J. Exp. Biol. (1965), **43**, 511–521
With 5 text-figures
Printed in Great Britain

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

I. ANATOMY, WATER EXCRETION AND OSMOREGULATION

By M. J. BERRIDGE
*Department of Zoology, University of Cambridge**

(*Received* 20 *May* 1965)

INTRODUCTION

In recent years there has been a considerable volume of research on insect excretion, but in most cases only descriptive accounts of the composition of the excreta have been given (Irreverre & Terzian, 1959; Powning, 1953; Terzian, Irreverre & Stahler, 1957; Nelson, 1958; Nation & Patton, 1961; Harington, 1961; Mitlin, Vickers & Hedin, 1964). The physiology of excretion in insects has also been studied with respect to the mechanisms involved in the formation of urine (Patton & Craig, 1939; Ramsay, 1951–54, 1955*a*, *b*, 1956, 1958; Phillips, 1961, 1964*a*, *b*, *c*).

Apart from a few studies (Wigglesworth, 1931*a*, *b*, *c*; Brown, 1936, 1938*a*, *b*), very little has been done on the relationship of excretion to other physiological processes and, more specifically, to changes associated with moulting. For this reason, excretion in the cotton stainer has been studied extensively; this paper is mainly concerned with the excretion of water.

MATERIAL

All experiments in this paper have been performed on female fifth-instar larvae. Adults have not been used because they mate 1–2 days after emergence and remain *in copula* until the first oviposition 6–8 days later. A more important reason for using larvae instead of adults will be discussed later.

Dysdercus colonies were reared in large glass jars on a diet of moist cotton seeds and water. It was found convenient to set up a fresh culture jar each week; twenty-five pairs of adults of approximately the same age were placed in a jar provided with a layer of moist peat in which the eggs were deposited. Animals in any one culture jar were all of approximately the same age, because the adults oviposited over a short period. Cultures were kept at 25° C.; moist cotton seeds and water were replaced at 2-day intervals.

When the insects in each stock culture reached the fourth instar, they were inspected at twelve hourly intervals; those larvae which had moulted were removed and placed in small glass dishes. The age of each batch was reckoned from the time when the animals were removed from the stock-culture jar; the ages of these fifth-instar larvae varied from 0 to 12 hr. This age variation is acceptable in the light of the small variations which have been recorded when various measurements are made on a batch of these

* Present address: Department of Biology, University of Virginia, Charlottesville, Virginia, U.S.A.

animals. The cotton seeds and water of these experimental animals, which were also kept at 25° C., were replaced at daily intervals.

METHODS

The general anatomy of the digestive and excretory systems was studied by dissection under Ringer solution.

Animals were weighed in a small container to 0·1 mg. on a Stanton Ultramatic balance. For the determination of water content larvae were dried to a constant weight at 80° C.; care was taken to weigh animals as soon as removed from the oven, because adsorption of water vapour from the atmosphere is extremely rapid. Graduated micro-pipettes were used to measure urine volume; each micro-pipette was calibrated by weighing the volume of mercury which it delivered.

Osmotic pressure determinations were made on haemolymph and urine immediately after removal from the animal, using the cryoscopic method of Ramsay & Brown (1955).

The vertical lines on the graphs represent ± twice the standard error of each mean.

RESULTS

Anatomy

The mid-intestine of *Dysdercus* may be regarded as made up of four distinct regions (Fig. 1). The oesophagus leads into a swollen first region of midgut (m_1), which is connected to an enlarged sac-like third region (m_3) by a long tubular part of midgut (m_2). The fourth region of midgut (m_4), which connects m_3 with hindgut, is relatively short, and, in the female only, bears a number of small caecae. m_4 and rectum are connected by a small ileum, to which the two pairs of Malpighian tubules are attached. The rectum is a large ovoid sac, with thin walls capable of great distension.

