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THE PHYSIOLOGY OF EXCRETION IN THE COTTON STAINER, *DYSDERCUS FASCIATUS*, SIGNORET

IV. HORMONAL CONTROL OF EXCRETION

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INTRODUCTION

Considering the close analogy between the insect median neurosecretory cell (m.n.c.)/corpus cardiacum (c.c.) complex, and the vertebrate hypothalamo-hypophyseal system (Scharrer & Scharrer, 1944), few studies have been made on the possible hormonal control of water metabolism in insects. In fact, our present knowledge of this aspect is confusing and, in some cases, contradictory (Wall & Ralph, 1962*a*). Some of the confusion may arise because the control of water balance in insects is achieved by a number of different mechanisms. More critical studies must be made if this situation is to be resolved.

When studying the hormonal control of physiological and biochemical mechanisms it is imperative that the mechanisms themselves be understood, and also that indirect effects, introduced by experimental procedures, can be recognized. Wrong conclusions may be reached if these elementary precautions are not observed. Such dangers are inherent in some techniques used to study neurosecretion in insects. For example, removal of m.n.c. is a common operative technique used to study the way in which these cells influence a particular physiological process. It is now known that these cells control a large number of physiological processes (Wigglesworth, 1964). Considerable caution must therefore be exercised when interpreting such experiments, especially in recognizing indirect effects caused by disturbance of related physiological events. For this reason a somewhat indirect method has been used to study the possible hormonal control of excretion in *Dysdercus*.

In this insect there are two distinct phases of excretion (Berridge, 1965*a*). The excretory phase may be considered as a period of diuresis; the Malpighian tubules generate a rapid flow of liquid and there is a large output of urine, because the rectum reabsorbs little of this liquid (Berridge, 1965*c*). Conversely, since no urine is excreted during the post-excretory phase, this may be considered as an antidiuretic period. The primary cause of this antidiuresis is complete inactivity of the Malpighian tubules (Berridge, 1965*b*). Clearly, the two phases of excretion are intimately connected with changes in Malpighian tubule activity. Investigations have been made on isolated Malpighian tubules in order to determine how these changes in activity may be controlled. The advantages of these isolated preparations are twofold: first, indirect effects are largely avoided, and secondly, because the rate of urine flow is easily measured, the isolated tubules can be used as a sensitive method of biological assay.

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MATERIAL AND METHODS

Female 5th-instar larvae of *Dysdercus* were obtained as described previously (Berridge, 1965*a*).

The technique used for setting up isolated Malpighian tubules was essentially the same as that described by Ramsay (1954) for *Dixippus morosus*. In all cases tubules were set up in serum which was obtained by removing proteins from haemolymph by heat coagulation (Ramsay, 1955). Streptomycin and penicillin were added to serum to prevent bacterial growth.

In *Dysdercus* the two Malpighian tubules on one side of the gut are joined together to form a continuous loop (Berridge, 1965*a*); for convenience the complete loop from one side of the insect was removed as a single unit. All dissections were performed under Ringer solution (NaCl, 6·3 g./l.; KCl, 5·2 g./l.; $NaHCO_3$, 0·18 g./l.; NaH_2PO_4-$2H_2O$, 0·13 g./l.; $CaCl_2$, 0·5 g./l.). The insect was pinned down in a dissecting dish with its ventral surface uppermost. The abdomen was opened and the Malpighian tubules, which form the loop on the left side of the abdomen, were carefully freed of surrounding fat body and tracheae. The tubules were then severed close to their attachment to the ileum and removed from the abdomen. A wide-mouthed pipette was used to transfer these tubules to a watch glass containing liquid paraffin. The tubules were pulled out of this drop of Ringer and placed in 10·0 μl. of serum. The two tubules were ligatured close to their cut ends with fine silk threads, which were used to pull these two ends a short distance out of the serum droplet. Urine was allowed to escape from the tubule through a cut made immediately behind the ligature. The rate of urine production was measured as described by Ramsay (1955). In all experiments the two tubules of each preparation have been treated separately. Unless otherwise stated, Malpighian tubules were always removed from 3-day-old female 5th-instar larvae.

The procedure adopted for extracting hormone from tissues has been designed so as to disrupt the membrane which bounds neurosecretory granules (Willey & Chapman, 1960; Scharrer, 1963). A hypotonic medium is extremely effective in disrupting neurosecretory granules (Pérez-González, 1957). The relevant tissue was rapidly dissected from the insect and homogenized in a small volume of distilled water. Since Malpighian tubule activity is markedly influenced by osmotic pressure, the extract was made isotonic with serum by adding an equal volume of 2N Ringer solution. The tissue brei was centrifuged at 3000 rev./min.; the resulting clear supernatant was assayed for hormone activity. To facilitate comparison and avoid confusion, hormone dosage has been represented in the same way as described by Maddrell (1963, 1964*a*), i.e. amount of tissue per 100 μl. of serum.

Osmotic pressure determinations were made by the cryoscopic method of Ramsay & Brown (1955).

The vertical lines on the graphs represent $\pm$ twice the standard error of each mean.

RESULTS

I. *Normal functioning of isolated Malpighian tubules*

The typical performance of an isolated tubule is illustrated in Fig. 1. The volume of urine produced increases almost linearly with time over a 10 hr. collecting period, the rate of urine flow remaining almost constant, with only a small decrease with time. These isolated tubules are still capable of secreting urine, albeit at a much reduced rate, 36 hr. after removal from the body.

