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EXCRETION IN THE BLOOD-SUCKING BUG, *RHODNIUS PROLIXUS* STÅL

II. THE NORMAL COURSE OF DIURESIS AND THE EFFECT OF TEMPERATURE

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INTRODUCTION

It has recently been shown that the diuresis of fed 5th-stage larvae of *Rhodnius* is brought about by a hormone that appears in the haemolymph (Maddrell, 1962, 1963). This diuretic hormone is released from the fused ganglionic mass in the mesothorax, probably from its posterior neurosecretory cells. This paper examines some of the features of this diuresis in the light of this discovery and includes a section on the effect of temperature on the process of diuresis.

MATERIALS AND METHODS

As previously, 5th-stage larvae of *Rhodnius prolixus* were used in the experiments. Rates of excretion by fed insects were measured in two ways; (1) Drops of urine were collected under liquid paraffin and the times at which they were produced were noted from a stop-watch. The sizes of the drops were measured by calculating their volumes from measurements of their diameters assuming them to be spherical (Maddrell, 1963). (2) The urine was collected in a weighed polythene tube half filled with liquid paraffin and the tube was reweighed at the end of a measured length of time. Since the specific gravity of urine produced in diuresis is 1·007 (Wigglesworth, 1931), these methods are approximately equivalent.

Sodium concentrations in samples of urine and haemolymph were measured using an EEL flame photometer, while osmotic concentrations were estimated using the cryoscopic method of Ramsay & Brown (1955). The sodium determinations were accurate to within ± 5–6 mM/l. and melting-point determinations could easily be made with an accuracy of $\pm 0{\cdot}005°$ C.

The experiments on the effects of temperature were carried out in a series of rooms maintained at different constant temperatures. The insects used were taken from a laboratory culture kept at 27° C.

RESULTS

The course of normal diuresis

(*a*) *The onset of diuresis*

It is reasonable to suppose that diuretic hormone released into the mesothorax must arrive at the Malpighian tubules at an effective concentration before diuresis

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can begin. Diuresis starts within 3 min. of the time that feeding begins (Maddrell, 1963) so that even if the hormone is released quickly, there must be a rapid circulation of the haemolymph. To investigate this, a small amount of neutral red dissolved in saline was injected through a leg into the mesothorax in each of five partially fed insects. The subsequent movements of the dye were readily followed by holding each insect over a bright light. In each case, the dye was found to reach the tubules within 30 sec., indicating that there is a brisk circulation of the haemolymph at this time. It was obvious from the movements of the dye that powerful peristaltic movements of the opaque mid-gut were a major factor in causing this fast circulation. These movements are infrequent and weak in unfed insects. To find out how soon they become vigorous, ten insects were each allowed to feed for 30 sec. and were immediately examined by transmitted light. In each insect, although no blood had yet reached the mid-gut, vigorous peristaltic waves were running down the mid-gut towards the rectum. In five of these insects the movements were timed at 1–2 per minute before feeding and at 9–10 per minute after feeding for 30 sec. It seems reasonable to assume that the arrival of the hormone at the tubules so soon after feeding starts can be attributed, at least in part, to the increased rate of circulation of the haemolymph caused by the peristalsis of the mid-gut.

Since diuresis starts promptly and feeding usually occupies 15 min., a considerable volume of urine must be produced during feeding. Voiding of the urine is under nervous control, however, and the urine is retained until the insect has finished feeding, with the consequence that the first drop is much bigger than subsequent drops (Fig. 1). This retention is perhaps of importance to the insect because by so doing it may avoid calling attention to itself while feeding. When a drop of urine is about to be eliminated, a short cylindrical structure is protruded from the anus, whereupon any increase of pressure in the abdomen, as with squeezing with thumb and finger, causes the voiding of the drop. The insect appears to provide this pressure by a powerful synchronous contraction of the vertical tergosternal muscles of the abdomen, for the patches of light-coloured cuticle over their insertions become indented every time a drop of urine is voided. A trio of these muscles occurs near each lateral edge of abdominal segments 2–7 inclusive. Contraction of the muscular rectal wall probably assists the extrusion of the drop.

(b) The rate of excretion

Although during diuresis the rectum constantly fills and empties, its average size, as observed through the transparent cuticle, remains constant. Since it is thought that the activity of the rectum is of negligible importance during diuresis (Maddrell, 1963), it has therefore been assumed that the rate at which the urine is voided from the anus is a direct measure of the rate of secretion by the Malpighian tubules. In the many cases studied the pattern of diuresis was always the same. Provided that the temperature was constant, clear urine was produced at a high and surprisingly constant rate from the end of feeding until it fell away rapidly at the end of diuresis (Fig. 1). The following experiments using preparations of isolated Malpighian tubules (Maddrell, 1963) have suggested an explanation of the constancy of the rate of diuresis. Different concentrations of hormone were prepared by adding different amounts of brei of mesothoracic ganglia to 4 μl. of haemolymph, and were tested on sets of resting

tubules. For each set of tubules, the highest rate of secretion reached was measured. After each experiment, before being used again, the tubules were rinsed twice in inactive haemolymph from unfed insects. From the results (Fig. 2), it is clear that the response increases with the dose up to a maximum. Further increase of the dose merely lengthens the time for which the maximum rate is maintained (Fig. 3). The activity of the hormone in the haemolymph of recently fed insects has been investigated by making dilutions of the haemolymph with isotonic Ringer solution and testing these dilutions on preparations of isolated tubules; in this way it has been shown that during diuresis the haemolymph contains the diuretic hormone at a concentration a little higher (0–50%) than that needed to elicit the maximum rate of secretion from the tubules. It seems likely that this is the explanation of the constancy of the rate of excretion during diuresis.