The arrangement of the four Malpighian tubules in *Dysdercus* is unusual in that the two tubules on each side of the animal are connected to each other at their distal ends to form a continuous loop (Fig. 1). *In situ* the major part of this loop lies posteriorly as a tangled mass which is closely associated with the posterior part of the aorta. The two ends of each loop lead out of this mass and run anteriorly to as far as m_1 before curving back to connect with the ileum. These two leads are held in position by a network of tracheae.

One of the most important aspects of intestinal anatomy, certainly as far as excretion is concerned, is that the intestine is discontinuous during the larval instars. The discontinuity occurs at m_4, which in all larvae is a small undifferentiated strand of tissue connecting m_3, with the ileum. m_4 only becomes functional in the adult when it develops a lumen, and food residues, which have accumulated in m_3 throughout the larval stages, pass through into the rectum.

These anatomical observations on the discontinuity of the gut have been substantiated by feeding animals with a suspension of Chinese black in place of the usual distilled water. The presence or absence of ink in the excreta was checked by keeping the animals over filter paper. Chinese black was completely absent from the excreta of larvae, which were always liquid and dried on the filter paper to give slightly yellowish spots. Dissection of a few fifth-instar larvae clearly indicated that Chinese black

had been ingested; the dye was present in all parts of the midgut, but the rectum was completely free of particulate matter. Phagocytosis of Chinese black by the cells of the alimentary canal was not evident. During the adult stage, when the gut became continuous, Chinese black began to appear in the excreta.

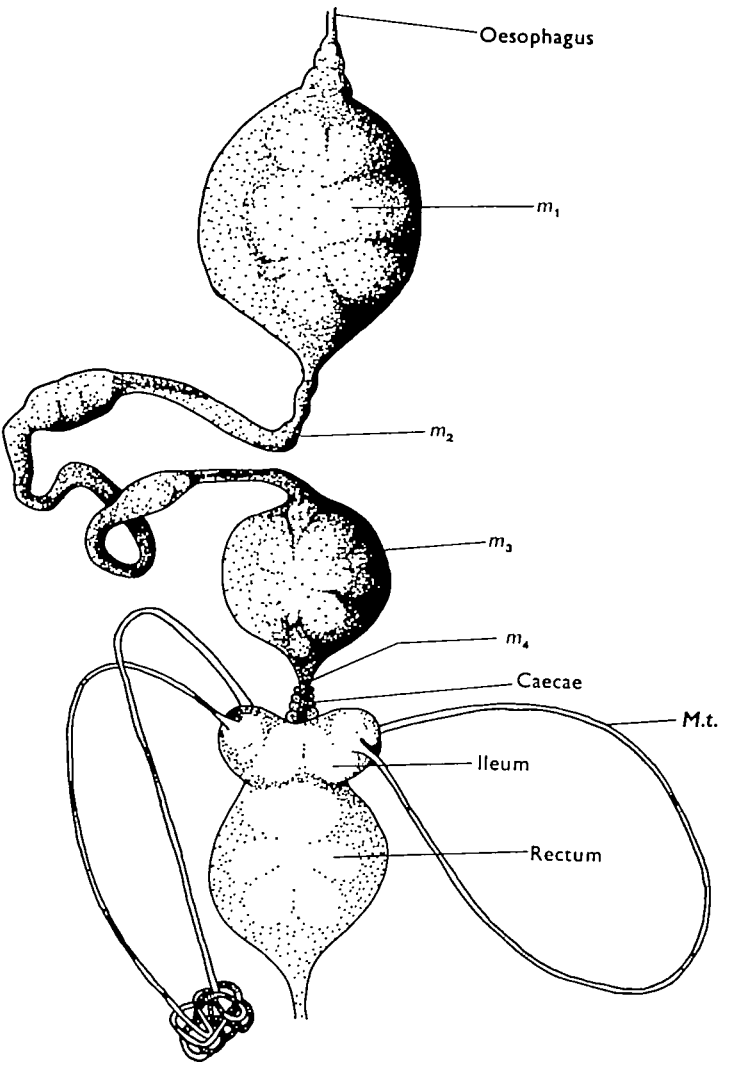

Fig. 1. The alimentary canal and excretory system of *Dysdercus*. The Malpighian tubules on one side have been freed of tracheal attachments to show that the distal ends are connected to each other to form a loop. m_1, m_2, m_3, m_4, Four regions of the midgut; *M.t.*, Malpighian tubules.