The rate of urine flow is markedly influenced by the osmotic pressure of serum (Fig. 2). Hypotonic serum was obtained by diluting normal serum with distilled water;

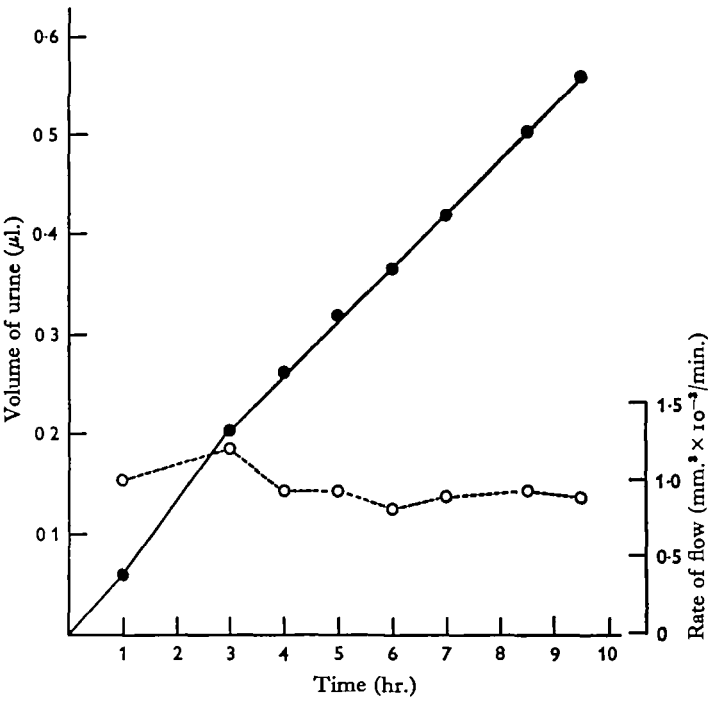

Fig. 1. The performance of a typical isolated Malpighian tubule; ●, volume of urine; ○, rate of urine flow.

hypertonic serum was produced by adding sucrose to normal serum. Urine flow is accelerated with increasing serum dilution, and vice versa (Fig. 2). The secretion of urine ceases if the osmotic pressure of serum is increased above 200 mM/l. NaCl. The tubules used in the above experiment produced urine which was almost isotonic with serum (Fig. 3), throughout the range of osmotic pressure of the latter. The average value of the difference, $O.P._{haemolymph} - O.P._{urine}$, for all the observations in Fig. 3, was only 3·0 mM/l. NaCl. The reaction of these isolated tubules of *Dysdercus* to changes in osmotic pressure of the medium bathing them is essentially similar to that already recorded for the isolated tubules of *Dixippus morosus* (Ramsay, 1954).

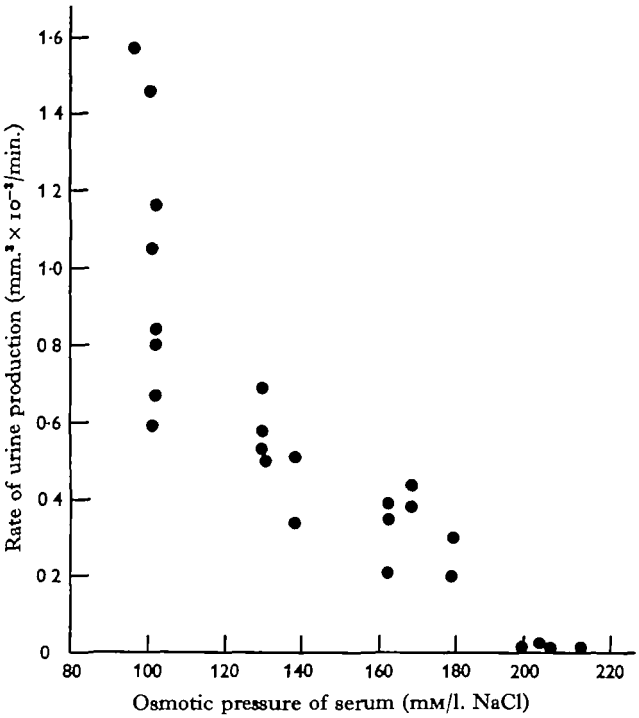

Fig. 2. The influence of osmotic pressure on the rate of urine formation by isolated Malpighian tubules.

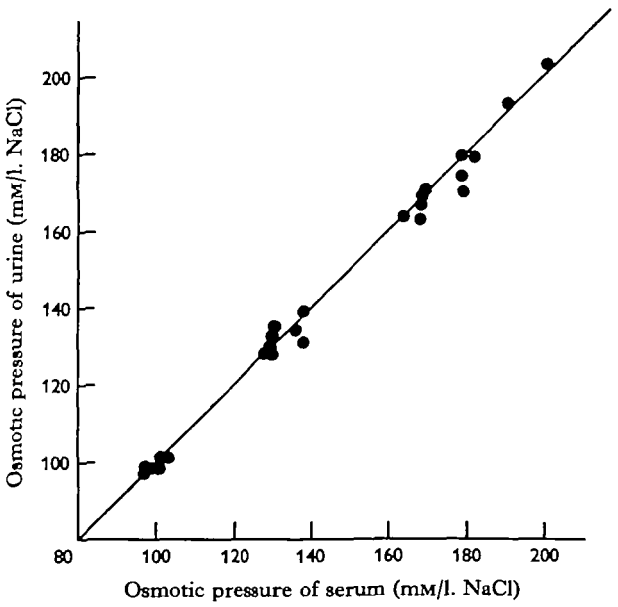

Fig. 3. Osmotic pressure of the urine as a function of the osmotic pressure of the serum. For convenience, the isotonic line has been included.