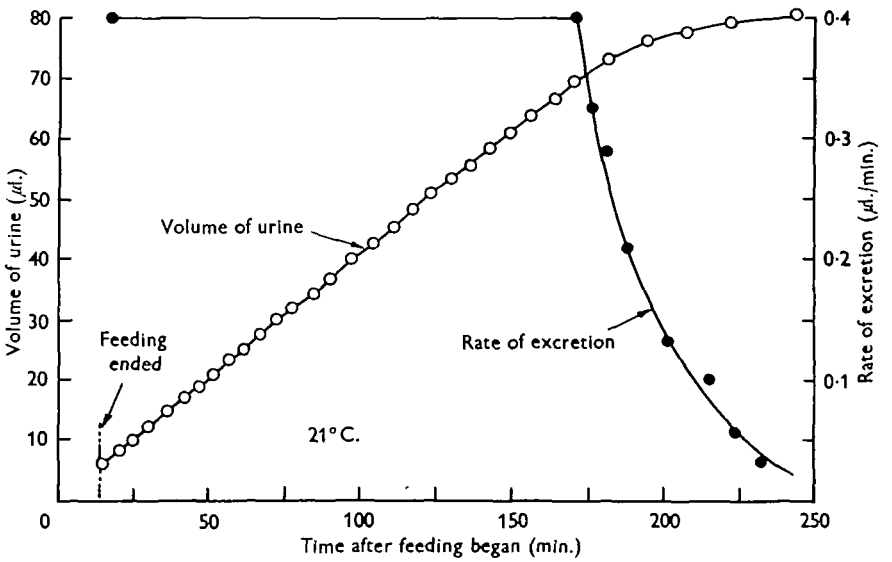

Fig. 1 The course of diuresis after feeding.

(*c*) *The extent of diuresis*

Most larvae evacuate the rectum on being handled. Only those insects which upon observation with transmitted light proved to have empty recta were used in the following experiments. The insects were weighed and allowed to take meals of different sizes and then were immediately weighed again to find out how much blood they had consumed. After diuresis, they were weighed once more, the loss in weight representing the amount of urine eliminated. They were then dissected and the volume of urine found in the rectum was added to that actually voided to give the total amount of urine produced. From the results (Fig. 4), it is clear that for meals larger than about 40 mg. of blood, about 40–50% by weight of the meal is excreted. For meals smaller than this there is considerable variation in the proportion excreted. Low values were obtained when insects were used that had been starved over a considerable period and high values when the insects were fed soon after moulting. It is probable that tissues of starved insects need to take up water to recover from

partial desiccation. It is known, for example, that the lower the water content of the tissues of the tsetse fly at the time of feeding, the less water is excreted afterwards (Bursell, 1960), while a similar explanation would fit observations on the bed-bug (Mellanby, 1935).

The extent of diuresis is dependent on the length of time during which the diuretic hormone is to be found in the haemolymph, for only haemolymph taken from insects

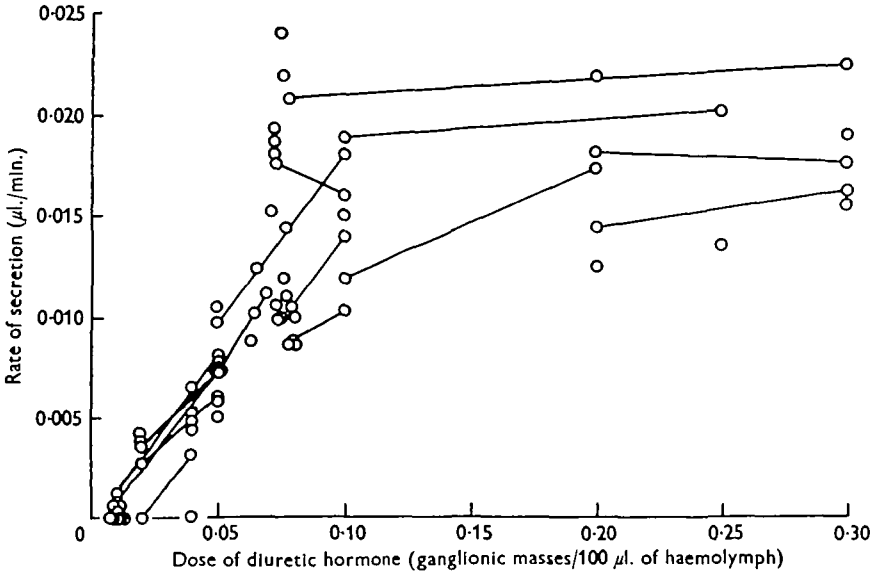

Fig. 2. The response of sets of isolated Malpighian tubules to different doses of diuretic hormone. The lines join determinations made on the same preparation.