Body weight and water content

The fifth instar lasts for 8 days. During this period dry weight increases fairly regularly up to the fourth day; the wet weight, however, continues to increase until the sixth day. These weight changes (Fig. 2*a*) suggest that, apart from liquid intake, feeding ceases after the fourth day.

The water content (Fig. 2*b*) shows a very marked fall during feeding to a minimum of 62% on the fourth day. There is a subsequent increase in the water content as the animal drinks in the latter part of the instar.

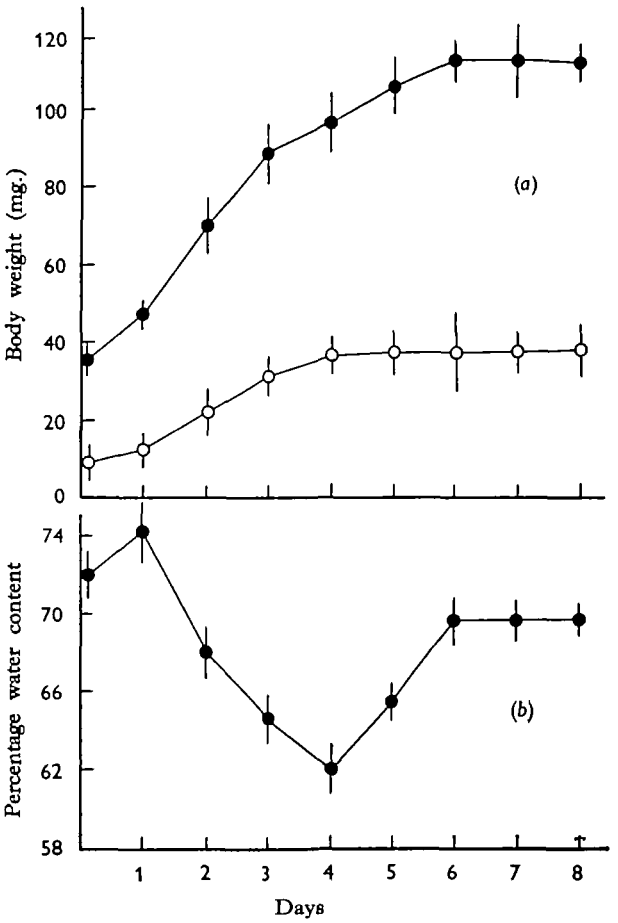

Fig. 2. (*a*) The increase in weight of female fifth-instar larvae. ●, Wet weight; ○, dry weight. (*b*) Water content of female fifth-instar larvae.

Rate of excretion during the fifth instar

One of the most striking features of excretion in *Dysdercus* is that the urine is completely liquid. This urine is not analogous to the honeydew produced by sap-sucking insects because, as already described, the gut of *Dysdercus* is discontinuous throughout the larval instars. The liquid excreted is produced solely by the Malpighian tubules. The rate of excretion of this liquid urine, expressed as μl./24 hr., was estimated by two methods.

(i) Individuals were kept over filter paper in small Perspex containers. The animals had access to cotton seeds and water through two small holes in the floor of the container. The filter paper was replaced daily, and the volume excreted was calculated from the size of the spots, which were clearly visible when the paper was

viewed under ultraviolet light. A calibration curve was used which related volume to diameter. (This calibration curve was obtained by spotting standard volumes of urine on filter paper, the diameter of these spots being then measured and used to construct the calibration curve.)