II. *The control of urine flow of isolated Malpighian tubules*

(*a*) *Variation in the 'hormone' content of the haemolymph*

Since Malpighian tubules do not receive nervous innervation, activity changes must be mediated via the haemolymph. The following working hypothesis was established: Malpighian tubules are activated by a factor which is released into the haemolymph during the feeding period. When release of this factor is withheld by a cessation of feeding, the Malpighian tubules stop functioning. Serum obtained from insects in the two phases of excretion would, therefore, be expected to exhibit differences in their ability to promote urine production by isolated tubules.

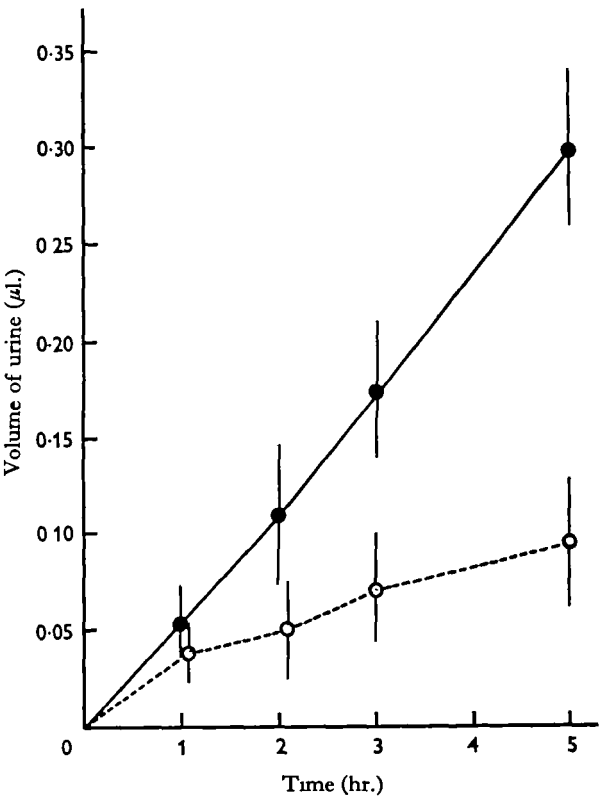

Fig. 4. The response of isolated Malpighian tubules to different sera; ●, 3-day serum; ○, 6-day serum.

Serum was obtained from insects on the 3rd and 6th day of the instar. Twelve isolated tubules, removed from 3-day-old insects, were set up individually, six in each serum, and urine production was followed for 5 hr. (Fig. 4). At the end of this period, tubules set up in 3-day serum had produced a significantly larger volume of urine (0·30 μl.) than those set up in 6-day serum (0·096 μl.). The difference between the two batches, however, only becomes significant after 2 hr. This initial delay in response of tubules set up in 6-day serum might represent the time required for these tubules to adjust to a new hormonal environment.

The tubules placed in 3-day serum showed a uniform response throughout the collecting period. Presumably they were set up in serum with a composition similar to that of the haemolymph from which they had just been taken.

These results are consistent with the original hypothesis that Malpighian tubules are activated by a factor released during the excretory phase. Experiments were then devised to try to locate the source of this factor.

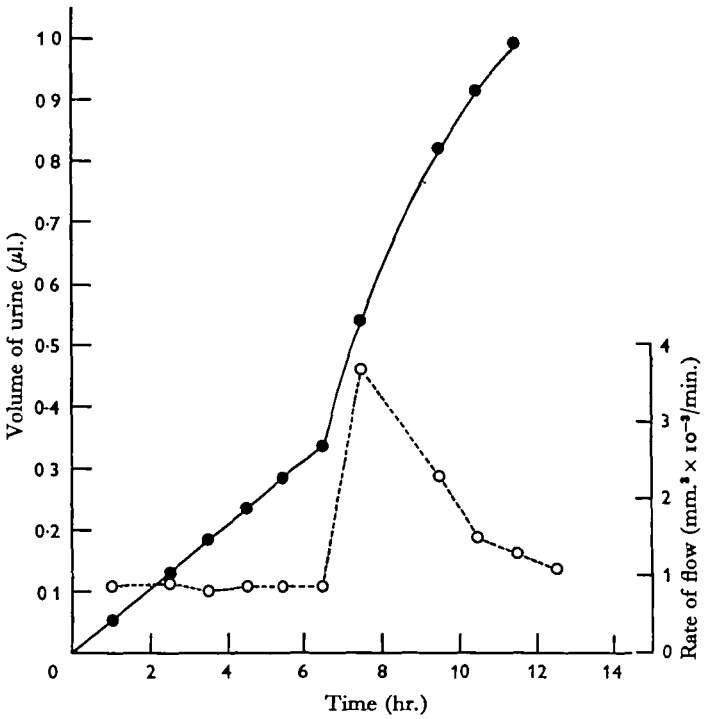

Fig. 5. The response of an isolated Malpighian tubule to the addition of 'hormone' (0·1 m.n.c./100 μl. of serum). Hormone was added to the serum bathing the tubules at 6½ hr.; ●, volume of urine; ○, rate of urine flow.