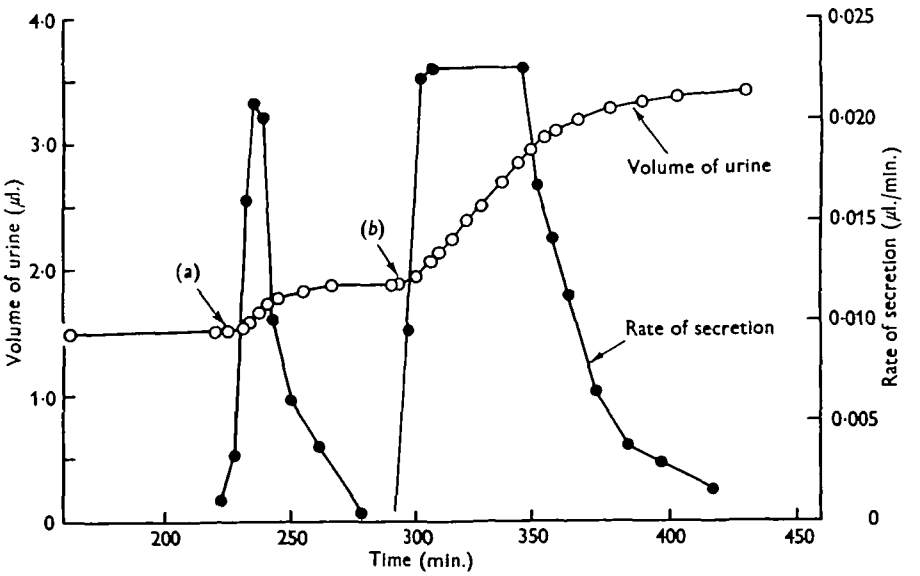

Fig. 3. The response of a set of isolated tubules to successive doses of the diuretic hormone of (*a*) 0·078, (*b*) 0·30 ganglionic masses/100 μl. of haemolymph.

during diuresis causes resting isolated tubules to resume secretion (Maddrell, 1963). This raises the question of the fate of the hormone. Its loss from the haemolymph might be a result of one of the following processes: the excretion of the hormone unchanged, the spontaneous decay of the hormone, the breakdown of the hormone by the Malpighian tubules or other tissues. The first possibility could be excluded because a large dose of hormone added to a preparation of tubules discharging their secretion into a drop of haemolymph bathing another set of tubules did not affect the rate of secretion of this second set (five experiments). That the second set was competent to respond was shown when hormone was later added directly to the haemolymph bathing the tubules. If the drop of secretion was returned to the haemolymph bathing a set of tubules there was no acceleration of their secretion (five cases).

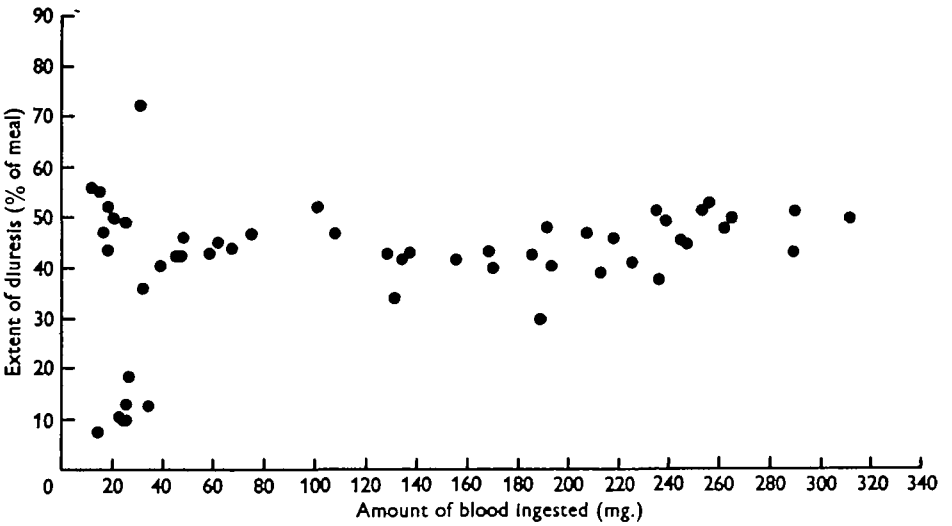

Fig. 4. The relation of the extent of diuresis to the size of the meal.

Spontaneous decay of the hormone does occur, for it was found that active samples of haemolymph lost their activity when kept under liquid paraffin for 1½–2 hr. This loss was not due to solution of the hormone in the liquid paraffin because drops of the same haemolymph of very different surface areas lost their activity at the same rate. It was still possible that the tubules might break down the hormone appreciably faster than it decayed. Fig. 5 displays the results of an experiment designed to test this possibility. Five sets of tubules were isolated from fed insect and the first, third, fourth and fifth sets (A, C, D and E in Fig. 5) were placed in one quarter of a large sample of active haemolymph, while the second set (B) in another quarter of the sample acted as a control. The two other parts of the sample were left unoccupied. From the secretory activity of each of the five sets of tubules it was clear that secretion is slower and is cut short much more quickly when several sets occupy a limited supply of active haemolymph. One of the unoccupied samples was scarcely less active at the end of an hour, as shown by the behaviour of a freshly isolated set of tubules (F) placed in it, whereas the haemolymph bathing several sets was very much reduced in activity at the end of 30 min. and was nearly inactive at 45 min. In order to show

that the sets of tubules last put into the test drop could have responded to diuretic hormone one of them (E) was moved into the last unoccupied sample of active haemolymph at 65 min. whereupon secretion was resumed. Two similar experiments gave essentially the same results. The possibilities that the different sets of tubules were interacting chemically or were producing a substance that neutralized the diuretic hormone could be discounted, because haemolymph taken from a drop bathing four sets of tubules had no more effect on the activity of a further active sample of haemolymph when added to it than did an equal volume of isotonic Ringer's solution. It is concluded that the diuretic hormone is quickly broken down by the activity of the Malpighian tubules. Since the concentration of the hormone in the haemolymph is never very much higher than that needed to cause the maximum response from the tubules, it follows (*a*) that the insect has a potential method for the precise control of diuresis, because once the release of the hormone stops, diuresis is quickly brought to a halt, and (*b*) that there must be a continued release of the hormone in order to maintain diuresis.

