J. exp. Biol. (1975), 63, 143–159
With 10 figures
Printed in Great Britain

HORMONAL CONTROL OF INTEGUMENTARY WATER-LOSS: EVIDENCE FOR A NOVEL NEUROENDOCRINE SYSTEM IN AN INSECT (*PERIPLANETA AMERICANA*

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(*Received* 9 *January* 1975)

SUMMARY

An accelerated water-loss was observed in decapitated individuals, no equivalent increase being obtained following severance of the nervous connectives in the neck. Injection of brain and, to a lesser extent, corpus cardiacum extract resulted in a significant reduction in the rate of loss of water from decapitated individuals. The accelerated water-loss observed following decapitation appeared not to result from significant increase in excretory output or loss of water through the spiracles. It is suggested that integumentary transpiration may be affected by a blood-borne factor, or factors, which originate in the brain and corpus cardiacum.

INTRODUCTION

The waterproofing of the integument of insects and arachnids is well known to result from the highly impermeable epicuticular lipid layer (cf. Beament, 1964) and, possibly, from permeability barriers associated with the membranes of the underlying epidermal cells (Berridge, 1970). The available evidence also indicates that regulation of integumentary water-loss can occur, being manifested, for example, by the decrease in the relative rates of water-loss at low humidities (Bursell, 1955; Loveridge, 1968). While this effect may well result from the closer packing of epicuticular lipid molecules under dry conditions (cf. Beament, 1964) it is also conceivable that the regulation of integumentary transpiration may be achieved by more complex mechanisms, perhaps involving regulation of the permeability of the epidermal cell membranes (Berridge, 1970).

The present investigation describes the results of experiments which suggest that the rate of integumentary water-loss in the cockroach may be under neuroendocrine control.

MATERIALS AND METHODS

Adult male cockroaches (*Periplaneta americana*) were used in this investigation. They were maintained in large tanks under normal daylight conditions at a room temperature of 27–29 °C. Water was freely available. The cockroaches were fed on a balanced standard diet (cf. Treherne, Buchan & Bennett, 1975).

All experiments were carried out at room temperature (18–20 °C) at ambient humidity, recorded for each set of experiments, unless otherwise stated. Insects maintained at low relative humidities were kept in large glass desiccators over dry $CaCl_2$. Relative humidities were measured using Edney paper hygrometers. Control and operated insects were kept individually in small glass pots, with gauze covers, for the duration of the experiments. Individual insects were weighed to an accuracy of 0·5 mg.

Decapitated animals were prepared by severing the neck, above a cotton thread ligature, and sealing with beeswax-resin mixture or with 'Newskin'. To sever the nervous connectives in the neck a loose, temporary, ligature was applied to the neck to restrict bleeding during the subsequent operation. A ventral incision was then made, the exposed connectives cut and the wound re-sealed with 'Newskin' or beeswax-resin mixture. Extracts of whole brain, of the corpus cardiacum and the corpus allatum were made by homogenizing the organs in 50 μl of physiological saline which contained: NaCl, 208·6 mM; KCl, 3·1 mM; $CaCl_2$, 5·4 mM; $NaHCO_2$, 2·0 mM (Callec and Sattelle, 1973). The 50 μl volumes of extracts were injected into the animals through a loose neck ligature, to minimize fluid loss. They were then decapitated as described above.

The activity of the thoracic spiracles was observed by placing insects on a mirror, under a glass cover, and viewing the reflexion with a Wild (M.5) binocular microscope. The general level of activity of groups of ten cockroaches was measured using an ultrasonic movement detector (P. B. Buchan, unpublished observations). Observations were carried out on normal and decapitated individuals over periods of from 3 to 5 days.

Blocking of the spiracles and the anus was carried out using beeswax-resin mixture, Eastman compound and 'Newskin'. The latter compound was found to be most satisfactory, in having no obvious side effects, and was used routinely in these experiments. Operated individuals were inspected at the end of the experiment to ensure that the spiracles were effectively blocked.

The specific resistance of the integument was measured using the method described by Scheie (1970). With this method an isolated pronotum was floated on a bath of 0·1 M-NaCl, the resistance of the integument being measured, between the bath and drops of 1·0 or 5·0 M-NaCl placed on the epicuticular surface, using a Keithley electrometer.

The rate of synthesis and incorporation of epicuticular lipids was measured using sodium ^{14}C-acetate according to the method of Diehl (1973). In these experiments 0·5 μCi of sodium ^{14}C-acetate (58 mCi/mM), in 50 μl physiological saline, was injected into the haemolymph. Triton X and toluene solvent (2 to 1 mixture) was used as the lipid solvent and, with the addition of 0·6 g POPOP per 3 l, as the scintillation fluid. The water content of the cuticle was measured by the method of Winston & Beament (1969) using the whole pronotum from each insect.

Potassium-induced extraneural potentials and compound action potentials were measured in intact penultimate connectives of the abdominal nerve cord using the mannitol-gap technique (Pichon & Treherne, 1970). The apparatus and techniques were as described by Pichon, Moreton & Treherne (1971).

RESULTS

(*a*) *Water-loss in normal and operated individuals*

Decapitation was found to cause an accelerated loss of weight in animals maintained at relative humidities of 54–55% (Fig. 1). This effect did not appear to result from the lack of direct nervous connexion with the brain, for accelerated weight-loss was not observed in individuals in which the neck connectives had been severed. This observation, plus the fact that the ligatured area was covered with 'Newskin' or wax, also suggests that increased water-loss did not arise from local 'wounding' effects in the neck region. It can be reasonably proposed, therefore, that the rapid weight-loss observed in decapitated insects could have resulted from the loss of a blood-borne factor which originates in the brain and which, in some way, affects the rate of loss of water from the body.

(*b*) *Injection of brain extract*

Injection of homogenized tissues (1 brain + 50 μl saline) produced a dramatic reduction in the apparent rate of water-loss in decapitated individuals, the decline in weight being strikingly similar to that recorded for normal insects (Fig. 2). As can be seen from Fig. 2 injection of saline into the haemolymph of decapitated individuals produced little effect, the decline in weight being not significantly different from that observed with untreated decapitated insects.

Injection of brain-extract 24 h prior to the application of a neck ligature produced no reduction in the apparent rate of water-loss (Fig. 3) such as was observed following injection of brain-extract into decapitated individuals (Fig. 2).

(*c*) *Injection of corpus cardiacum and corpus allatum extracts*

Injection of homogenized corpora allata (2 c.a. in 50 μl saline) produced no significant effect on the apparent rate of water-loss in decapitated individuals (Fig. 4). Injection of corpus cardiacum extract, on the other hand, resulted in an appreciable reduction, the rate of decrease in weight being significantly slower than that observed with untreated decapitated insects. The corpus cardiacum extract appeared to be less effective than that of the brain (Fig. 2) in reducing the apparent rate of water-loss from decapitated individuals.