Table 1. *Effect of diuretic hormone on the osmotic pressure of urine produced by isolated Malpighian tubules*

	Before hormone addition	After hormone addition
Rate of urine flow mm.³ × 10⁻³/min. (mean ± S.E.)	0·65 ± 0·13	4·12 ± 0·73
Osmotic pressure of urine (mM./l. NaCl) (mean ± S.E.)	174·0 ± 1·61	173·2 ± 1·1

(*b*) *Source of the diuretic factor*

Initially it was important to know whether or not tubule activity could be influenced by extracts of neurosecretory origin; in preliminary experiments only extracts of m.n.c. were tested on isolated Malpighian tubules. A series of six isolated tubules were allowed to produce urine under normal conditions for a period of 4 hr., during which

time the rate of urine production was almost constant. At the end of 4 hr., 0·2 μl. of m.n.c. extract was added to the 10 μl. of serum bathing each pair of isolated tubules, to give a final dosage of 0·1 m.n.c./100 μl. of serum. Thirty minutes after the addition of hormone the rate of urine flow had increased from a normal value of 0·87 to a maximum of 3·1 mm.^{3} × 10^{-3}/min. (Fig. 5). After this peak there was a gradual decline towards the original rate.

The rate of urine flow was unaffected by the addition of extracts from the brain, from which m.n.c. had been removed. Clearly, a factor produced in the m.n.c. has

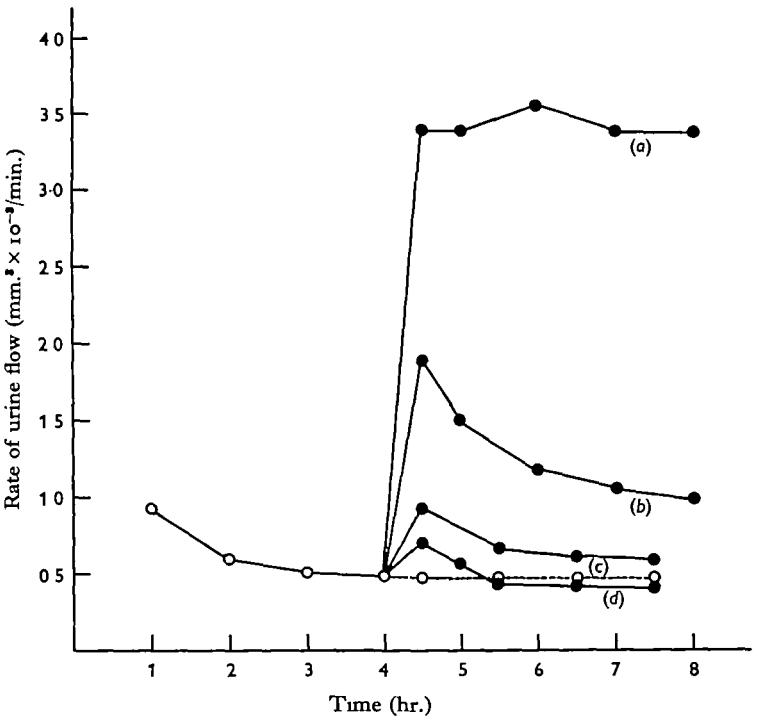

Fig. 6. The response of isolated Malpighian tubules to different doses of diuretic hormone. (*a*) 0·5; (*b*) 0·2; (*c*) 0·08; (*d*) 0·04 m.n.c./100 μl. of serum. O- - -O, Rate of flow after addition of 0·2 μl. Ringer. Hormone was added to serum at 4 hr.

a marked effect on the rate of urine production by isolated tubules. In another series of experiments it was shown that the osmotic pressure of the urine was unchanged even though the rate of urine flow had increased enormously (Table 1), which probably indicates that the hormone acts simply by accelerating the normal process of urine formation.

In order to assay other parts of the nervous system for hormone activity it was necessary to determine whether hormone dosage was linearly related to rate of urine production by isolated tubules. Different doses of hormone were tested on isolated tubules; six tubules were used for each determination. The change in rate of urine flow after the addition of different amounts of m.n.c. extract is shown in Fig. 6. The rate of urine flow before addition of hormone was the same in all cases; the subsequent response of the tubules appeared to depend on dosage. This relationship is

shown more clearly if the percentage increase in rate of flow, 30 min. after addition of hormone, is plotted against dosage (Fig. 7). There is a linear relationship between the percentage increase in the rate of urine flow and dosage, over the range 0·02–0·20 m.n.c./100 μl. of serum. There appears to be a definite maximum dosage, probably limited by the secretory processes of the cell, above which there is no further increase in rate, but the time for which the maximum rate is maintained is lengthened (Fig. 6). Although different tubules vary considerably in their response to any one dosage, they can be used as a rough assay to determine the diuretic hormone activity of different tissues.