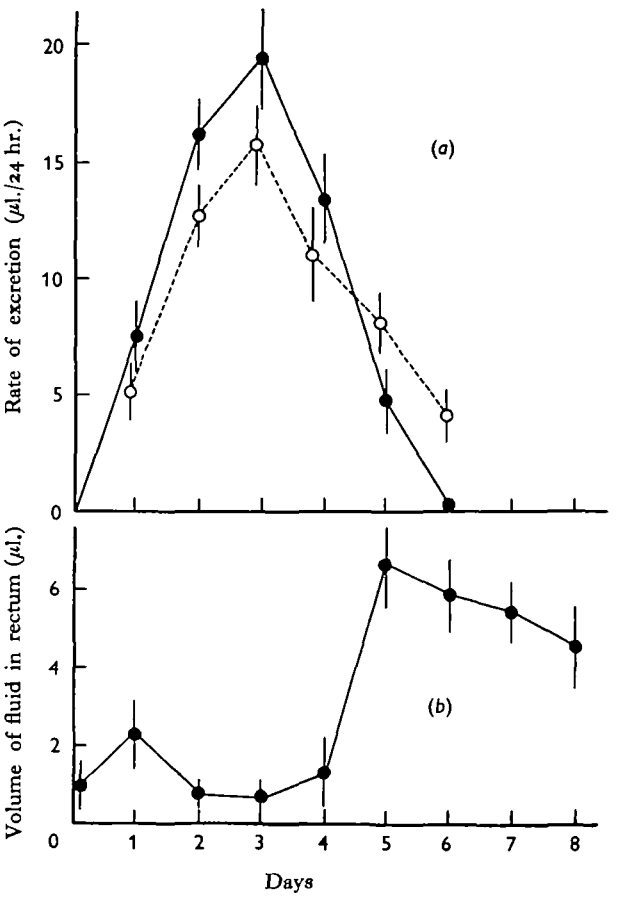

Fig. 3. (*a*) Rate of urine excretion throughout the fifth instar. ●, Collection method (i); ○, collection method (ii). For further explanation see text. (*b*) Volume of fluid in rectum.

(ii) The second method for measuring rate of excretion entailed blocking the anus. The rectum was emptied of fluid by gently squeezing the abdomen; the anus was closed off by applying a little molten wax over the external aperture. The animal was then returned to its feeding dish. After 24 hr. the wax seal was removed with a pair of forceps; the fluid which had accumulated in the rectum was then squeezed out and measured.

These two methods gave essentially the same results (Fig. 3 *a*). The rate of excretion reaches a peak on the third day, which is followed by a sharp decline. There is no further excretion of fluid after the fifth day. The very high rate of excretion which occurs from the second to the fourth day may well account for the marked decrease in water content over this period (Fig. 2 *b*). The subsequent increase in water content

in the latter part of the instar is possibly correlated with the cessation of excretion on the fifth day.

If the anus is not blocked, the volume of fluid in the rectum during the period 0–4 days is very low (Fig. 3*b*); this is the period when the animals are actively excreting. The explanation for this is probably behavioural. Animals in this period excrete urine immediately if disturbed or handled. After the fourth day, however, a large