(*d*) *Effects of decapitation on excretory water-loss*

The water-loss induced by decapitation appeared not to result from any appreciable increase in the rate of elimination of water by the excretory system. This conclusion is based on the observation that blocking of the anus (with either a beeswax-resin mixture, Eastman compound or 'Newskin') did not affect the rapid decline in weight of decapitated individuals maintained at 48–50% R.H. (Fig. 5).

(*e*) *Effects of decapitation on spiracular water-loss*

Decapitation was found to drastically reduce spiracular activity observed with a binocular microscope, the first two thoracic spiracles being seen to remain in an apparently closed position for very much longer periods than with normal insects, during quiescence and locomotory activity (Fig. 6). Locomotory activity was also

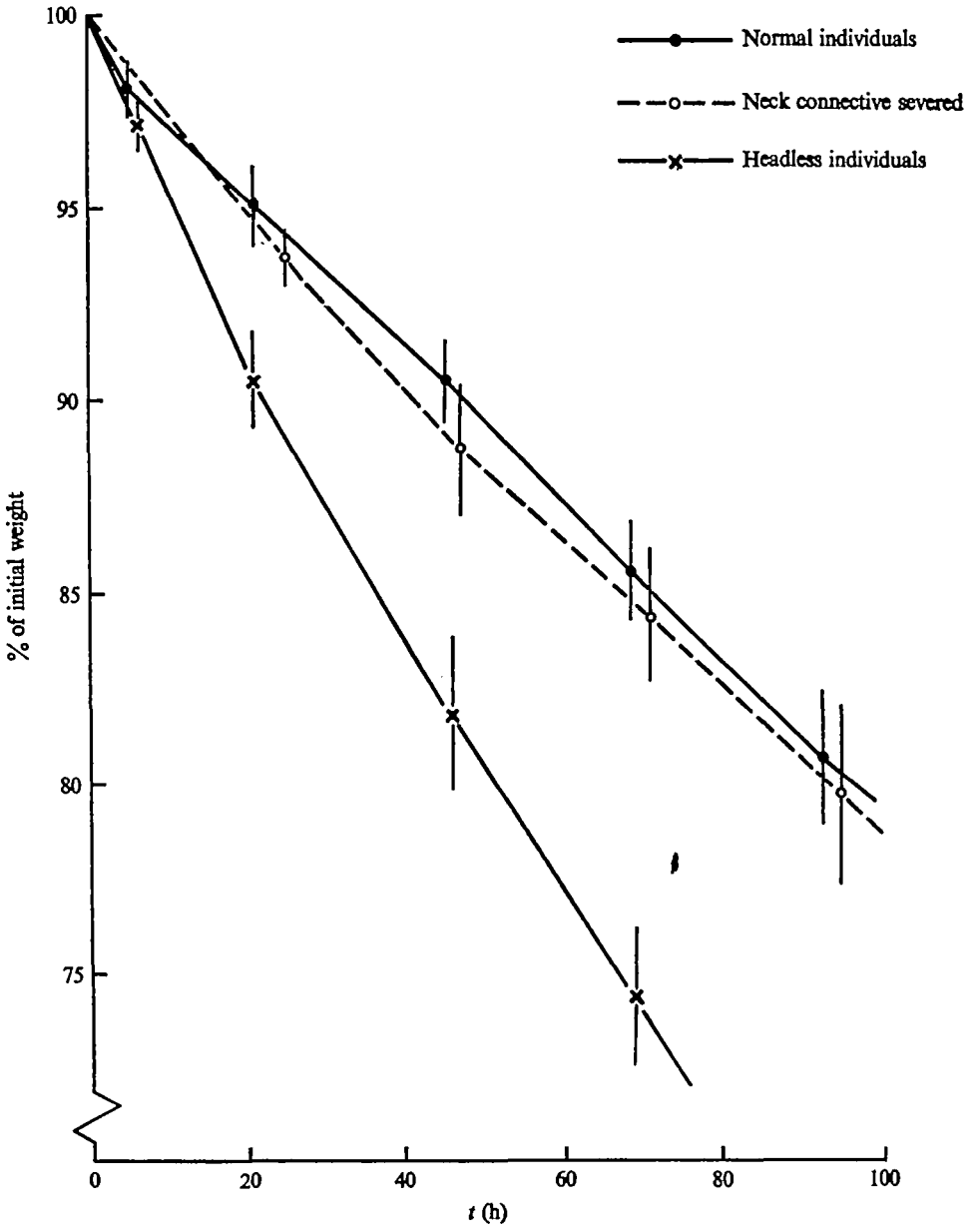

Fig. 1. Effects of decapitation and severance of the neck connective on the decline in weight of individuals maintained at 54–55% R.H. In this, and subsequent diagrams, the symbols indicate the mean of measurements made on ten individuals, the vertical lines indicating the extent of twice the standard error of the mean.

substantially reduced in decapitated individuals as compared with unoperated ones (Fig. 7). The percentage spiracular opening for normal individuals ($n = 15$) was: $0{\cdot}46 \pm 0{\cdot}22\%$ and $6{\cdot}96 \pm 2{\cdot}08\%$ for the first and second thoracic spiracles respectively. The equivalent values for decapitated individuals were: $0{\cdot}046 \pm 0{\cdot}04\%$ and $1{\cdot}61 \pm 1{\cdot}20\%$.