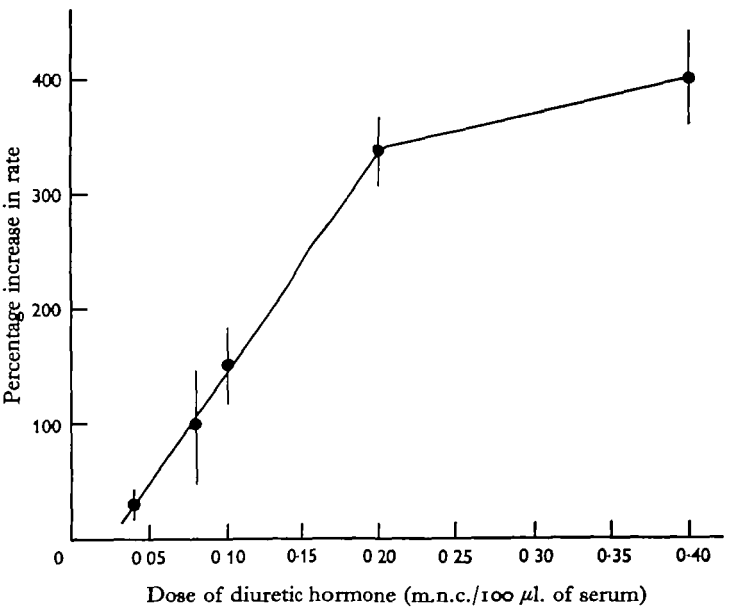

Fig. 7. The response of isolated Malpighian tubules to different doses of 'hormone'. (Data taken from Fig. 6; the increase in rate 30 min. after addition of hormone is expressed as a percentage of the initial rate).

In a preliminary experiment approximately equal amounts of salivary gland, muscle, gut, Malpighian tubule and supraoesophageal ganglion were assayed; only the latter possessed hormone activity. The distribution of hormone activity in various parts of the nervous system and retrocerebral glands is set out in Table 2.

It is extremely difficult to draw any positive conclusions from such a comparison, because there is a large disparity, both in the size and number of neurosecretory cells, between the tissues tested. The small difference in hormone content between the various parts makes it impossible to decide on the exact source of the diuretic factor. Since a large part of the activity is concentrated in the m.n.c./c.c. complex, it would appear that this system would represent the most likely source of diuretic hormone.

All the experiments reported so far have been performed on Malpighian tubules removed from insects in the middle of the excretory phase (3 days). Tubules were therefore removed from insects in the post-excretory phase in order to determine whether they were inactive as predicted earlier (Berridge, 1965*b*). Malpighian tubules

Table 2. *The occurrence of diuretic activity in various parts of the nervous and retrocerebral systems*

Tissue	% increase in rate of urine flow after addition of tissue extract (0·1 tissue/100 μl. of serum)
m.n.c.	163·0
c.c.	62·0
Corpus allatum	10·0
Optic lobe	6·0
Suboesophageal ganglion	12·0
Prothoracic ganglion	13·5
'Mesothoracic ganglion'	79 0

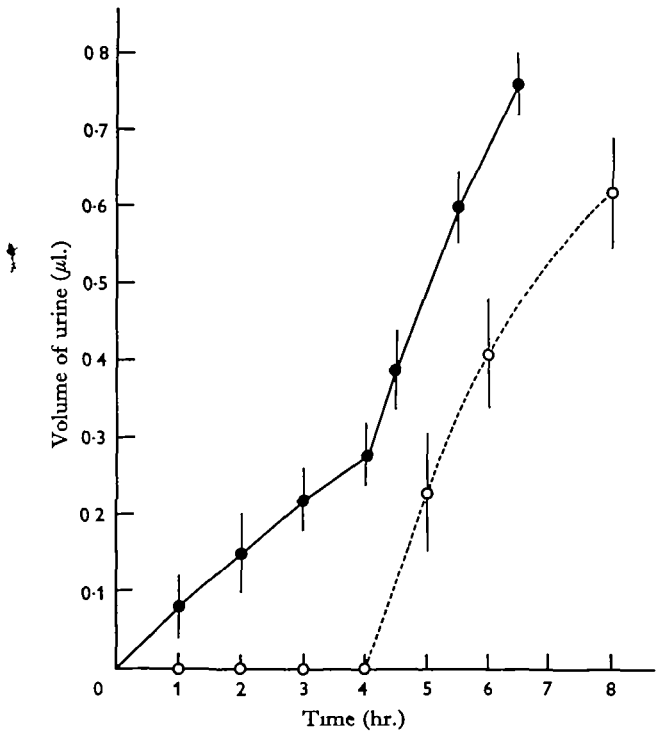

Fig. 8. The secretory behaviour of isolated Malpighian tubules taken from 3-day-old animals (closed circles) and 6-day-old animals (open circles). 'Hormone' was added to the serum bathing the tubules at 4 hr.

isolated from 6-day-old insects were set up in the usual way; for comparison, isolated tubules were prepared from 3-day-old insects.

Tubules taken from 6-day-old insects were completely inactive (Fig. 8), whereas those taken from 3-day-old insects were immediately active and produced urine at a constant rate as already described (Fig. 1). The hypothesis which proposed that tubule activity is controlled hormonally gains further support from the observation that these inactive tubules can be activated by the addition of an extract from m.n.c.

(Fig. 8). This experiment clearly indicates that, although the Malpighian tubules are inactive during the post-excretory phase, they are physiologically capable of secreting urine when supplied with a hormonal stimulus.