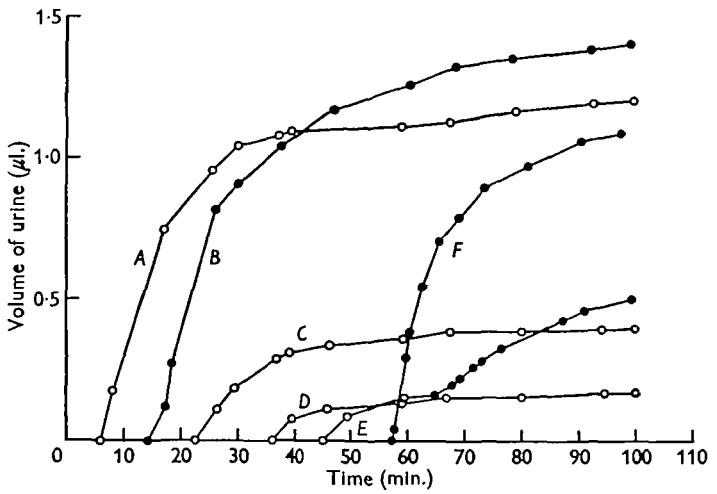

Fig. 5. The secretory behaviour of sets of tubules placed singly (closed circles) or together (open circles) in samples of active haemolymph. For further explanation see text.

This last idea is supported by the results of experiments in which substantial amounts of isotonic Ringer's solution were injected through a leg into the haemocoel of each of several fed insects. When this was done soon after feeding, the flow of urine was only temporarily slowed (Fig. 6), but in insects near the end of diuresis, this treatment stopped the flow prematurely (Fig. 7). Presumably, in the first case, the high rate of release of the hormone was sufficient to allow the hormone concentration to recover to that value necessary to cause the tubules to secrete at the maximum rate; while the slower release near the end of diuresis precluded such a recovery in the face of a sudden increase in the volume of the haemolymph.

(*d*) *The concentrations of the haemolymph, urine and gut contents*

The osmotic concentrations of the haemolymph, urine and gut contents vary in a related fashion during diuresis. Fig. 8 illustrates this point. Each determination of

the concentration of the gut contents involved the sacrifice of the insect, hence this figure necessarily comprises the results of many individual experiments. Each of the salient points was confirmed on at least five insects. After a meal the concentrations

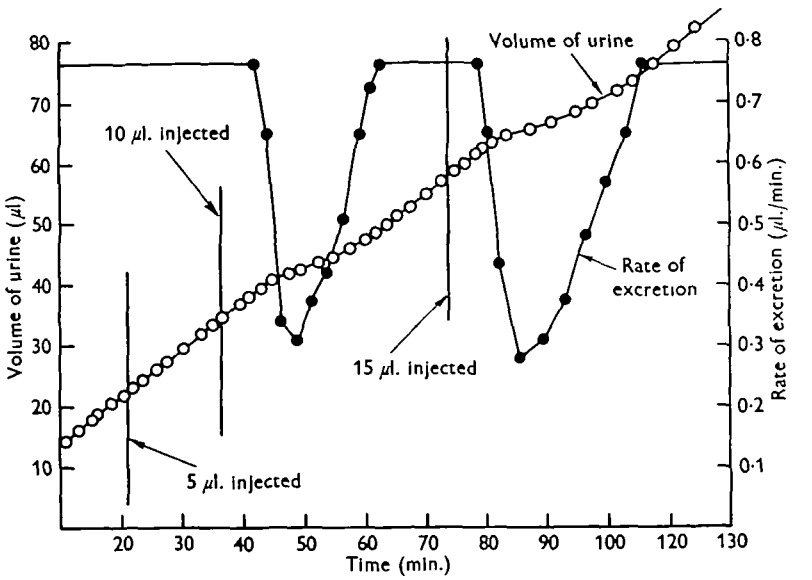

Fig. 6. The effect of injecting various quantities of isotonic Ringer's solution into the haemocoel of a freshly fed insect.