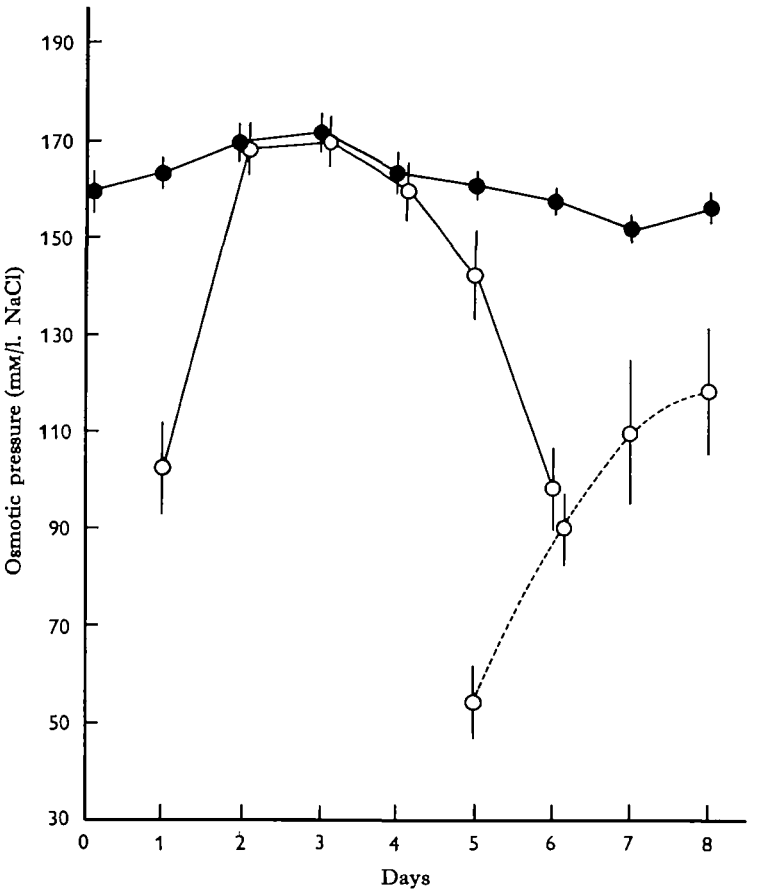

Fig. 4. The osmotic pressure of urine and haemolymph. ●——● , haemolymph; ○——○, urine (excretory phase); ○ – – – – ○, urine (post-excretory phase).

volume of fluid is retained in the rectum (Fig. 3*b*). This fluid can only be removed by applying considerable pressure to the abdomen. The volume of this retained fluid appears to reach a maximum on the fifth day, then decreases as the animal progresses towards the final moult. The degree of water reabsorption, as will be shown later, is dependent on the amount of water lost by transpiration during this period.

From the foregoing results, it is evident that excretion in *Dysdercus* may be divided into two distinct phases.

(i) *Excretory phase* (0–5 days). This phase, correlated with feeding activity, is characterized by a high rate of excretion.

(ii) *Post-excretory phase* (5–8 days). Fluid is not voided, but retained in the rectum.

Osmotic pressure of the haemolymph and urine

Probably the most important fact to emerge from the measurements of osmotic pressure is that at no time in the fifth instar is the urine hypertonic to the haemolymph (Fig. 4). The osmotic pressure of the latter remains relatively constant throughout the instar. In the excretory phase the most concentrated urine is produced in the