DISCUSSION

It has been shown that the isolated Malpighian tubules of *Dysdercus* are markedly influenced by extracts prepared from various endocrine centres. Further evidence, summarized below, seems to suggest that this influence might be part of a mechanism controlling excretion in this insect. Haemolymph taken from insects in the excretory phase can support urine production by isolated tubules better than that taken from insects in the post-excretory phase. Isolated Malpighian tubules removed from insects in the post-excretory phase remain inactive, but can be stimulated to produce urine by the addition of an m.n.c. extract. Inactivity of the Malpighian tubules, therefore, appears to be related to absence of some factor which is only present in the haemolymph during the excretory phase. The Malpighian tubules are probably directly responsible for decreasing the haemolymph content of this factor as the post-excretory phase approaches. When low hormone dosages were added to a series of isolated tubules, the rate of urine flow decreased after an initial peak; with high hormone dosages, however, it remained at a high level (Fig. 6). In the latter case, urine flow remained constant because the tubules could not significantly reduce the hormone titre, whereas at low hormone dosages, the tubules inactivate the hormone causing decreased urine production. Such a mechanism may occur *in vivo*. Urine and allantoin excretion of 2- or 3-day-old larvae was reduced to zero 12 hr. after removing water from the diet (Berridge, 1964). When water is unavailable *Dysdercus* stops feeding and presumably release of hormone also ceases; the decrease in excretion can then be explained if the tubules inactivate the hormone which remains in the haemolymph. Excessive water loss is prevented by rapidly switching off the excretory system. The Malpighian tubules of *Rhodnius* are also thought to degrade the diuretic hormone secreted, immediately after a blood meal, from the fused ganglionic mass in the mesothorax of this insect (Maddrell, 1964*a*). Breakdown of hormone by its target organ also occurs in vertebrates, where vasopressin is known to be inactivated by the kidney (Sawyer, 1961).

All this evidence, direct and circumstantial, has been incorporated into a tentative hypothesis of the hormonal control of excretion in *Dysdercus*. When the insect feeds, a 'diuretic hormone', which is probably synthesized in the m.n.c., is released into the haemolymph from the corpus cardiacum, to activate the excretory system. It is well known that feeding can initiate hormone release in insects (Wigglesworth, 1934; Dadd, 1961; Clarke & Langley, 1963; Maddrell, 1964*b*). The Malpighian tubules begin to generate a flow of urine containing waste nitrogen and excess inorganic ions. When feeding ceases, however, release of hormone is withheld; the remaining hormone being mopped up by the Malpighian tubules which consequently stop functioning and the insect passes into the post-excretory phase. Such an hypothesis dovetails nicely with our present knowledge of the hormonal control of metabolism in insects. It would appear that some aspect of feeding activates the m.n.c. to trigger a chain of hormone-controlled processes which are geared to dealing with indigest foodstuffs. The gut is

activated to digest the food (Dadd, 1956, 1961; Thomsen & Møller, 1963), the fat body is stimulated to metabolize the products of digestion (Hill, 1963), and, by activating other endocrine centres, such as the prothoracic gland or corpus allatum, the m.n.c. also control the utilization of the fat-body products. Apparently these cells also stimulate the excretory system to eliminate the waste products which accrue during these metabolic events. As yet, there is no indication whether all these processes are controlled by a single hormone or by several hormones. The multiplicity of neurosecretory cell types present in the brain (Fraser, 1959; Herman & Gilbert, 1965) suggests that the latter may prove correct.

The occurrence of a diuretic hormone in *Rhodnius* was recently reported by Maddrell (1962, 1963, 1964*a*, *b*) who has provided valuable information on the control of excretion in insects. By head-ligation and nerve-transection experiments, Maddrell has demonstrated that a hormone which is released into the haemolymph from the fused ganglionic mass in the mesothorax is responsible for the rapid diuresis which immediately follows a blood meal. The hormone, which can be extracted from this ganglion, will also increase the rate of urine flow of isolated Malpighian tubules (Maddrell, 1963, 1964*a*). Hormone production, when thus assayed, has been localized in neurosecretory cells which lie in the posterior part of this fused ganglionic mass. Only very slight hormone activity was found in the brain, suboesophageal or prothoracic ganglia (Maddrell, 1963). In *Dysdercus*, the difference in hormone concentration between the different tissues assayed is not large enough to allow identification of the precise source of the diuretic hormone. The combined hormone content of the m.n.c. and c.c. is considerably larger than that of the other tissues tested; this complex is therefore the most likely source of hormone in this insect. It would seem that the source of hormone in *Dysdercus* is different from that in *Rhodnius*, which is substantiated by the observation that the hormone from *Dysdercus* is heat-stable (unpublished observation), whereas that from *Rhodnius* is not (Maddrell, 1962). But such a difference will only take on significance once it is shown that these two factors share a similar physiological function. The possibility remains that the mechanism controlling excretion in *Rhodnius* may represent a special adaptation which has been developed to deal with the sudden intake of a large volume of fluid. It might be of considerable significance that the control of excretion proposed for *Dysdercus* has many similarities to that already advanced by Núñez (1956) for the beetle *Anisotarsus cupripennis*. In the normal insect water intake occurs not only by feeding, but also through the general body surface. The rate of excretion of this excess water is regulated so that the water content of the insect remains constant. If the neck is ligated or if the upper part of the brain is ablated, excretion stops and the insect increases in weight. It appears that the brain secretes a diuretic hormone which activates the Malpighian tubules to excrete the excess water. This view is certainly consistent with the observation that injection of brain extracts restores water balance by increasing excretion (Núñez, 1956).