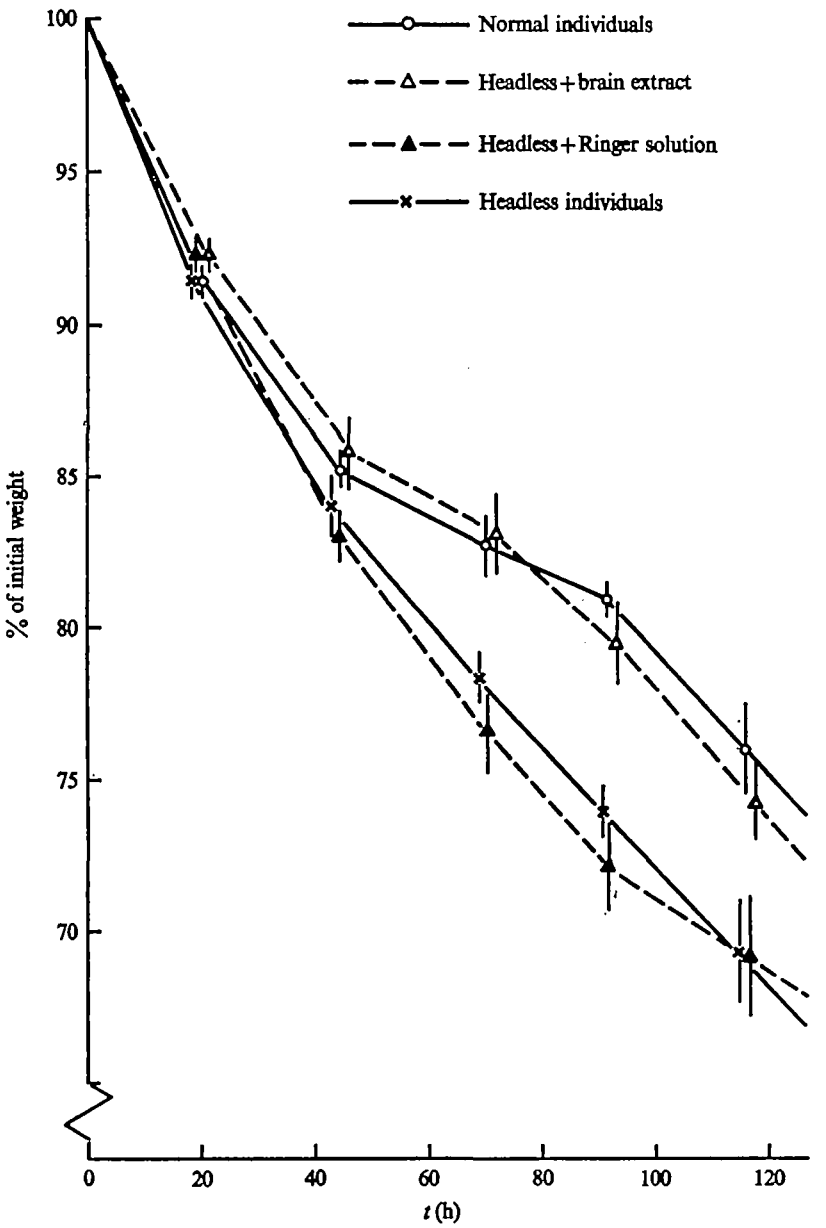

Fig. 2. The effects of injection of brain extract (1 brain + 50 μl extract) on the decline in weight of decapitated insects compared with that of normal and control individuals maintained at 48–50% R.H.

In the presence of an atmosphere containing 5% CO_2 the decline in weight of decapitated individuals (at 50% R.H.) was still observed to occur significantly more rapidly than with normal insects (Fig. 8). The thoracic spiracles of decapitated individuals, as well as those of normal ones, were observed to be held in the open position during the period of hypercapnia.

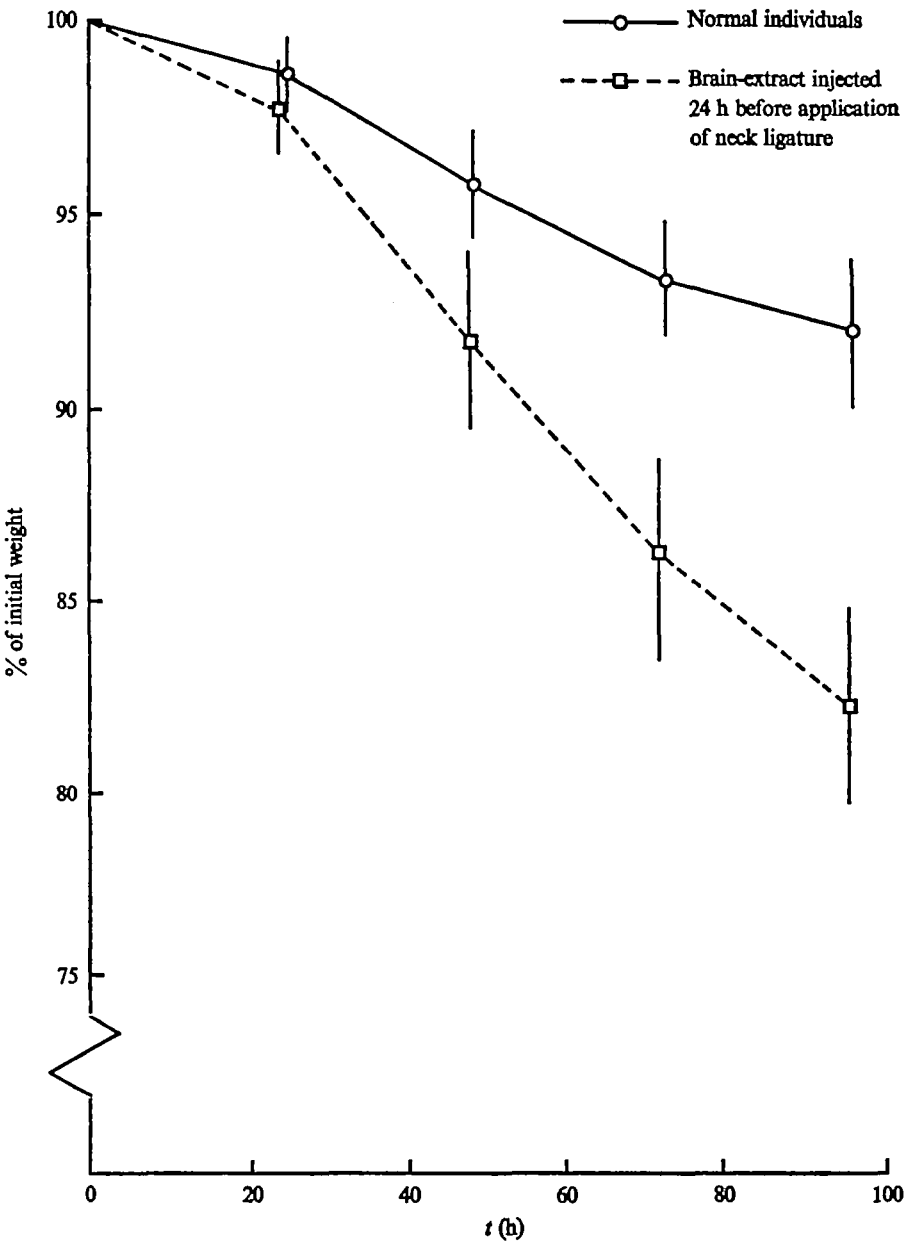

Fig. 3. The effect of the application of a neck ligature 24 h after the injection of brain extract (1 brain + 50 μl saline) on the decline in weight at 48–50% R.H.

