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EVIDENCE AGAINST HORMONAL CONTROL OF INTEGUMENTARY WATER LOSS IN *PERIPLANETA AMERICANA*

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SUMMARY

The effect of decapitation on water loss from adult male cockroaches was studied in order to re-examine the possibility of hormonal modulation of integumental permeability. We found no effect of decapitation on the permeability or water content in measurements made on the pronotum. Although overall weight losses of the decapitated animals always exceeded those of intact animals, loss rates and their rate of decline depended on the apparent severity of handling. There was a small but significant difference between the controls and all decapitated and sham-operated animals, and this difference persisted for at least 96 h. We attribute these results principally to the differential effects of cuticle damage, though increased respiratory water loss may also be a factor. Our results draw attention to the complex practical problems involved in obtaining accurate, representative cuticle permeabilities for *Periplaneta,* and form a basis for questioning the notion of inhibition of cuticular water loss by hormones from the head.

INTRODUCTION

Hormonal inhibition of integumentary water loss has been proposed on the basis of increased weight loss following decapitation in *Periplaneta americana* (Treherne & Willmer, 1975*a*,*b*). Some form of neuroendocrine control of integumentary permeability was inferred from the lessening of the increase in water loss in decapitated individuals after injection of extracts of the brain or corpus cardiacum. Control experiments suggested that the effect was not due to alterations in the rates of excretory or spiracular water loss. Edney (1980) reported that the unpublished work of P. Franco essentially confirmed these observations with the cockroach *Leucophaea*.

The proposal of neuroendocrine control of integumentary water loss has been discussed in many reviews and monographs (Beament, 1976; Edney, 1977, 1980; Machin, 1980; Maddrell, 1980; Gilby, 1980; Mullins, 1982; Willmer, 1982). The

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functional significance of such control resides in the possibility for modulation of cuticle waterproofing characteristics in response to changing external conditions. Treherne & Willmer (1975*b*) disussed several possible mechanisms by which this control might be achieved. The permeability of either the epidermis or the epicuticular lipid layer might be directly modulated. Alternatively, ancillary processes such as the release of the antioxidant which prevents degradation of cuticular lipids (Atkinson & Gilby, 1970) might be altered by decapitation.

Of fundamental importance to Treherne & Willmer's argument is their claim that excretory or spiracular water loss does not increase after decapitation. This conclusion has been based primarily on the finding that water loss rates in 5% CO_2, which causes the spiracles to remain continuously open, were also higher in decapitated animals relative to control animals (Treherne & Willmer, 1975*b*). However, there was a remarkable variability in rates of mass loss from normal animals from experiment to experiment. In figs 1, 2 of Treherne & Willmer (1975*b*) mean weights at 96 h after the start of the experiment were close to 80% of the initial weight. However, in five other weight loss graphs (Treherne & Willmer, 1975*b*) weights of normal animals were 90% of their initial weight at 96 h. The variability among the controls greatly exceeded the maximum difference of 4% initial weight between normal and decapitated animals in the crucial CO_2 experiments.

Treherne & Willmer also discounted an increase in spiracular water loss after decapitation because blockage of the spiracles in these animals did not alter the rate of weight loss. However, a recent review (Loveridge, 1980) has addressed the difficulties of sealing insect spiracles. The consensus seems to be that effective sealing is impossible without concomitant damage to cuticle waterproofing.

It is important to note that decapitation will alter a variety of physiological processes, many of which may affect the rate of water loss from the animals. For example, the corpus cardiacum of *Periplaneta* contains 12 factors with physiological effects (Tobe & Stay, 1982). It might be difficult, therefore, to separate direct neuroendocrine effects upon integumentary water loss from more general effects of metabolism, heart rate, etc., which might indirectly affect respiratory water loss. Both Penzlin & Stolzner (1971) and Keeley (1975) have reported neuroendocrine effects on water loss that were attributed to diuretic effects. However, neither of these studies determined the contribution of spiracular water loss. We note also that a single exposure to CO_2 increases the activity of *Periplaneta* for several days (Ralph, 1959); such a response could differ in headless compared to normal animals.

We are also puzzled by a third aspect of the Treherne & Willmer study, namely their data on cuticle water content. Although, based on our present understanding (Machin & Lampert, 1985), the observed drop in water content after decapitation would be consistent with an increase in cuticle permeability, higher values at 35% relative humidity (RH) compared with 60% RH are difficult to understand. Moreover, their cuticle water contents are unduly high, compared with the data of other workers (Winston & Beament, 1969; Machin, Lampert & O'Donnell, 1985).