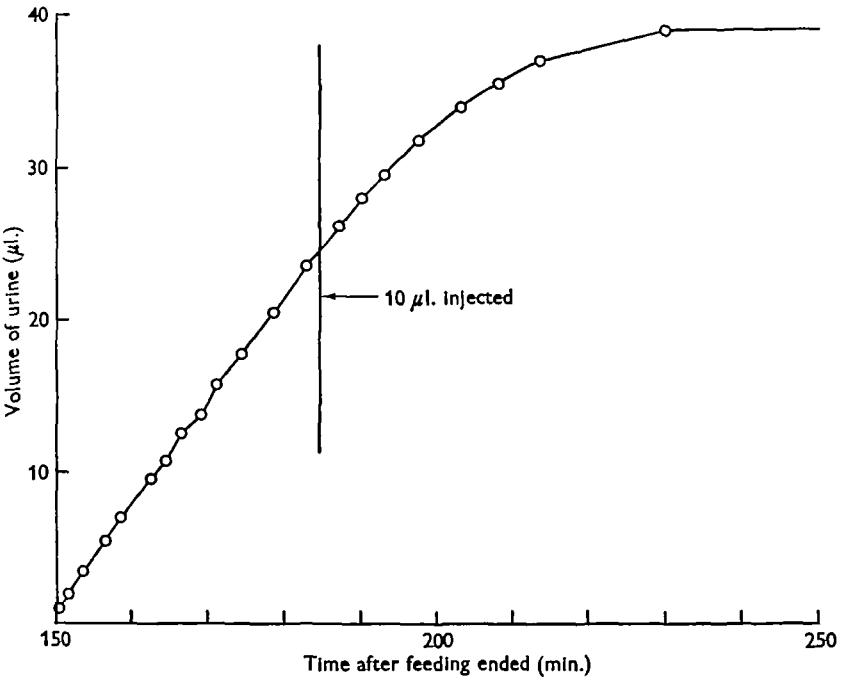

Fig. 7. The effect of injecting isotonic Ringer's solution into the haemocoel of an insect near the end of diuresis.

of the haemolymph and of the contents of the mid-gut soon reach the same value, presumably because the distended gut is permeable and the blood meal is able to equilibrate with the haemolymph. The subsequent steady rise in concentration of these two fluids would seem at first sight to be a direct result of the synthesis of a hypotonic urine. However, from the concentrations and volumes of the various fluids, calculation shows that only about half the observed increase can be explained in this way. Five weighed fully fed insects each with its anus plugged with wax lost less than 2 mg. each in 5 hr. at 29° C. and about 20% R.H., so that evaporation is not sufficient to account for the extra rise in concentration. In freshly fed insects the colour of the ingested blood, as seen through the transparent cuticle, changes rapidly from light red to dark brown, whereas in insects killed just after they have fed, it remains bright red for several days. So, although digestion does not begin in earnest until some time after diuresis has ended (Wigglesworth, 1943), there may be some change occurring much earlier involving an increase in the number of soluble molecules. It is, of course, also possible that the cells take up water from the haemolymph or lose salts to it or both; all these changes would contribute to a rise in the concentration of the haemolymph and gut contents.

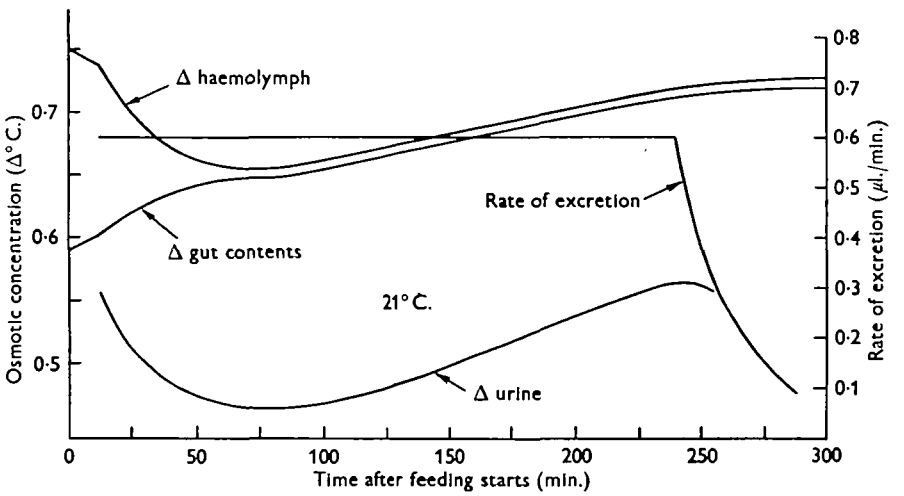

Fig. 8. Changes in the concentrations of the haemolymph, gut contents and urine during feeding and subsequent diuresis.

The tubules produce a urine whose concentration is lower than that of the haemolymph by a more or less constant amount, so that the changes in the concentration of the urine reflect changes in that of the haemolymph (Fig. 8). The concentration of sodium in the urine behaves similarly to the total osmotic concentration (Fig. 9); this is to be expected because sodium is the principal cation in the urine of *Rhodnius* (Ramsay, 1952).

As a result of these changes, the concentration of the haemolymph at the end of diuresis is scarcely lower than it was before feeding.

The effect of temperature on diuresis

(*a*) *The effect of temperature on the rate of excretion*

An increase in temperature quickly causes the rate of excretion to increase to a new high level, while the rate soon falls in response to a drop in temperature (Fig. 10). There seems to be little short-term regulative or compensatory change in the rate of excretion at any one temperature, for the rates once established remained constant over the lengths of time involved. The time taken to reach a new steady rate of excretion is probably that needed for the temperature of the insect to attain that of the surroundings. It was assumed that the excretory organs were at air temperature. The rates of excretion determined for various insects at different temperatures are plotted in Fig. 11, and, as shown, it was possible to fit an S-shaped curve to the observations. The significant fact is that between 12 and 25° C. a change in temperature has a marked effect on the rate of excretion; Q_{10} in this range varies from 2·3 to 2·8. It was often possible, for example, to detect small changes in the room temperature from the changes in the rate of excretion.