The above results indicate that the increased water-loss induced by decapitation is unlikely to have resulted from a substantial increase in spiracular opening. It is also relevant to note that the rate of apparent water-loss recorded with blocked spiracles did not differ from that observed with untreated decapitated individuals (Fig. 9). It is conceivable, however, that the variation obtained for decapitated individuals with abdominal spiracles blocked might have concealed a real difference. In this case it

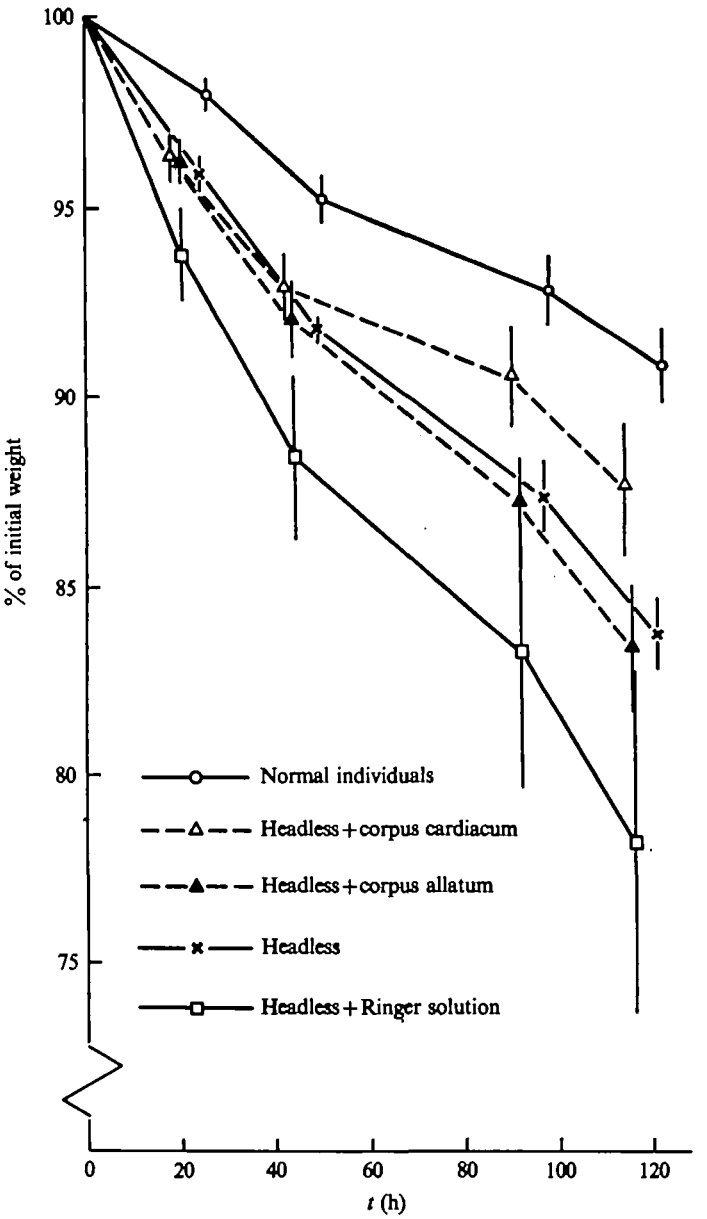

Fig. 4. Effects of injection of corpus cardiacum and corpus allatum extracts (2 organs + 50 μl saline) on the decline in weight of decapitated individuals at 48–50% R.H.

would be necessary to suppose that a proportion of the increased water-loss from decapitated individuals occurred through the tracheal surfaces.

(*f*) *Measurement of cuticle resistance*

The resistance of pronotal cuticle taken from decapitated individuals was found not to differ significantly from that taken from normal ones. The resistances of

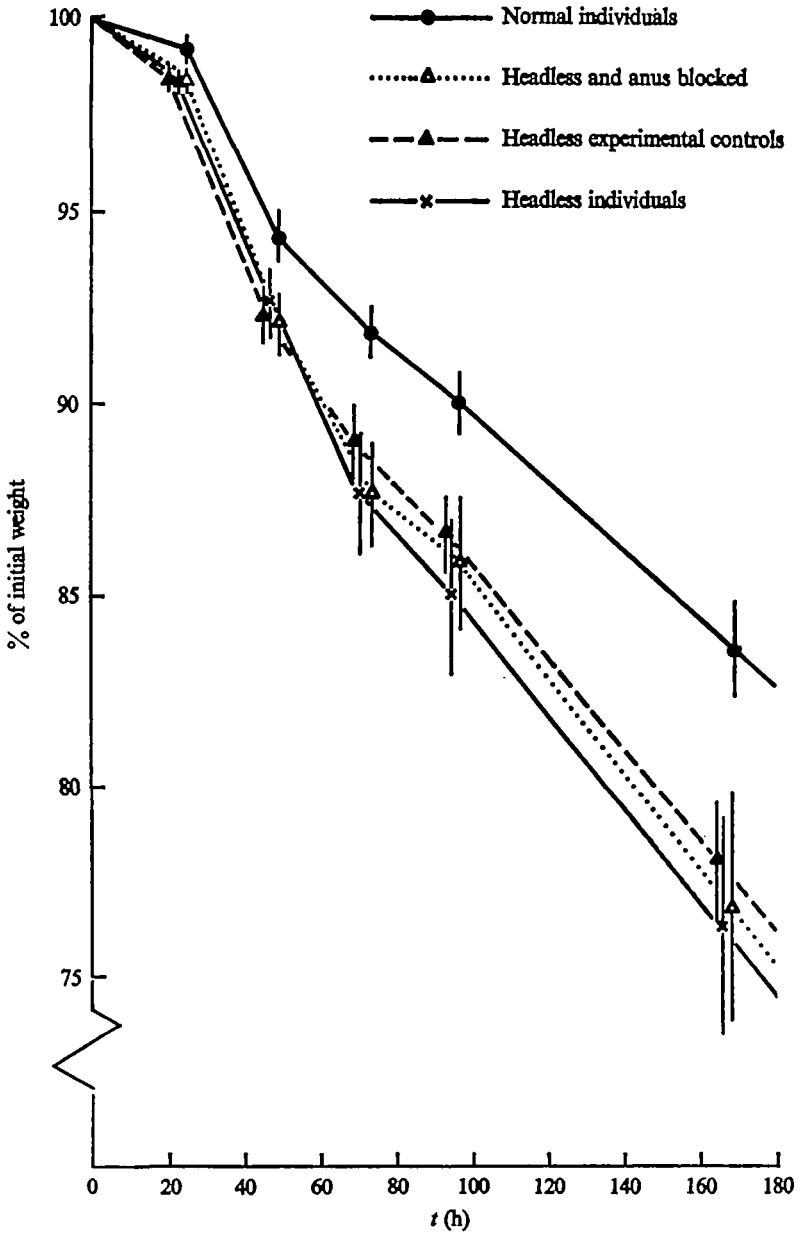

Fig. 5. Effects of blocking of the anus, with 'Newskin', on the decline in weight of decapitated individuals. The experimental controls were headless individuals in which 'Newskin' was placed adjacent to the anus, but without blocking it. Experiments carried out at 48–50% R.H.

pronotal cuticle taken from individuals 70–162 hr after decapitation averaged $3571 \pm 1324\,\Omega\,cm^2$ as compared with the value of $3661 \pm 1020\,\Omega\,cm^2$ for normal insects.