These doubts about the original Treherne & Willmer study lead us to re-examine some of the critical experiments on which this potentially important discovery is

based. Because of the controversy concerning the effectiveness of spiracle sealing, our examination of Treherne & Willmer's experiments will concentrate on the differences between normal and headless individuals, both with unblocked spiracles. The sources of variability in water loss rates of normal cockroaches, such as the effect of handling stress on increased respiratory water loss, will also be examined.

MATERIALS AND METHODS

Following the techniques of Treherne & Willmer (1975*b*) all experiments were performed using adult male cockroaches. They were taken from colonies maintained at 23–27 °C, 43–45 % RH and fed *ad libitum* on lab. chow and water.

Before experimentation, cockroaches were held overnight with water but no food (to reduce defecation). The handling procedure used by Treherne & Willmer to determine water loss was unclear and so the following techniques, having different stress levels, were tried.

Group 1. Caught with no special care, held individually in glass jars and removed to a cage for daily weighing (to 0·1 mg).

Group 2. Caught to minimize stress. Cockroaches were gently captured with large blunt forceps; animals which avoided capture were rejected. Cockroaches were kept in individual glass chambers (5 cm diameter×6 cm deep) closed with metal gauze lids, and were not removed for weighing.

Group 3. Taken after immobilization by chilling the culture to about 1 °C. Animals were removed as soon as they succumbed and normal mobility was regained following the operation in about 15 min. Held and weighed as in group 2.

Between weighings all experimental animals held at 20 °C and 40–45 % RH.

Treherne & Willmer (1975*b*) describe the decapitation procedure itself but give no other details of handling. We followed their technique as far as possible but found it necessary to restrain or chill the cockroaches for the operation. In groups 1 and 2, cockroaches were restrained in an inverted position on a glass plate with a strip of modelling clay. Care was taken not to move the animal while in contact with the glass plate. Some group 2 animals were sham-operated by restraining in this manner but stopping short of decapitation. The chilled animals in group 3 were decapitated without mechanical restraint.

A further series of *in vivo* pronotal permeability measurements was performed using a ventilated cup apparatus described in Machin *et al.* (1985). A water vapour-sensitive electrode (Panametrics 35501-PR moisture sensor, Shannon, Ireland) reading dew points from −80 °C to 20 °C was used to measure the vapour released from the pronotum. The method permitted air to be used as the carrying gas, but to detect dew point differences between the animal and control streams with sufficient precision, extremely dry air of dew point around −70 °C had to be used, direct from a pressurized cylinder. Unfortunately, equilibration of the air stream with the copper tubing of the apparatus took up to 24 h, thus preventing immediate permeability determinations. After stable values with normal cockroaches were reached, the animals were decapitated without removing them from the cup. For the purposes of

comparison, pronotal permeabilities were converted to different ambient humidities using an empirical equation from Machin *et al.* (1985). Roughly speaking, permeability varies inversely with the reciprocal of ambient vapour pressure lowering.

At the end of whole animal weighing experiments *in vitro* permeabilities were determined using pronotal discs, without removing the epidermis (Machin *et al.* 1985). Further animals were taken from the culture and used for both *in vivo* and *in vitro* pronotal water content determinations (Machin *et al.* 1985). Control and decapitated specimens were equilibrated to constant ambient conditions for 6 h before measurement. Water contents, which involve removing the epidermis, were measured after equilibration with a variety of ambient water activities (Machin *et al.* 1985).

Comparison of whole animal and pronotal water losses in differing humidities requires that they be expressed in terms of area and gradient specific flux or apparent permeability. For calculating unit area flux, we checked, then converted Edney & McFarlane's (1974) formula from nymphal to adult male surface area. For adults with wings folded we found it was necessary to increase the proportionality constant slightly, thus:

$$\text{Adult surface area } (\text{cm}^2) = 14{\cdot}5 \times \text{body mass } (\text{g})^{0{\cdot}63}.$$

The surface area of the head, 0·4 cm^2, was subtracted in decapitated individuals.

Without prejudicing the differences between normal and decapitated animals, the data of Treherne & Willmer were transformed in the same way by assuming a representative body mass for adult male *Periplaneta* of 0·9 g.