Throughout the studies described in this paper there was no evidence for the existence of an antidiuretic factor, although such a factor is thought to occur in insects. In *Iphita limbata* neurosecretory material accumulated in the A-cells of the m.n.c. when the insects were hydrated, but was considerably depleted if they were dehydrated or fed on salt solution. It was inferred from these observations that the A-cells elaborate a secretion which has an antidiuretic effect (Nayar, 1960). The same author

has also shown, by a similar experimental procedure, that the m.n.c. of *Periplaneta americana* probably also produce an antidiuretic factor (Nayar, 1962). This observation is in agreement with the finding that extracts of the corpus cardiacum of the cockroach cause a decrease in the rate of excretion of indigo carmine by Malpighian tubules *in vitro* (Wall & Ralph, 1962*b*). Similarly, A-cells of the third thoracic and all the abdominal ganglia of the locust *Schistocerca gregaria* are depleted of secretion under conditions of water loss; it is suggested that a factor from these cells is involved in water conservation (Delphin, 1963*a*, *b*).

The presence of an antidiuretic hormone has also been postulated from experiments where extracts of various endocrine glands have been injected into insects and/or vertebrates. Stutinsky (1953) found that extracts of the m.n.c. and corpus cardiacum of *Blaberus* had an antidiuretic effect when injected into the rat. Similarly, extracts of the corpus cardiacum, when tested on the honeybee, resulted in decreased water excretion (Altmann, 1956).

Although there appears to be considerable evidence favouring the existence of an antidiuretic factor in insects, it might be worthwhile to await more critical evidence before the existence of such a factor may be accepted as certain. Many of the observations are based on inference which can often be very misleading. For example, when salt solutions are injected into *Blaberus* (Wall & Ralph, 1962*a*), or into *Periplaneta americana* (Nayar, 1962), to induce antidiuresis, the possibility of osmotic diuresis was not considered. If the terms diuresis and antidiuresis are used, conditions in the excretory system must be considered. For example, it would be of value to know if the antidiuretic factor can induce a change in the excretory system resulting in a marked retention of water. In *Dysdercus*, it appears that a decrease in excretion during the post-excretory phase is brought about by withholding release of the diuretic hormone rather than by releasing an antidiuretic substance.

SUMMARY

1. The preparation of isolated Malpighian tubules is described. The rate of urine flow increases with increasing serum dilution, and vice versa. Urine is almost isotonic with haemolymph over a wide range of osmotic pressure.

2. Serum collected from 3-day-old insects promotes urine formation, whereas that from 6-day-old insects does not.

3. A factor which was extracted from the m.n.c. accelerates the rate of urine flow, from a normal value of 0·87 to 3·1 mm.^{3} × 10^{-3}/min. The osmotic pressure of the urine, however, remains unchanged.

4. The hormone concentration of different parts of the nervous system was assayed with these isolated tubules. Most activity occurs in the m.n.c., but some activity is present in extracts from the c.c. and the fused ganglionic mass in the mesothorax.

5. Malpighian tubules isolated from 6-day-old insects remain inactive, but after the addition of hormone they immediately begin to produce urine.

6. These observations have been incorporated into a tentative hypothesis on the control of excretion in *Dysdercus*.

This work formed part of a thesis submitted to the University of Cambridge for the degree of Ph.D. It is a great pleasure to thank my supervisor, Prof. Sir Vincent Wigglesworth, for his help and encouragement during this work, and Dr Dietrich Bodenstein for reading the manuscript. The work was carried out during tenure of a Commonwealth Scholarship, and I am most grateful for this support.

REFERENCES

ALTMANN, G. (1956). Die Regulation des Wasserhaushaltes der Honigbiene. *Insectes soc.* **3**, 33–40.

BERRIDGE, M. J. (1964). The physiology of excretion in the cotton stainer, *Dysdercus fasciatus* Signoret (Hemiptera, Pyrrhocoridae). Ph.D. Thesis, Cambridge University.

BERRIDGE, M. J. (1965*a*). The physiology of excretion in the cotton stainer, *Dysdercus fasciatus* Signoret. I. Anatomy, water excretion and osmoregulation. *J. Exp. Biol.* **43**, 511–521.

BERRIDGE, M. J. (1965*b*). The physiology of excretion in the cotton stainer, *Dysdercus fasciatus*, Signoret. II. Inorganic excretion and ionic regulation. *J. Exp. Biol.* **43**, 523–533.

BERRIDGE, M. J. (1965*c*). The physiology of excretion in the cotton stainer, *Dysdercus fasciatus* Signoret. III. Nitrogen excretion and excretory metabolism. *J. Exp. Biol.* **43**, 535–552.

CLARKE, K. U. & LANGLEY, P. A. (1963). Studies on the initiation of growth and moulting in *Locusta migratoria migratorioides* R. & F. IV. The relationship between the stomatogastric nervous system and neurosecretion. *J. Insect Physiol.* **9**, 423–30.

DADD, R. H. (1956). Proteolytic activity of the midgut in relation to feeding in the beetles *Tenebrio molitor* L. and *Dytiscus marginalis* L. *J. Exp. Biol.* **33**, 311–24.

DADD, R. H. (1961). Evidence for humoral regulation of digestive secretion in the beetle, *Tenebrio molitor* L. *J. Exp. Biol.* **38**, 259–266.