(*g*) *Incorporation of* ^{14}C*-acetate into cuticular lipids*

It seemed possible that the increased cuticular water-loss observed in decapitated insects could result from interference with lipid metabolism which thus affected the

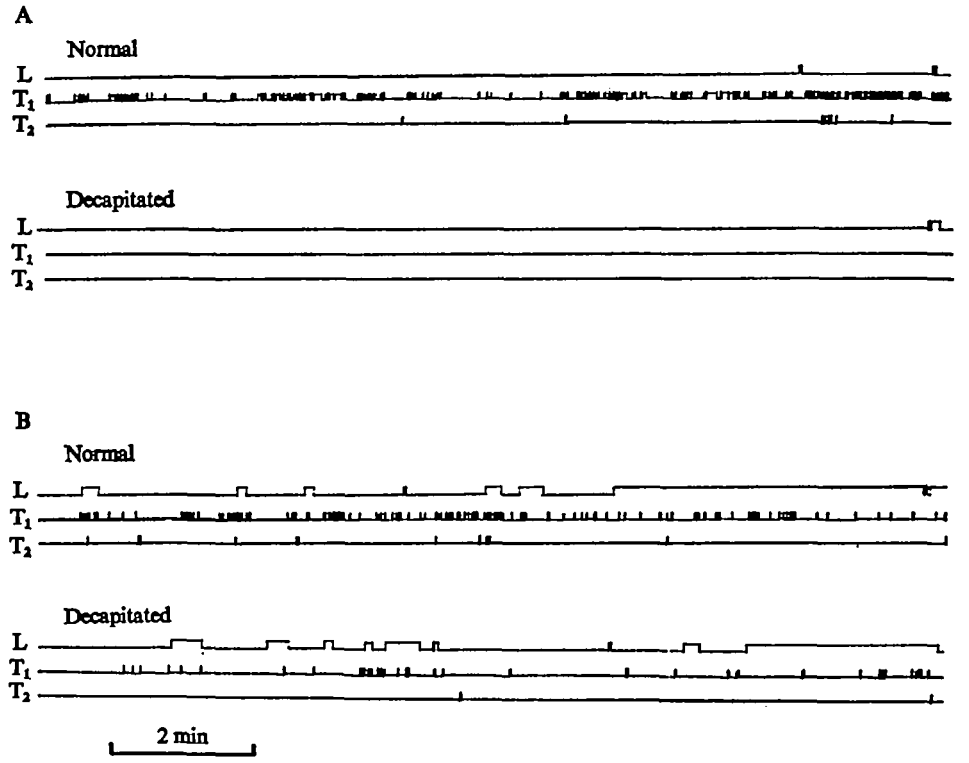

Fig. 6. The effects of decapitation on the opening of the thoracic spiracles (T_1 and T_2) in quiescent individuals (A) and those showing locomotory activity (B). Spiracular opening and locomotory activity (L) are indicated by upward deflexion of the traces.

synthesis and deposition of cuticular lipids. To test this possibility the technique of Diehl (1973) was used to measure the rate of incorporation of radioactivity into cuticle lipids following injection of ^{14}C-acetate into the haemolymph. The data showed that a significant proportion of the radioactivity washed from the cuticular surfaces was also contaminated by that derived from the anus (Table 1). To eliminate this contamination experiments were performed on cockroaches in which the anuses were blocked with 'Newskin'. In these individuals it was found that decapitation produced no significant reduction in the apparent rate of incorporation of radioactivity into the epicuticular lipids (Table 1).

(*h*) *Effects of decapitation on the permeability of the perineurium*

It is conceivable that the absence of a blood-borne factor in decapitated insects could produce unspecific effects on cellular function. Such unspecific effects could change the permeability properties of the epidermal layer of the integument and, thus, increase integumentary transpiration. It could be, for example, that intercellular diffusion is increased due to changes in the lateral junctional complexes of the epidermal cells.

To test the above possibility the effects of decapitation on permeability properties of another relatively impermeable cellular layer was measured. The cellular layer chosen

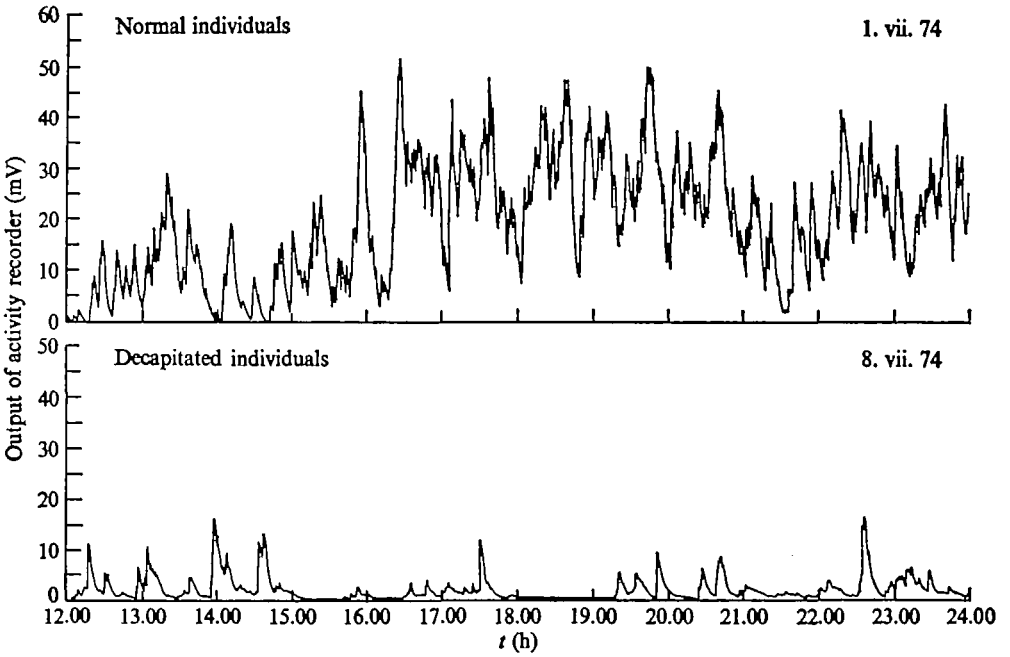

Fig. 7. Activity of normal and decapitated individuals, during 12 h periods, measured with an ultrasonic movement detector.

for these experiments was that surrounding the central nervous system; the perineurium, which is known to confer the properties of the insect blood–brain barrier (cf. Treherne, 1974). As in normal preparations it was found that exposure of intact nervous connectives, from decapitated individuals, to high-potassium saline resulted in relatively large extra-neuronal potentials with no detectable decline in the amplitude of the recorded action potentials (Fig. 10). It is concluded, therefore, that intercellular access to the axon surface, which is thought to result from junctional complexes at the inner margin of the perineurial clefts (cf. Lane & Treherne, 1972), was not significantly affected by decapitation. The magnitude of the extra-neuronal responses to elevated potassium saline also suggests that the permeability properties of the outwardly-directed perineurial surfaces was unaffected by the absence of the blood-borne factor in decapitated insects. These experiments do not, therefore, provide evidence for appreciable unspecific effects on epithelial permeability.

