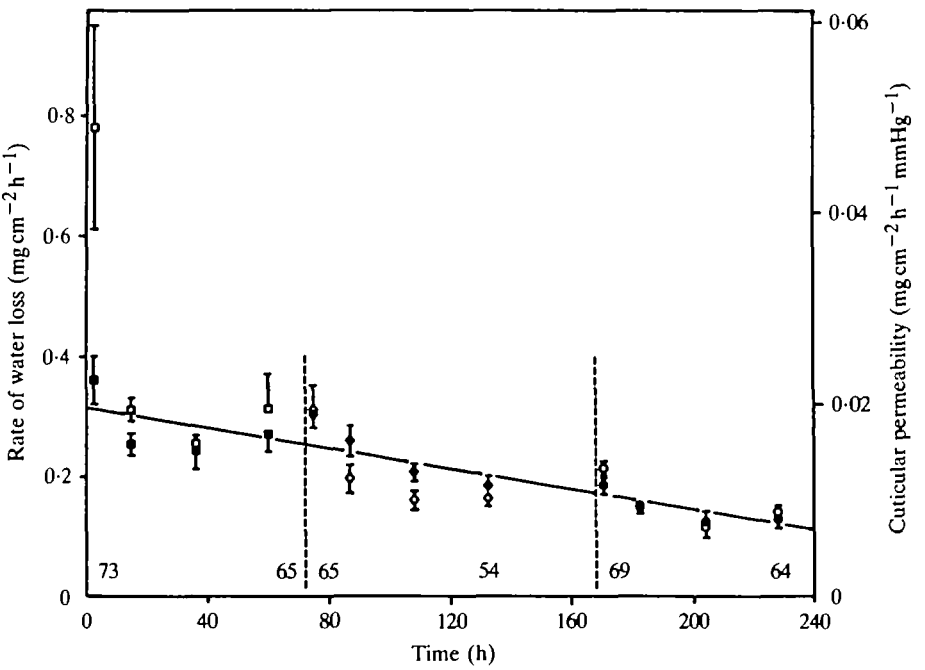

Fig. 5. Rates of water loss and cuticular permeability plotted as a function of cumulative time under desiccation and water-stress. The continuous line is the regression line fitted to all points except the initial reading for the unpretreated, decapitated cockroaches. Symbols as for previous figures. The vertical dashed lines separate the measurement phases for the different sets of cockroaches. The figures under the data show the water content expressed as a percentage of the total body mass at the beginning and end of each measurement phase.

commencement of the measurement of water loss. This physiological state can be altered by pretreatment, deliberate or incidental.

We deliberately placed the insect under water-stress before commencing the experimental phase of water loss measurements. This physiological state of water-stress was created in either one of two ways. Some insects were predesiccated for 3 days over silica gel at room temperature. Other insects were kept under dry culture conditions for a week. Both methods gave essentially similar results, with only the degree of change in water loss rate being different. Since they parallel the experimental treatment of desiccation used to determine the rate of water loss, it is to be expected that their effects will parallel those in the experimental measurement phase. Our results clearly bear this out.

In cockroaches taken from normal culture conditions (with food and water *ad libitum*) subsequent water loss under desiccation is at first erratic and high compared with measurements performed later. In the intact insect, the rate of water loss gradually falls with time of desiccation, with decreasing variability, suggesting a physiological tightening of control over water loss. In decapitated insects, a similar pattern is shown initially but much potentiated, with a much higher mean and with greater variability both among individuals and with time in an individual. Lowering of the loss rate in response to desiccation takes considerably longer than in the intact insect. This suggests that the principal effect of decapitation is on the initial response and not on the later responses. The initial erratic pattern suggests a lack of physiological control rather than an initial stress response which gradually wanes with time.

Intact cockroaches predesiccated for 3 days show water loss rates over the first 5 h of the experimental regime ($0{\cdot}31\,mg\,cm^{-2}\,h^{-1}$) that are intermediate between the initial ($0{\cdot}34\,mg\,cm^{-2}\,h^{-1}$) and later ($0{\cdot}26\,mg\,cm^{-2}\,h^{-1}$) rates for insects drawn direct from normal culture conditions. Later rates for the predesiccated insects are lower still ($0{\cdot}20\,mg\,cm^{-2}\,h^{-1}$). Pretreatment of 1 week in dry culture conditions confirms this trend of decreasing water loss rates with increasing water-stress, with an initial rate of $0{\cdot}19\,mg\,cm^{-2}\,h^{-1}$ and a later rate of $0{\cdot}12\,mg\,cm^{-2}\,h^{-1}$. Increased water-stress also reduces the variability.

The pretreatment effect is most noticeable in the results obtained with decapitated cockroaches (decapitated after pretreatment, at the start of the experimental measurement phase). Without pretreatment, initial water loss rates are high ($0{\cdot}72\,mg\,cm^{-2}\,h^{-1}$) and widely variable. Following pretreatment, initial rates ($0{\cdot}27\,mg\,cm^{-2}\,h^{-1}$ for 3-day desiccation pretreatment; $0{\cdot}20\,mg\,cm^{-2}\,h^{-1}$ for dry culture pretreatment) are as low as in intact insects and variability is also low. Our conclusion is that physiological adjustment had already taken place in the intact state during pretreatment. There is no evidence of handling- or stress-related elevation of water loss rate, which Machin *et al.* (1986) suggest may be responsible for the initially high water loss rate in decapitated cockroaches (see also below).