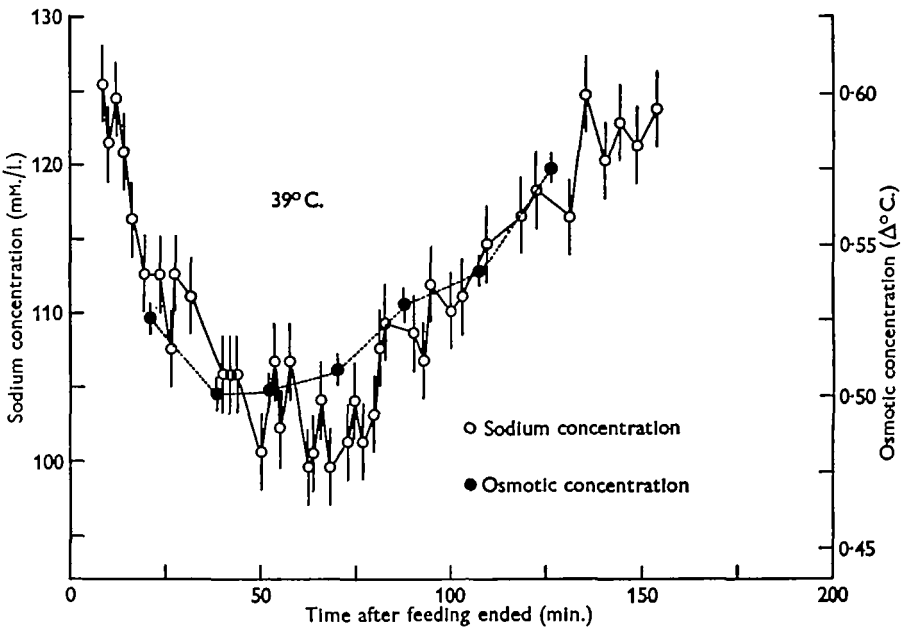

Fig. 9. The sodium concentration and osmotic concentration of the urine during diuresis. (The vertical line attached to each point represents the size of the probable error in each determination.)

(*b*) *The effect of temperature on the concentration of the urine*

The effect of a temperature change on the urine concentration was examined by taking measurements of the osmotic concentration and the sodium concentration of the urine of more than 20 insects before and after a change in temperature. Figs. 12 and 13 illustrate typical results of such experiments. When the concentration changes that would have occurred at a constant temperature are taken into account (thick

dotted lines in Figs. 12 and 13), it is clear that the effect of an increase in temperature is to raise the concentration of the urine and vice versa. It takes $\frac{1}{2}$–$\frac{3}{4}$ hr. for the concentration of the urine to adjust to a level characteristic of the new temperature, although the rate of excretion alters within 15 min. This is at least partly due to the presence in the rectum of some urine which is at a concentration characteristic of the first temperature. From the changes in the concentration of the haemolymph after a change in temperature (Tables 1 and 2), it is clear that the concentration of the haemolymph is only affected slightly by a change in temperature. The much larger changes in the concentration of the urine are, therefore, to be attributed to the activity of the excretory organs, in particular to the Malpighian tubules, since it is thought that the activity of the rectum contributes little during diuresis (Maddrell, 1963).

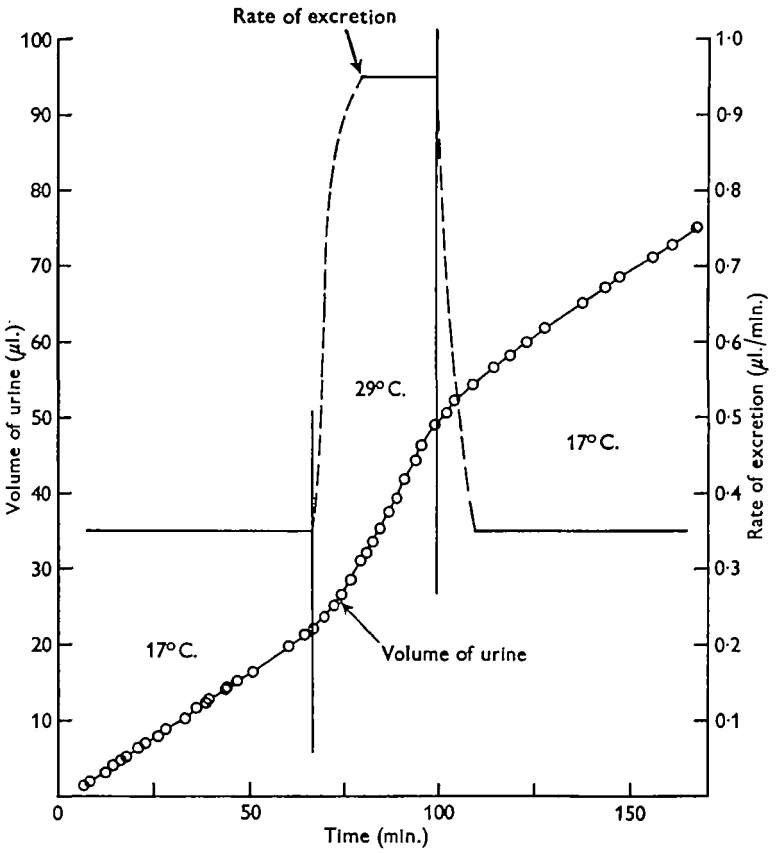

Fig. 10. The effect of an increase and a decrease in temperature on the rate of excretion during diuresis.