(*j*) *Measurements of cuticular water content*

The results summarized in Table 2 indicate that the water content of the cuticle in normal insects was lower in those maintained at 35% than at 60% relative humidity. At both humidities decapitation resulted in a significant decline in the water content of the cuticle. The decline induced by decapitation was, however, proportionally greater at the higher humidity.

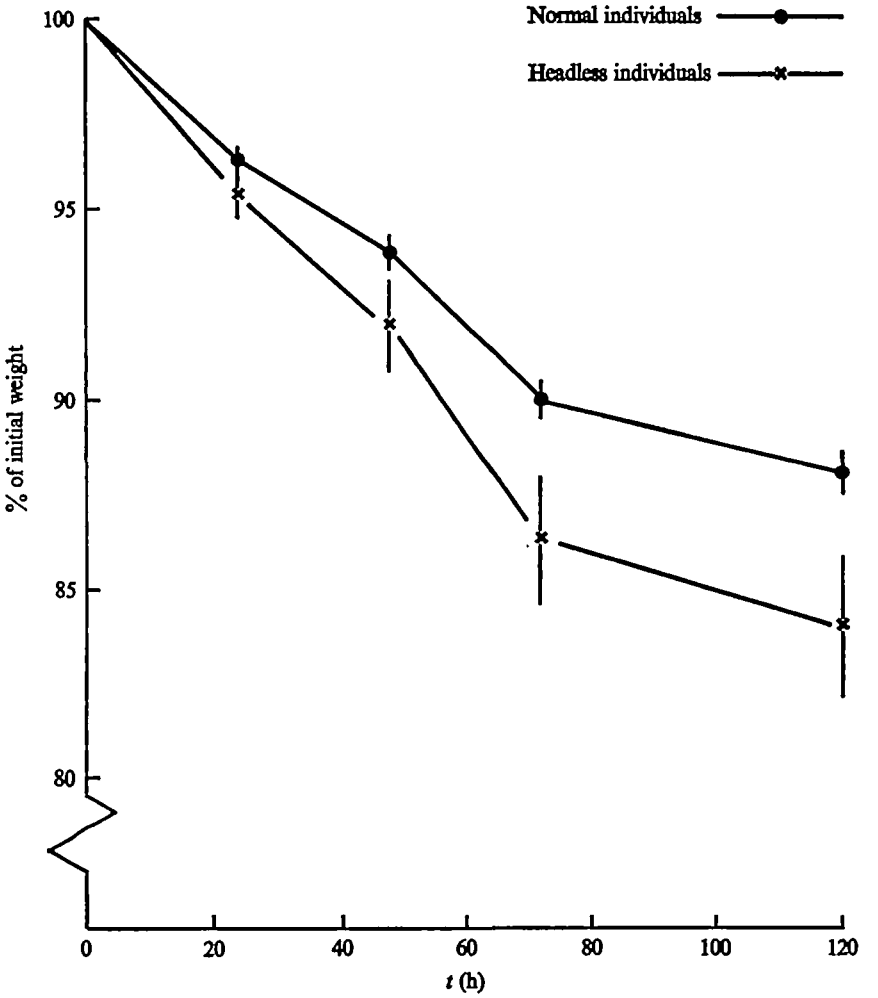

Fig. 8. Decline in weight of normal and decapitated individuals in the presence of 5 % CO_2 at 50 % R.H.

DISCUSSION

The accelerated water-loss, induced by decapitation, appears not to result from a significant increase in excretory output or from a more rapid escape of water molecules due to increased spiracular opening. It can be reasonably concluded, therefore, that decapitation induces a significant increase in integumentary transpiration.

The increased transpiration, resulting from decapitation, appeared not to result from severance of nervous connexion with the brain, for no significant differences were observed in the rate of water-loss from normal insects and those in which the neck connectives had been cut. These observations can, however, be conveniently explained by the assumption that the rate of integumentary water-loss is profoundly affected by a blood-borne factor, or factors, which originate in the head. This postulation is further supported by the observation that the injection of brain extract dramatically reduces the rate of loss of water from decapitated individuals. An

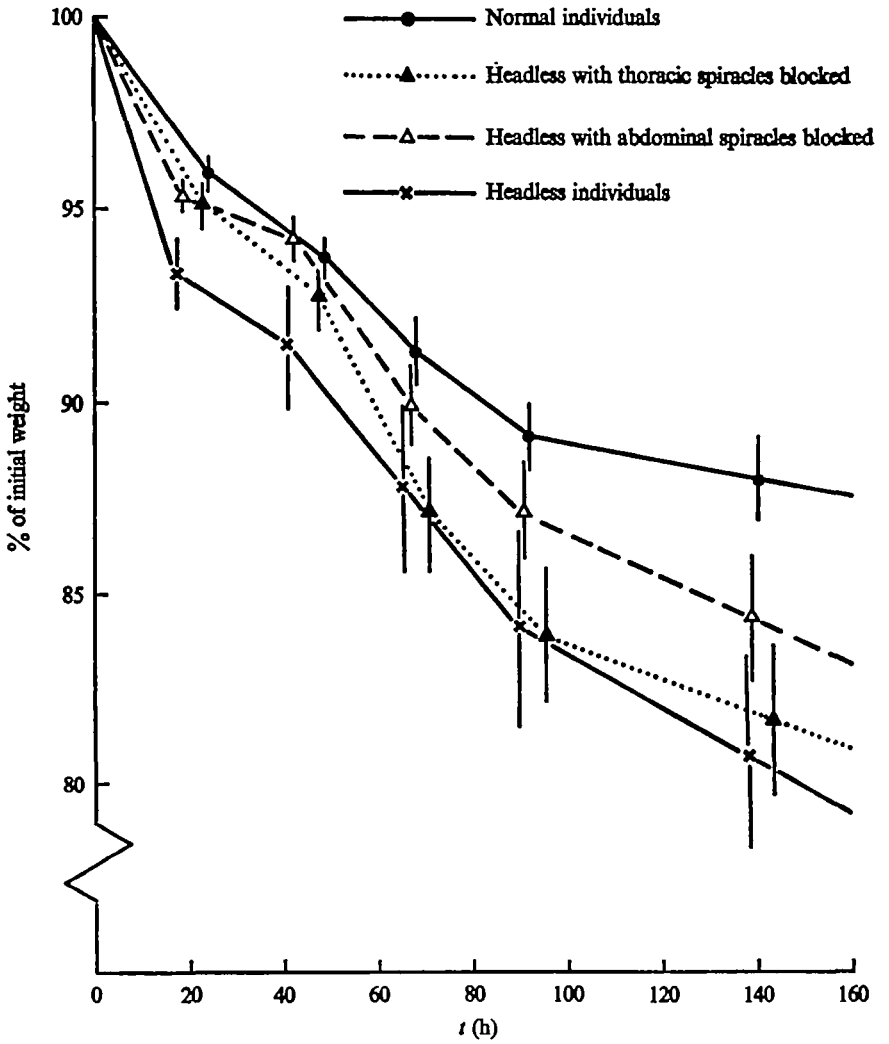

Fig. 9. The effects of spiracular blocking on the decline in weight of decapitated individuals at 50% R.H.