RESULTS

The ventilated cup experiments show no significant effect of decapitation on permeability (Table 1). Whole animal permeabilities of group 2 and 3 controls (Table 2) were less than *in vivo* pronotal values in Table 1, related to 0·43 ambient water activity. Errors in calculating whole animal surface area, regional permeability differences, respiratory (Kestler, 1985; Miller, 1982), anal and buccal losses (Coenen-Stass & Kloft, 1976; Edney, 1977, 1980; Hadley & Quinlan, 1982) and slight cuticle damage could all contribute to the small discrepancy.

Table 1. In vivo *pronotal permeabilities in normal and decapitated cockroaches measured by means of a ventilated cup apparatus*

	Ambient activity	N	Permeability ± S.E. ($\text{mg h}^{-1}\,\text{cm}^{-2}\,\text{mmHg}^{-1}$)	P	Estimated permeability at ambient activity 0·43
Normal	0·0001	5	0·00286 ± 0·00038	>0·9	0·00502
Decapitated	0·0001	5	0·00292 ± 0·00037		0·00512

Estimated permeabilities at 0·43 ambient activity (referred to in the text) are also given.

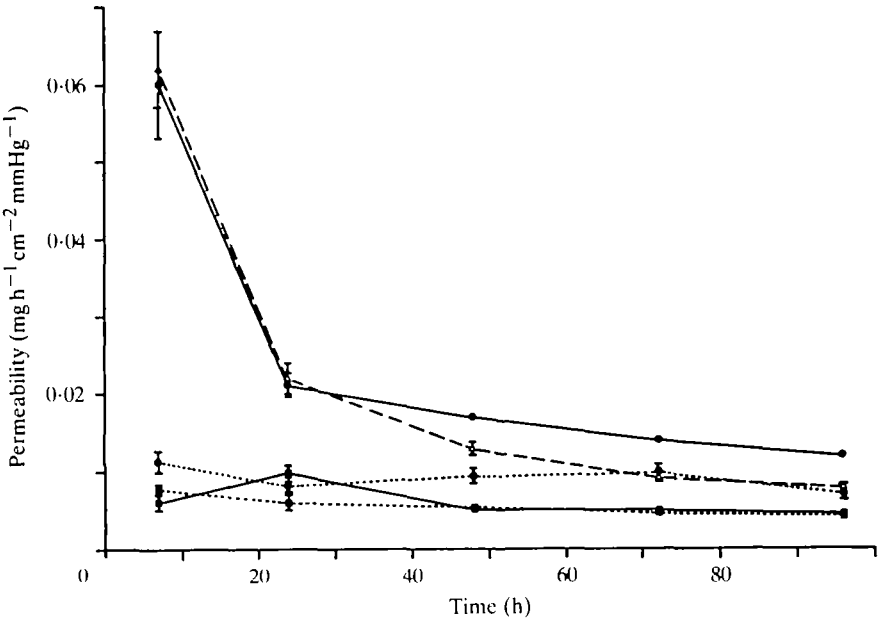

Fig. 1. Graph showing the apparent permeability with time with different handling procedures. Group 2 in Materials and Methods (not removed for weighing): (■——■) control, (△---△) sham operated, (●——●) decapitated. Group 3 in Materials and Methods (chilled): (■-----■) control, (●-----●) decapitated.

It is clear from Fig. 1 and Table 2 that the amount of handling associated with capture and or decapitation can have a profound effect on water loss as well as the rate at which the animal recovers. For example, animals captured and operated after anaesthesia by chilling show practically no handling effect whereas even carefully caught unchilled specimens initially lost mass six times as fast. Furthermore, animals caught and transferred daily for weighing (Table 2) show greater weight losses than animals which were not removed from their containers. Animals from the latter group all showed a progressive decline in the rate of weight loss (Fig. 1) in the series; normal, sham-operated and decapitated. This decline had clearly not been completed by 96 h in the more slowly recovering sham-operated and decapitated animals.

It is also apparent (Table 2) from the experiments involving chilled animals that decapitation has a lasting elevating effect on whole animal weight loss with little evidence of adjustment. In experiments where the handling effects were small or where there was sufficient time for recovery, control permeabilities are relatively low, with the permeabilities of decapitated and sham-operated animals nearly twice as high or greater. With the more stressful weighing technique (group 1), control permeabilities exceed the decapitated values for groups 1 and 2. Moreover, the differential between control and operated animals disappears or even reverses. Rather than confirming the results of the ventilated cup experiments, pronotal permeabilities *in vitro* (Table 2) all exceeded corresponding whole animal values with a pattern of variability suggesting cuticle damage. Mean permeabilities calculated from Treherne & Willmer's data are intermediate between groups 2 and 3 with a lower decapitated/control ratio.