It follows from these results that a fed insect kept at high a temperature will be left at the end of diuresis with a relative excess of water and an insect kept at a low temperature with a relative excess of salts. It was, therefore, of some interest to follow the course of subsequent excretion in such insects. Table 3 sets out the results of an experiment in which two fed insects were kept at different temperatures during diuresis and were brought to the same temperature at the end of it. It is clear that

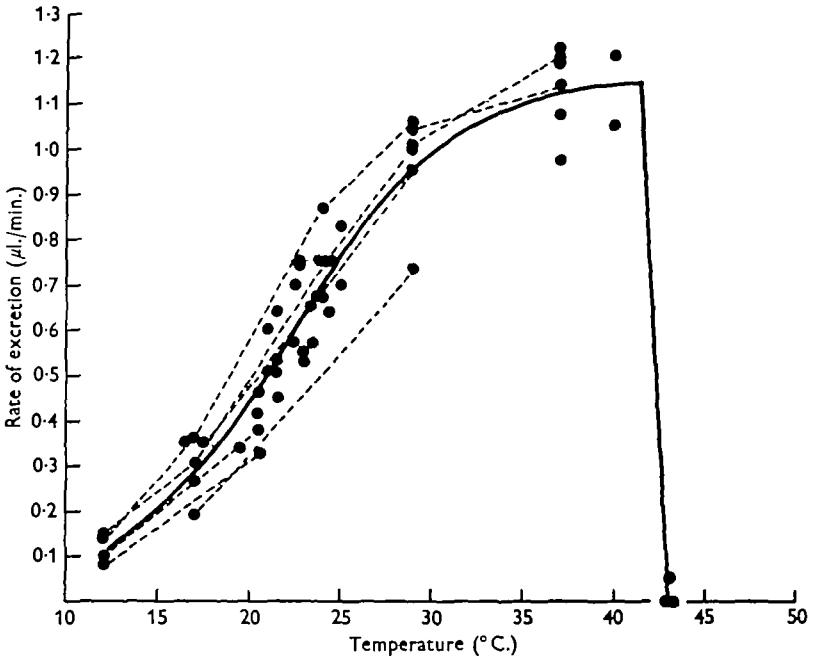

Fig. 11. The rates of excretion observed for various insects at different temperatures. The broken lines join determinations made on the same insect. The continuous line was fitted by eye.

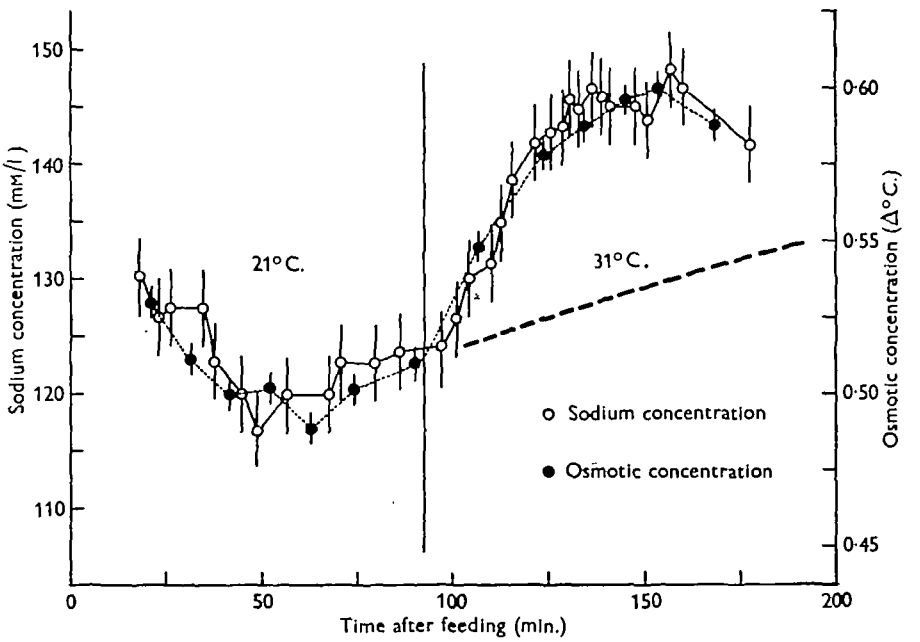

Fig. 12. The effect of an increase in temperature upon the concentration of the urine. (The vertical line attached to each point represents the size of the probable error in each determination.)

there was some regulation; the insect left with a relative excess of water (insect *B*) subsequently produced a large volume of dilute urine and the other insect a smaller quantity of much more concentrated urine. Two similar experiments gave essentially the same results.

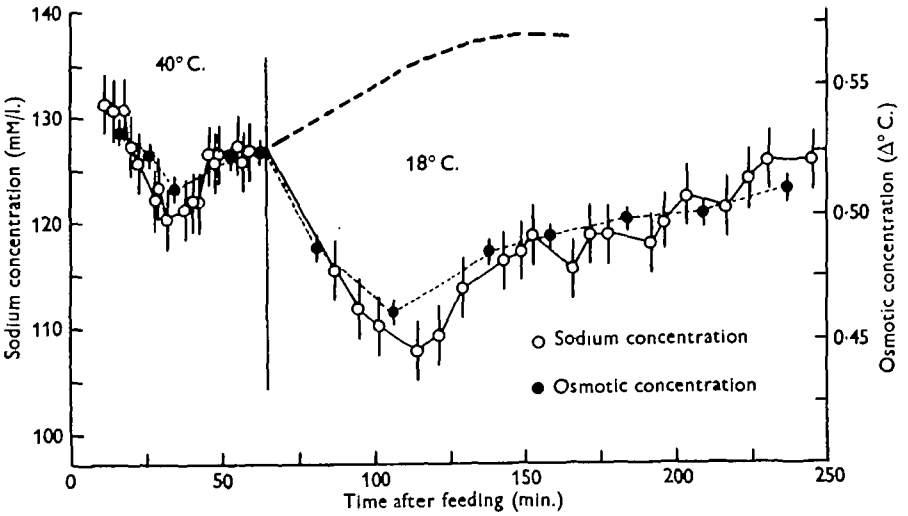

Fig. 13. The effect of a decrease in temperature upon the concentration of the urine. (The vertical line attached to each point represents the size of the probable error in each determination.)