Table 1. *Radioactivity from the body surfaces of normal and decapitated individuals 24 h after the injection of* 0·5 *μCi of* ^{14}C*-acetate into the haemolymph*

(Decapitation was carried out 24 h prior to injection.)

Preparation	Date	Epicuticular radioactivity c.p.m. ± 2 × S.E. Normal	Decapitated
Anus unblocked	14. viii. 1974	1980 ± 174 (n = 8)	2408 ± 250 (n = 7)
Anus blocked	9. viii. 1974	1539 ± 156 (n = 6)	1434 ± 260 (n = 5)

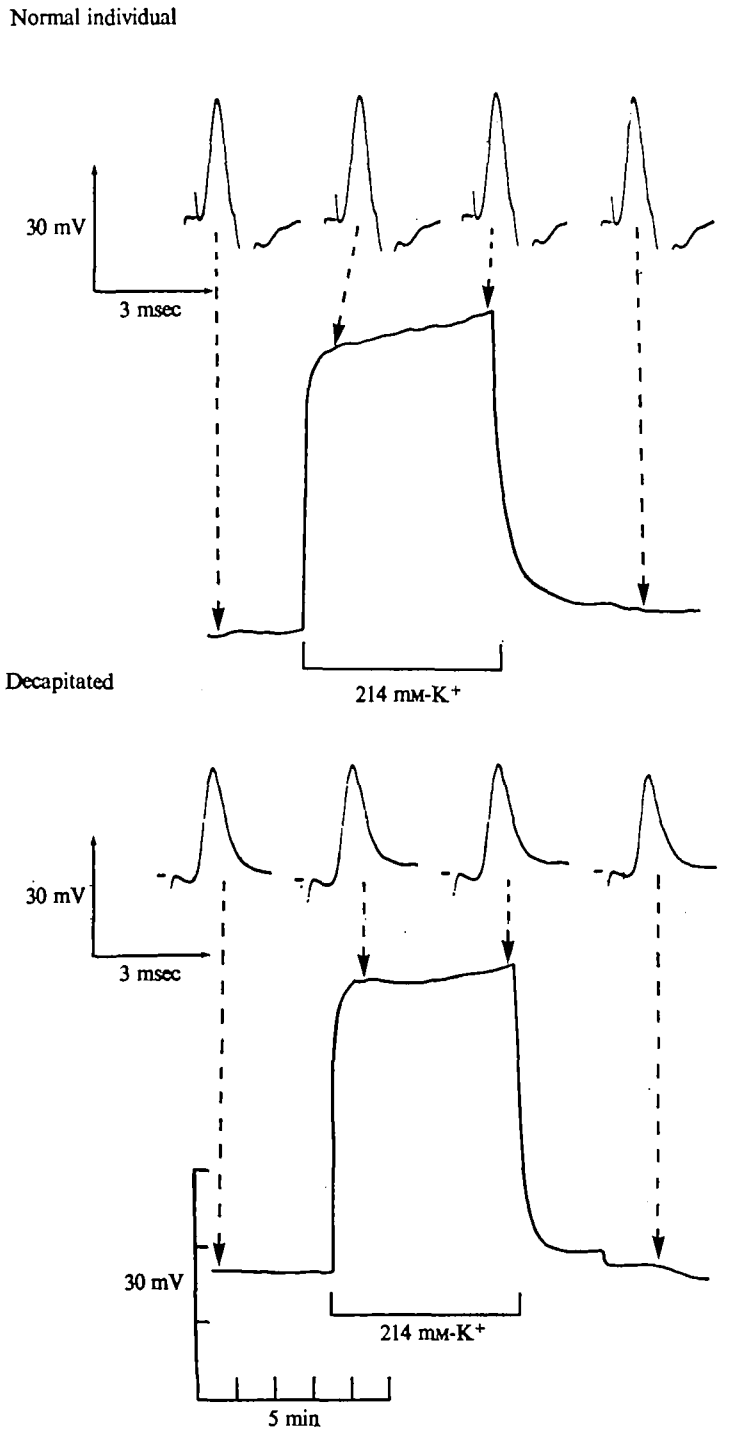

Fig. 10. The effects of elevated potassium concentration (214 mM) on the amplitude of the action potentials and the d.c. potentials recorded in intact penultimate connectives in a normal individual and one decapitated 28 h prior to the experiment.

Table 2. *Water content of pronotal cuticle of normal and decapitated individuals after 72 h at relative humidities of 35 and 60%*

Relative humidity	Date	Cuticular water content (%)	
		Normal ± 2 × S.E.	Decapitated ± 2 × S.E.
60%	7. x. 1974	57·09 ± 1·96 (n = 10)	37·87 ± 5·66 (n = 9)
35%	11. xi. 1974	51·14 ± 1·72 (n = 11)	44·66 ± 4·56 (n = 9)

involvement of the corpus cardiacum can also be postulated, for injection of fluid extract of this organ produced a partial, but significant, reduction in the rate of water loss induced by decapitation.

It is, thus, difficult to avoid the conclusion that the accelerated water-loss results from the decline in the concentration of some critical factor, or factors, in blood of decapitated insects. Such a conclusion contains the assumption that the waterproofing properties of the integument are normally controlled by a hormone, or hormones, originating in the brain and corpus cardiacum.

It is known that an accelerated water-loss can be induced by removal of the frontal ganglion or severance of the frontal connectives in *Periplaneta* (Penzlin & Stölzner, 1971). It is conceivable, as suggested by Penzlin & Stölzner, that the frontal ganglion receives impulses from osmoreceptors and could, thus, be involved in mediating the release of neurosecretory material from the brain.

The involvement of both the brain and the corpus cardiacum in the apparent control of integumentary transpiration suggests a similarity with other neurosecretory systems. It is well-established, for example, that neurosecretory material is synthesized within neuronal cell bodies, in the brain, and is then transported along specialized axons to the corpus cardiacum (cf. Maddrell, 1974).