Table 2. *Final whole animal permeabilities obtained in time course experiments compared with* in vitro *pronotal permeabilities taken from the same animals*

Experimental protocol	Whole animal permeability *in vivo* ($mg\,h^{-1}\,cm^{-2}\,mmHg^{-1}$)	N	P	Decapitated/ normal	Protonal permeability *in vitro* ($mg\,h^{-1}\,cm^{-2}\,mmHg^{-1}$)	N	*In vitro/ in vivo*
Group 1. Removed for weighing							
Normal	0·0165 ± 0·0014	6					
Decapitated	0·0190 ± 0·0012	10	>0·2	1·15	0·0246 ± 0·0033	9	1·29
Group 2. Not removed for weighing							
Normal	0·0042 ± 0·0003	6			0·0180 ± 0·0040	6	4·29
Decapitated	0·0120 ± 0·0005	6	<0·001	2·86	0·0045 ± 0·0060	12	3·75
Sham-operated	0·0077 ± 0·0003	6	<0·001	1·83	0·0460 ± 0·0100	6	5·97
Group 3. Chilled							
Normal	0·0038 ± 0·0004	6			0·0508 ± 0·0086	6	13·37
Decapitated	0·0074 ± 0·0005	6	<0·010	1·95	0·0189 ± 0·0016	6	2·55
Data of Treherne & Willmer (1975*b*)							
Normal	0·0095	6					
Decapitated	0·0120	4		1·26			

Temperature 20°C, ambient water activity 0·40–0·45.

In vivo and *in vitro* water contents obtained by this study (Table 3) are lower than those of Treherne & Willmer and show the qualitative trends of Machin & Lampert's (1985) model in declining with ambient activity. Differences between water contents from control and decapitated animals *in vivo* were not significant in two of three ambient water activities. The reason for the difference in the third is unknown. Significant differences between the two groups *in vitro* may reflect further cuticle damage connected with decapitation. Differences between *in vivo* and *in vitro* water contents already reported in Machin *et al.* (1985) are significant and may reflect the cuticle damage suggested above.

DISCUSSION

On the basis of three types of permeability determination and of cuticular water content, we question Treherne & Willmer's suggestion that cuticular permeability in *Periplaneta* is controlled by hormones from the head.

First, *in vivo* measurements of pronotal permeability by the ventilated cup method show no effect of decapitation. Importantly, these measurements were based on a single cuticular plate, with no risk of interference from other pathways of water loss. Furthermore, if attaching the cup resulted in cuticle damage, there is no reason why it would be different for decapitated and normal animals since the cockroaches were first immobilized by chilling as in group 3. Secondly, although permeability increased after decapitation in groups 2 and 3, sham operations also had the same effect. Many more factors than had been realized evidently contribute to weight loss, because permeabilities were higher still in both normal and decapitated animals subjected to the stress associated with initial capture and periodic weighing (group 1). Thirdly, pronotal permeabilities of decapitated animals, measured *in vitro*,

Table 3. *Pronotum water content*

Ambient activity	Water content (% dry weight ± S.E.)				Normal and decapitated compared
	N	Normal	*N*	Decapitated	*P*
Present study					
In vivo					
0·850	18	35·7 ± 1·1	12	37·3 ± 1·7	>0·300
0·560	17	35·7 ± 1·4	10	33·3 ± 2·3	>0·300
0·046	18	28·1 ± 1·2	12	36·0 ± 1·5	<0·001
In vitro					
0·850	18	30·7 ± 0·8	12	27·9 ± 1·3	<0·050
0·560	17	29·5 ± 1·2	10	25·3 ± 1·4	<0·050
0·046	18	22·0 ± 0·8	12	28·5 ± 0·7	<0·001
Treherne & Willmer (1975*b*)					
0·600	10	133·0	9	61·0	
0·350	11	104·7	9	80·7	

in groups 2 and 3, were, in fact, less than in the controls. With the greater degree of handling involved in removing cuticle samples for study, cuticle damage seems more likely. We suggest that decapitated animals may not be damaged as much because they are more quiescent and easier to handle.

Finally, in only one experiment was there any evidence for an effect of decapitation upon cuticular water content. We believe that water content values in Treherne & Willmer (1975*b*) were higher than those in Winston & Beament (1969) and Machin *et al.* (1985) probably because of the influence of hydrated tissue within marginal folds of the pronotum. The use of a circular punch, to obtain a central disc from the pronotum, eliminates this source of error.