Table 1. *The effect of an increase in temperature on the osmotic concentration of the haemolymph and urine of a fed insect*

	Urine	Haemolymph
Osmotic concentration after 90 min. at 17° C. (mM/l. NaCl)	115·8	169·5
Osmotic concentration after a further 40 min. at 37° C. (mM/l. NaCl)	142·6	179·0
Percentage increase	23·3	5·5

Table 2. *The effect of a change in temperature on the sodium concentrations of the haemolymph and urine of fed insects*

	$[Na]_{urine}$ (mM/l.) (mean ± S.E.)	$[Na]_{haemolymph}$ (mM/l.) (mean ± S.E.)
Samples taken from 9 insects kept at 17° C. for 2 hr.	130·4 ± 2·6	173·1 ± 3·5
Samples taken from 9 further insects kept at 37° C. for 1 hr. after 2 hr. at 17° C.	148·0 ± 2·6	175·8 ± 2·9

DISCUSSION

The excretion of hypotonic urine at the high rate which is characteristic of diuresis in the larva of *Rhodnius* is perhaps surprising in view of the high rate of energy expenditure which this must entail. The advantage to the insect is that it quickly overcomes the handicap of its large size after feeding and that its tissues are bathed

Table 3. *The effect on subsequent excretion of keeping two insects at different temperatures during diuresis*

Diuresis at different temperatures

	Insect *A*	Insect *B*
Weight unfed (mg.)	39·7	42·2
Weight of meal (mg.)	344·5	360·9
Kept at 100% R.H. and at	17° C.	37° C
During	420 min.	240 min.
Weight of urine produced (mg.)	135·3	146·7
% of meal excreted	39·3	40·8
$[Na]_{urine}$ (mM/l.)	129·9	145·1
O.P.$_{urine}$ (mM/l. NaCl)	145·2	162·7

Subsequent excretion at 27° C.

A				*B*			
		Concentration				Concentration	
Time (hr.)	Volume (μl.)	sodium (mM/l.)	osmotic (mM/l. NaCl)	Time (hr.)	Volume (μl.)	sodium (mM/l.)	osmotic (mM/l. NaCl)
7–12	5·0	181	288	4–7	5·8	178	197
12–18	4·6	194	328	7–18	5·4	143	170
18–21	3·0	199	348	18–21	7·0	125	177
40–48	3·6	203	366	40–48	2·6	95	217
48–64	2·6	—	423	48–64	3·5	98	225
64–112	3·2	270	880	64–112	4·5	95	232
				112–136	5·8	—	691
	22·0			136–160	3·9	Semi-solid	
					38·5		

in a fluid of abnormally low concentration for as short a time as possible. The speed at which diuresis gets under way and the fact that the concentration of the diuretic hormone in the haemolymph is more than adequate to elicit the fastest possible rate of secretion by the Malpighian tubules combine to speed the whole process. At the end of diuresis the fed insect has lost about 40% in weight and the osmotic concentration of its haemolymph is approximately the same as it was before feeding.

SUMMARY

1. The course of diuresis in *Rhodnius* is described and interpreted in terms of the underlying mechanism.

2. The rapid onset of diuresis is attributable to the prompt release of the diuretic hormone into the haemolymph and to an acceleration of the circulation of the haemolymph caused by peristaltic movements of the mid-gut.

3. Diuresis proceeds at a surprisingly constant rate. This is shown to be a reflexion of the fact that the concentration of the diuretic hormone in the haemolymph at this time is always higher than that which causes the maximum response by the Malpighian tubules.

4. The diuretic hormone is quickly destroyed by the activity of the Malpighian tubules and possibly other tissues. Consequently, the extent of diuresis is controlled by the length of time during which diuretic hormone is released into the haemolymph.

5. Excretion is very sensitive to changes in temperature; both the rate of excretion and the composition of the urine are affected.

This work is part of a thesis submitted to the University of Cambridge for the degree of Ph.D. It is a pleasure to record my thanks to Prof. V. B. Wigglesworth for his encouragement and supervision, to Drs J. E. Phillips and E. H. Anthony for reading and criticizing the manuscript and to the Department of Scientific and Industrial Research for generous financial support.

REFERENCES

BURSELL, E. (1960). Loss of water by excretion and defaecation in the Tsetse fly. *J. Exp. Biol.* **37**, 689–97.

MADDRELL, S. H. P. (1962). A diuretic hormone in *Rhodnius prolixus* Stål. *Nature, Lond.*, **194**, 605–6.

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.

MELLANBY, K. (1935). A comparison of the physiology of the two species of bed-bug which attack man. *Parasitology*, **27**, 111–22.

RAMSAY, J. A. (1952). The excretion of sodium and potassium by the Malpighian tubules of *Rhodnius*. *J. Exp. Biol.* **29**, 110–26.

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**, 372–5.

WIGGLESWORTH, V. B. (1931). The physiology of excretion in a blood-sucking insect, *Rhodnius prolixus* (Hemiptera, Reduviidae). I. The composition of the urine. *J. Exp. Biol.* **8**, 411–27.

WIGGLESWORTH, V. B. (1943). The fate of haemoglobin in *Rhodnius prolixus* (Hemiptera) and other blood-sucking arthropods. *Proc. Roy. Soc.* B, **131**, 313–39.