The present observations suggest that a similar state of affairs may exist with this apparent waterproofing factor, that is, a synthesis within neuronal cell bodies in the brain with axonal transport to the corpus cardiacum. The greater activity of the brain extract, as compared with that of the corpus cardiacum, suggests that relatively large amounts of neurosecretory material occur in an active form in central neurone cell bodies as is the case, for example, with diuretic hormone in *Rhodnius* (Maddrell, 1966).

The prolonged effect of injected brain-extract in reducing water-loss suggests that the blood-borne factor was not rapidly metabolized in decapitated individuals. A rapid inactivation in normal individuals must, however, be inferred from the observation that no reduction in water-loss occurred when a neck ligature was applied 24 h after the injection of brain-extract. There is no obvious explanation for the absence of inactivation in decapitated insects. However, in the tsetse fly it has been shown that breakdown of the diuretic hormone is achieved by an enzyme which, it is proposed, is released into the haemolymph from the thoracic ganglion (Gee, 1974). It is thus envisaged that both the hormone and the inactivating enzyme originate from the same ganglion. The presence of an inactivating factor and the waterproofing factor in the brain of *Periplaneta* could, thus, explain the lack of effect of brain-extract when

injected 24 h before the application of neck ligatures. In this case it would be necessary, in addition, to argue that the inactivating factor was not effective when introduced into the haemolymph with brain-extract. Such an effect could result from instability of the inactivating factor following the extraction procedure. Alternatively, it could be that the synthesis and release of the inactivating factor depend upon the level of the waterproofing factor in the haemolymph. The latter possibility would explain both the breakdown of the waterproofing factor, when injected into normal individuals, and the absence of inactivation in decapitated ones.

Regulation of integumentary transpiration could be achieved by variation of the permeabilities of the epicuticular lipid layer, of the cell membrane or junctional complexes of the epidermal layer (Berridge, 1970).

It is arguable that the increased water-loss obtained following decapitation could have resulted merely from an unspecific effect on transcellular or intercellular epithelial permeability caused by the absence of the blood-borne factor. This interpretation would imply that the permeability of the epidermal layer of the integument was also critically affected by such an unspecific effect in these experiments. However, the observation that the permeability of the perineurial layer of the nerve cord was unaffected by decapitation would not accord with this hypothesis.

It is also conceivable that the apparent blood-borne factor demonstrated in these experiments might be involved in the control of lipid metabolism. In this case the effects of decapitation on integumentary transpiration could be reasonably postulated to result, secondarily, from changes in the synthesis and deposition of epicuticular lipids. Such an effect might, for example, be inferred from the work of Locke (1965*a*) who showed that wax secretion in *Calpodes ethlius* appears to be 'hormonally' controlled. This possibility is not, however, supported by the demonstration that the incorporation of injected ^{14}C-acetate into epicuticular lipids (Diehl, 1973) was not, apparently, affected by decapitation. Furthermore, the present results differ from those of Locke (1965*a*) with *Calpodes* who showed that wax secretion could not be induced by implantations of the brain and corpus cardiacum/corpus allatum into isolated abdomens.

The available evidence does not, therefore, conflict with the idea that the blood-borne factor, or factors, exert a relatively specific effect on the waterproofing properties of the integument. Unfortunately, this evidence sheds little light on the nature of the processes likely to be involved in the apparent hormonal control of integumentary water-loss. In particular, it is not possible to distinguish, with any degree of confidence, between possible effects on the permeability of the epicuticular lipid layer and those, as suggested by Berridge (1970), which occur at the level of the epidermis.

The observation that the electrical resistance of the integument is not changed appreciably by decapitation suggests that the increased transpiration is associated with relatively subtle changes in structure. This observation would, for example, accord with the concept that increased transpiration results from changes in restricted aqueous channels in an oriented epicuticular lipid layer of the type proposed by Beament (1964). Such changes could be readily envisaged as being insufficient to admit particles of the size of the inorganic ions which would be necessary to achieve an increase in the current flow across the integument.

The apparent decline in the water content of pronotal cuticle in decapitated

individuals can also be tentatively interpreted in terms of an increased permeability of the epicuticular lipid layer in response to a decline in the level of a blood-borne factor, or factors, originating in the brain and corpus cardiacum. For example, such an alteration in permeability could, as suggested by Locke (1965*b*), result from changes in the lipid water/liquid-crystalline phases of the epicuticular lipids.

The physiological role of an apparent neuro-endocrine control of integumentary water-loss is not immediately apparent. However, it can be conjectured that such a control of water-loss might confer some advantages on small terrestrial arthropods. In extremely dry atmospheric conditions or in the event of water deprivation it would obviously be essential to reduce integumentary water-loss to an absolute minimum. On the other hand, under conditions of excessive water intake, such as might occur with a liquid or semi-liquid diet of low nitrogen content, it would be physiologically advantageous to increase the rate of loss of water through the integument. This method for the elimination of water would be more efficient than an equivalent output through the excretory system, which would necessarily be associated with a loss of physiologically valuable solutes (cf. Maddrell, 1971). Furthermore, the elimination of water through the excretory system would involve a considerable expenditure of energy in ion transport, both in primary secretion and in reabsorption.

The ability to regulate integumentary transpiration could, conceivably, also be regarded as a thermoregulatory device which might control the extent of evaporative cooling. The importance of evaporative cooling in maintaining an appreciable difference between the temperature of the air and that of the body surface has been demonstrated by Beament (1958). However, a neuroendocrine control system would be unlikely to be effective at temperatures approaching the lethal range, for at temperatures of above about 30 °C evaporative cooling would largely result from the increased water permeability caused by disorganization of the epicuticular lipid layer (Beament, 1958). Furthermore, at slightly lower temperatures the use of increased integumentary water-loss to reduce the body temperature might be a suicidal form of thermoregulation, especially in conditions of water deprivation.

Alternatively, it may well be a misconception to interpret these results in terms of a neuroendocrine mechanism which can regulate integumentary waterproofing so as to achieve variations in the rate of water-loss from the body surface. It is conceivable that this apparent neuroendocrine control merely ensures that the organization of the epicuticular lipids is maintained so as to achieve maximal waterproofing of the integument. The epicuticular lipids of the cockroach are, to choose a single specific example, known to undergo autoxidation on exposure to air (Atkinson & Gilby, 1970). It could be that a controlled release of the antioxidant, protocatechuic acid, is necessary to prevent degradation and polymerization of the epicuticular lipids and, thus, to maintain the effective waterproofing of the integument.

As amateurs, in the fields of both integumentary permeability and insect endocrinology, we have greatly benefited from the kind advice and encouragement of Professor J. W. L. Beament, Drs M. J. Berridge, B. L. Gupta, S. H. P. Maddrell, J. Noble-Nesbitt and Sir Vincent Wigglesworth. We express our gratitude and thanks to these long-suffering gentlemen. We are also grateful to Mr John Rodford for preparing the illustrations used in this article.

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