Our data indicate that weight loss in *Periplaneta* is extremely sensitive to slightly different types of experimental treatment. There seems, in fact, to be a clear correlation between both the elevation of the initial weight loss and the rate of its subsequent decline with the amount of stress involved in capture, transfer and experimental treatment. Elevated metabolic rate and increased spiracular water loss are known to result from stress in normal cockroaches (Kestler, 1985) but as yet the effects of decapitation cannot be predicted. The reduced locomotory activity and spiracular frequency described by Treherne & Willmer could lead to less water loss. Alternatively, the effectiveness of the spiracular water conservation mechanism might be impaired.

The sensitivity of the waterproof layer to damage is frequently emphasized in the literature dealing with cuticle permeability measurement (Richards, 1951; Beament, 1961; Edney, 1977; Loveridge,1980) and it appears that the cuticle may only require surface contact for damage to occur. It may be significant that the highest *in vitro* permeabilities are very similar to those observed initially in group 2 decapitated and sham-operated animals (Fig. 1). The fact that healthy insects normally restore the original waterproof characteristics of the cuticle (Wigglesworth, 1945) and that the effectiveness of the repair process might vary with the extent of the damage is entirely consistent with the early pattern of weight loss seen in most experimental groups.

With the exception of group 2 decapitated cockroaches, reasonably stable permeabilities are reached by at least 96 h, with sham-operated and decapitated groups being more permeable than the controls. The difference between the two permeabilities is consistent in both studies, amounting to an increased water loss of about $0{\cdot}4\,mg\,h^{-1}\,animal^{-1}$. Since decapitated and sham-operated cockroaches, but not the controls, were inverted for operation, there is a basis for the permeability differences referred to above. It may also be significant that the only truly stable permeabilities towards the end of our experiments are seen in normal animals, whereas all operated groups show evidence of at least slight decline in permeability. If the cuticles of the experimentally manipulated groups are indeed damaged, it would be expected that the permeability at least of sham-operated animals would continue to decline to the control level. It is not known if the loss of the head would interfere with the ability to repair cuticle damage.

It is important to note that we have not repeated all of the types of control experiment which supported the original hypothesis of hormone control of cuticle

permeability. Treherne & Willmer (1975*b*) found that severing the neck connectives did not increase water loss. Although this procedure might be expected to result in cuticle damage, a simultaneous drop in metabolism and associated spiracular water loss might compensate for this. Similarly, the decrease in weight loss rate in decapitated animals after injection of extract of the brain or corpus cardiacum could be explained by a depression of metabolism. Treherne & Willmer rule out respiratory water loss as the reason for the decapitation effect because blocking the spiracles had no effect and decapitation depressed their frequency of opening. Our explanation for these results is that the blocking may be incomplete; in fact, opener muscles may break the seal as soon as the molten wax has solidified. On the other hand, there is reason for believing that sealing the neck is successful, because it is first mechanically sealed with thread and the wax is applied to both the thread and the wound. Again cuticle damage is a possibility and would add to the 'decapitation effect'.

In conclusion, we suggest that cuticle damage may account for our results as well as providing a plausible alternative explanation for many of the observations of Treherne & Willmer. In general support of this claim, we point out that *in vivo* permeabilities obtained in this study are at least 4·6 times lower than any comparable value for *Periplaneta* at 20°C, reported in the literature ($0{\cdot}027\,mg\,h^{-1}\,cm^{-2}\,mmHg^{-1}$, Mead-Briggs, 1956; $0{\cdot}013\,mg\,h^{-1}\,cm^{-2}\,mmHg^{-1}$, Edney & McFarlane, 1974), values which were obtained from dead animals with 'sealed' spiracles. Although we have no confirmatory evidence at present, we think it possible that changes in spiracular water loss may also be a contributory factor. Our work has shown that *Periplaneta* is exquisitely sensitive to any form of handling and emphasizes the extraordinary difficulty in obtaining accurate, representative integumental water permeabilities.

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REFERENCES

ATKINSON, P. W. & GILBY, A. R. (1970). Autoxidation of insect lipids: inhibition on the cuticle of the American cockroach. *Science* **168**, 992.

BEAMENT, J. W. L. (1961). The water relations of insect cuticle. *Biol. Rev.* **36**, 281–230.

BEAMENT, J. W. L. (1961). The ecology of cuticle. In *The Insect Integument* (ed. H. R. Hepburn), pp. 359–374. New York: Elsevier.

COENEN-STASS, D. & KLOFT, W. (1976). Transpirationmessungen an den beiden schabenarten *Periplaneta americana* und *Blaberus trapezoideus*. *J. Insect Physiol.* **22**, 945–950.