DELPHIN, F. (1963*a*). Studies on neurosecretion in *Schistocerca gregaria* Forskål. (Orthoptera: Acrididae). Ph.D. Thesis, London.

DELPHIN, F. (1963*b*). Histology and possible functions of neurosecretory cells in the ventral ganglia of *Schistocerca gregaria* Forsk. *Nature, Lond.*, **200**, 913–15.

FRASER, A. (1959). Neurosecretion in the brain of the sheep blowfly, *Lucilia caesar*. *Quart. J. Micr. Sci.* **100**, 377–94.

HERMAN, W. S. & GILBERT, L. A. (1965). Multiplicity of neurosecretory cell types and groups in the brain of the Saturniid Moth, *Hyalophora cecropia* L. *Nature, Lond.*, **205**, 926–7.

HILL, L. (1963). Endocrine control of protein synthesis in female desert locusts. (*Schistocerca gregaria*). *J. Endocrin.* **26**, xvii–xviii.

MADDRELL, S. H. P. (1962). The regulation of excretion in *Rhodnius prolixus* Stål. Ph.D. Thesis, Cambridge University.

MADDRELL, S. H. P. (1963). Excretion in the blood-sucking bug, *Rhodnius prolixus* Stål. I. The control of diuresis. *J. Exp. Biol.* **40**, 247–56.

MADDRELL, S. H. P. (1964*a*). Excretion in the blood-sucking bug, *Rhodnius prolixus* Stål. II. The normal course of diuresis and the effect of temperature. *J. Exp. Biol.* **41**, 163–76.

MADDRELL, S. H. P. (1964*b*). Excretion in the blood-sucking bug *Rhodnius prolixus* Stål. III. The control of the release of the diuretic hormone. *J. Exp. Biol.* **41**, 459–72.

NAYAR, K. K. (1960). Studies on the neurosecretory system of *Iphita limbata* Stål. VI. Structural changes in the neurosecretory cells induced by changes in water content. *Z. Zellforsch. mikrosk. Anat.* **51**, 320–4.

NAYAR, K. K. (1962). Effects of injecting juvenile hormone extracts on the neurosecretory system of adult male cockroaches (*Periplaneta americana*). *Mem. Soc. Endoc.* **12**, 371–8.

NÚÑEZ, J. A. (1956). Untersuchungen über die Regelung des Wasserhaushaltes bei *Anisotarsus cupripennis* Germ. *Z. vergl. Physiol.* **38**, 341–54.

PÉREZ-GONZÁLES, M. D. (1957). Evidence for hormone-containing granules in sinus glands of the fiddler crab, *Uca pugilator*. *Biol. Bull., Woods Hole*, **113**, 426–41.

RAMSAY, J. A. (1954). Active transport of water by the Malpighian tubules of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae). *J. Exp. Biol.* **31**, 104–13.

RAMSAY, J. A. (1955). The excretion of sodium, potassium and water by the Malpighian tubules of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae). *J. Exp. Biol.* **32**, 200–216.

RAMSAY, J. A. & BROWN, R. H. J. (1955). Simplified apparatus and procedure for freezing-point determinations upon small volumes of fluid. *J. Sci. Instrum.* **32**, 372–75.

SAWYER, W. H. (1961). Neurohypophysial hormones. *Pharmac. Rev.* **13**, 225–77.

SCHARRER, B. (1963). Neurosecretion. XIII. The ultrastructure of the corpus cardiacum of the insect *Leucophaea maderae*. *Z. Zellforsch. mikrosk. Anat.* **60**, 761–96.

SCHARRER, E. & SCHARRER, B. (1944). Neurosecretion. VI. A comparison between the intercerebralis-cardiacum-allatum system of the insect and the hypothalamo-hypophyseal system of the vertebrates. *Biol. Bull., Woods Hole*, **87**, 242–251.

STUTINSKY, F. (1953). Mise en évidence d'une substance antidiuretique dans le cerveau et le complexe retrocérébral d'une Blatte (*Blabera fusca* Brunn). *Bull. Soc. Zool. France*, **78**, 202–4.

THOMSEN, E. & MØLLER, I. (1963). Influence of neurosecretory cells and of corpus allatum on intestinal protease activity in the adult *Calliphora erythrocephala* Meig. *J. Exp. Biol.* **40**, 301–21.

WALL, B. J. & RALPH, C. L. (1962*a*). Responses of specific neurosecretory cells of the cockroach, *Blaberus giganteus*, to dehydration. *Biol. Bull., Woods Hole*, **122**, 431–8.

WALL, B. J. & RALPH, C. L. (1962*b*). Factors from the nervous system of the cockroach that influence excretion rates of Malpighian tubules. *Amer. Zool.* **2**, 455–6.

WIGGLESWORTH, V. B. (1934). The physiology of ecdysis in *Rhodnius prolixus* (Hemiptera). II. Factors controlling moulting and 'metamorphosis'. *Quart. J. Micr. Sci.* **77**, 191–222.

WIGGLESWORTH, V. B. (1964). The hormonal regulation of growth and reproduction in insects. *Advanc. Insect Physiol.* **2**, 247–336.

WILLEY, R. B. & CHAPMAN, G. B. (1960). The ultrastructure of certain components of the corpora cardiaca in Orthopteroid insects. *J. Ultrastructure Res.* **4**, 1–14.