Recently Appel & Rust (1985) have drawn attention to long-term acclimatization effects on whole-body water loss in *Periplaneta fulginosa*. Following 7-day acclimatization with plentiful food and drinking water at different ambient humidities, water

loss of HCN-killed adult male cockroaches during 4 h of desiccation was less in those cockroaches acclimatized to lower ambient humidities. Total body water content and haemolymph volume were not affected by the 7-day acclimatization at any of the ambient humidities used, which ranged from 11 to 98 %. The lowering of the rate of water loss following acclimatization cannot, therefore, be restriction caused by reduced body water content or haemolymph volume. It indicates some physiological adjustment during acclimatization.

The relatively high loss rate of *P. fulginosa* may mean that acclimatization involves processes unrelated to regulating processes involved in restriction of water loss in the relatively less permeable *P. americana*.

In a subsequent paper (Appel, Reierson & Rust, 1986), it was shown that water loss from HCN-killed *P. fulginosa* over the first 4 h of desiccation was approximately twice that of living *P. fulginosa*. The majority of this early loss in both dead and living cockroaches occurred within the first 2 h of desiccation. The magnitude of these early losses (over 10 % of total body water in living cockroaches and over 20 % of total body water in dead cockroaches) could mean that subsequent water loss was restricted by depletion of body water. Appel *et al.* do, in fact, show that the rate of loss is much less after 2 h of desiccation in both living and dead cockroaches. This emphasizes the need to carry out measurements of water loss before the body water reserves are depleted to this extent, as many earlier workers have recorded (Wigglesworth, 1945; Beament, 1959; Mead-Briggs, 1956; Loveridge, 1968, 1980; Edney, 1971, 1977; Schmidt, 1955). *P. americana* fortunately shows much lower loss rates and our experiments with fully hydrated cockroaches were conducted well within the span of water loss which could lead to restriction by depletion of body water.

Appel *et al.* (1986) also reported results of tritium-labelled water flux measurements carried out over 24 h, which they interpreted as showing that cuticular permeability is not affected by ambient humidity, despite the acclimatization results mentioned above. However, the fluxes at near 100 % RH depart markedly from the trend up to 75 % RH, and this could indicate an alteration in permeability between 75 and 100 % RH. Data at more ambient humidities, especially in the 75–100 % RH range, are needed to clarify this.

Machin *et al.* (1986) have argued that cuticular abrasion and the trauma which may result from handling, restraint and decapitation may be the cause of the observed higher initial water loss. The crucial evidence they present in support of their argument derives from control insects which were subjected to identical handling and restraint as their decapitated insects, but stopping short of actual decapitation. The patterns of water loss with time were closely parallel in both control and decapitated cockroaches, and after 96 h the rates were only slightly above those of less-handled controls. They also found that prechilling to 4°C before handling and decapitation eliminated the initial excessive water loss. This they ascribed to the likelihood of less cuticular abrasion because the chilled insects were easier to handle. The severity of handling is clearly an important factor (Beament, 1954, 1958, 1961*a*; Loveridge, 1980; Richards, 1951).

However, Treherne & Willmer's (1975) results do not support Machin *et al.*'s interpretation. Their homogenate-injected, decapitated insects were subjected to similar handling and restraint trauma, but showed lower water loss than either decapitated or Ringer-solution-injected, decapitated insects. In other experiments which we will publish separately, we have confirmed that the procedures involved in injection to not themselves affect the rate of water loss. Treherne & Willmer's operation to sever the neck connectives did not increase water loss above unoperated controls. Machin *et al.*'s attempt to explain this on the basis of a balance between an increase in water loss consequent on handling and restraint and a decrease due to reduced metabolism is unconvincing. Equally, their suggestion that handling and restraint trauma may elevate tracheal water loss through increased metabolic activity and gaseous exchange is not supported by Treherne & Willmer's observations on spiracular opening and locomotor activity, which are both less in decapitated than in intact cockroaches. Machin *et al.*'s handled control results are isolated departures from the pattern of results accrued from several types of experiment. Nevertheless, they indicate that the normal response of intact insects may be seriously affected by handling that falls short of the care which is necessary.

In our experiments, techniques devised to reduce this handling effect to a minimum were used. We found elevated water loss only in decapitated cockroaches that had not been subjected to predesiccation or water-stress before decapitation. If cuticular abrasion and handling and restraint trauma are the reasons for elevated water loss, these factors ought to apply equally to predesiccated or water-stressed insects.

Physiological adjustment in the intact state during pretreatment suggests an explanation for Machin *et al.*'s results with prechilled cockroaches. During prechilling, adjustments to a state of lower water loss probably take place, giving rise to low loss rates during the subsequent measurement phase. Similarly, adjustments would have already taken place by the time the measurements were made in their other reported experiments. In view of our results, it is scarcely surprising that they did not find differences between intact and decapitated insects.

Results from a parallel series of experiments, which we will publish separately, indicate that the nature of the superficial lipids of the cuticle changes as the rate of water loss falls, suggesting that control of cuticular water loss is mediated in this way. Decapitation and brain homogenate injection affect this initial reaction. Homogenate injection also affects the rate of water loss in intact cockroaches.

The balance of experimental evidence favours the interpretation that the cephalic neuroendocrine system plays an important part in the initial regulation of cuticular water loss, lowering it in response to desiccation and water-stress. Decapitation at this stage prevents this initial response. As desiccation or water-stress continue, regulation becomes tighter, expressed as lowered variability in the water loss rate, which continues to fall gradually, and the continued integrity of the cephalic neuroendocrine system becomes less important. Once this stage has been reached, decapitation has little effect.

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