EDNEY, E. B. (1977). *Water Balance in Land Arthropods*. New York, Heidelberg, Berlin: Springer-Verlag.

EDNEY, E. B. (1980). The components of water balance. In *Insect Biology in the Future* (ed. M. Locke & D. S. Smith), pp. 39–58. New York: Academic Press.

EDNEY, E. B. & MCFARLANE, J. (1974). The effect of temperature on transpiration in the desert cockroach, *Arenivaga investigata,* and in *Periplaneta americana*. *Physiol. Zool.* **47**, 1–12.

GILBY, A. R. (1980). Transpiration, temperature and lipids in insect cuticle. *Adv. Insect Physiol.* **15**, 1–33.

HADLEY, N. F. & QUINLAN, M. (1982). Simultaneous measurement of water loss and carbon dioxide production in the cricket, *Acheta domesticus*. *J. exp. Biol.* **101**, 343–346.

KEELEY, L. L. (1975). Neuroendocrine deficiency effects in trophic metabolism and water balance in the cockroach *Blaberus discoidalis*. *J. Insect Physiol.* **21**, 501–510.

KESTLER, P. (1985). Respiration and respiratory water loss. In *Environmental Physiology and Biochemistry of Insects* (ed. K. H. Hoffmann), pp. 137–183. Berlin: Springer-Verlag.

LOVERIDGE, J. P. (1980). Cuticle water relations techniques. In *Cuticle Techniques in Arthropods* (ed. T. A. Miller), pp. 301–366. New York: Springer-Verlag.

MACHIN, J. (1980). Cuticle water relations; towards a new cuticle waterproofing model. In *Insect Biology in the Future* (ed. M. Locke & D. S. Smith), pp. 79–103. New York: Academic Press.

MACHIN, J. & LAMPERT, G. J. (1985). A passive two layer permeability-water content model for *Periplaneta* cuticle. *J. exp. Biol.* **117**, 171–179.

MACHIN, J., LAMPERT, G. J. & O'DONNELL, M. J. (1985). Component permeabilities and water contents in *Periplaneta* integument: role of the epidermis re-examined. *J. exp. Biol.* **117**, 155–169.

MADDRELL, S. H. P. (1980). The control of water relations in insects. In *Insect Biology in the Future* (ed. M. Locke & D. S. Smith), pp. 179–199. New York: Academic Press.

MEAD-BRIGGS, A. R. (1956). The effect of temperature upon the permeability to water of arthropod cuticles. *J. exp. Biol.* **33**, 737–750.

MILLER, P. L. (1982). Respiration. In *The American Cockroach* (ed. W. J. Bell & K. G. Adiyodi), pp. 87–116. London: Chapman & Hall.

MULLINS, D. E. (1982). Osmoregulation and excretion. In *The American Cockroach* (ed. W. J. Bell & K. G. Adiyodi), pp. 117–149. London: Chapman & Hall.

PENZLIN, H. & STOLZNER, W. (1971). Frontal ganglion and water balance in *Periplaneta americana* L. *Experientia* **27**, 390–391.

RALPH, C. L. (1959). Modifications of activity rhythm of *Periplaneta americana* (L.) induced by carbon dioxide and nitrogen. *Physiol. Zool.* **32**, 57–62.

RICHARDS, A. G. (1951). *The Integument of Arthropods*. Minneapolis: University of Minnesota Press.

TOBE, S. S. & STAY, B. (1982). Neurosecretion and neurohormones. In *The American Cockroach* (ed. W. J. Bell & K. G. Adiyodi), pp. 305–342. London: Chapman & Hall.

TREHERNE, J. E. & WILLMER, P. G. (1975*a*). Evidence for hormonal control of integumentary water loss in cockroaches. *Nature, Lond.* **254**D, 437–439.

TREHERNE, J. E. & WILLMER, P. G. (1975*b*). Hormonal control of integumentary water loss: evidence for a novel neuroendocrine system in an insect (*Periplaneta americana*). *J. exp. Biol.* **63**, 143–159.

WILLMER, P. G. (1982). Microclimate and the environmental physiology of insects. *Adv. Insect. Physiol.* **16**, 1–57.

WIGGLESWORTH, V. B. (1945). Transpiration through the cuticle of insects. *J. exp. Biol.* **21**, 97–113.

WINSTON, P. W. & BEAMENT, J. W. L. (1969). An active reduction of water level in insect cuticle. *J. exp. Biol.* **50**, 541–546.