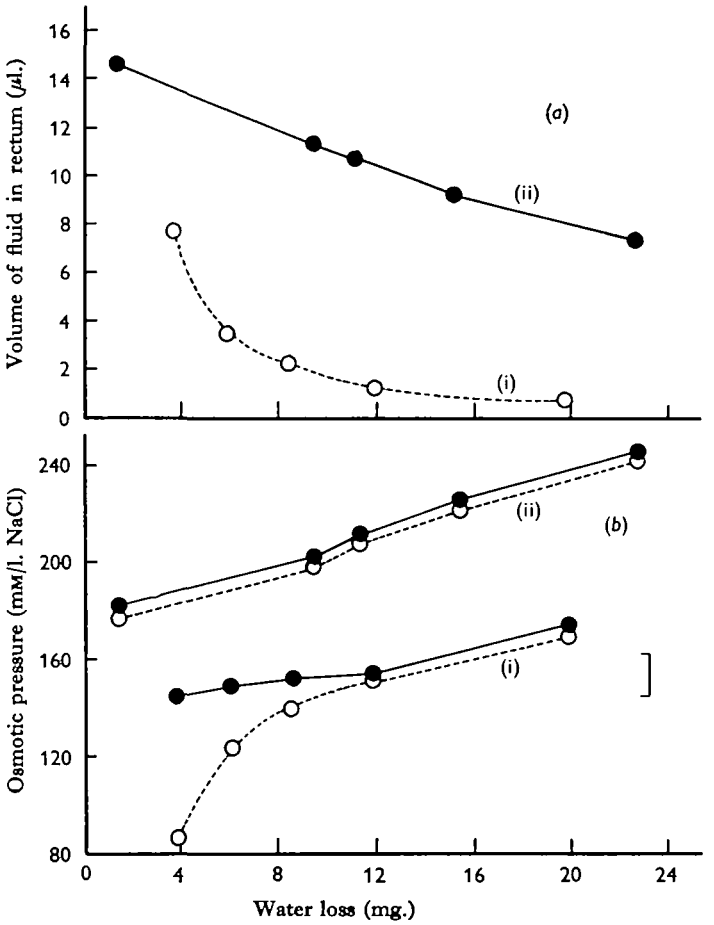

Fig. 5. (*a*) Volume of rectal fluid in relation to water loss. (i) Post-excretory phase; (ii) excretory phase. (*b*) Osmotic pressure of urine and haemolymph in relation to water loss. ●, Haemolymph; ○, urine. (i) and (ii) as above. Vertical line represents the normal range of osmotic pressure.

middle of the feeding period. As feeding activity declines, however, the urine becomes progressively more hypotonic to the haemolymph. Similarly, the urine which is stored in the rectum in the post-excretory phase is extremely hypotonic. The decrease in volume of this fluid is accompanied by an increase in its osmotic pressure.

The very considerable hypotonicity of this retained urine suggests that the fluid may function as a water store. The gradual decrease in volume throughout the post-excretory phase probably indicates water reabsorption from the rectum. This idea

has been tested by studying the osmoregulation of these animals in varying states of dehydration. By way of comparison, osmoregulation was also studied in animals from the excretory phase. The experimental procedure for the two groups is outlined below:

(i) *Post-excretory phase*

Fifth-instar larvae were allowed to feed normally until the sixth day, when they were placed in a series of graded humidities until the eighth day. The weight decrease (used as a measure of water loss), the volume of rectal fluid and the osmotic pressure of the haemolymph and urine were then determined on individuals from each of the humidities.

(ii) *Excretory phase*

Since the volume of fluid in the rectum during this phase is extremely small (Fig. 3*b*), measurements had to be made on urine that accumulated after the anus had been blocked with wax on the second day. The animals were allowed to feed normally until the third day, whereupon they were placed in the humidity chambers. Two days later these animals were treated as described in (i). In this group the main difference from (i) was that the animals started off with rectal fluid which was almost isotonic with the haemolymph.

Osmoregulation during the post-excretory phase will be considered first. The ability to reabsorb water from the rectum to compensate for varying degrees of water loss is shown in Fig. 5*a*(i). The volume of rectal fluid shows a marked decrease with increasing transpiration. The decrease in volume of the rectal fluid is reflected in an increase in its osmotic pressure (Fig. 5*b*(i)); the osmotic pressure of the haemolymph remains normal under moderate water loss. Only under very severe dehydration is the osmotic pressure of the haemolymph elevated above its normal range, and in this case the rectal fluid remains isosmotic with the haemolymph. The animal appears to be incapable of extracting water from rectal fluid once this becomes isosmotic with the haemolymph. Nevertheless, it is clear that the very considerable osmoregulatory ability of these animals can be attributed, at least in part, to reabsorption of water from the hypotonic urine which is stored in the rectum.

By contrast, however, animals in the excretory phase are unable to osmoregulate even under conditions of little water loss. There is some decrease in the volume o rectal fluid with increasing transpiration (Fig. 5*a*(ii)), but this occurs only at the expense of increasing the concentration of the haemolymph (Fig. 5*b*(ii)). The osmotic pressure of the haemolymph increases regularly with increasing water loss. The rectal fluid remains isotonic with the haemolymph throughout the whole range. Even under conditions of severe dehydration when the osmotic pressure of the haemolymph has increased as much as 50% of its normal value, the rectum still contains 8·0 μl. of urine. Clearly, this inability of the rectal epithelium to reabsorb water against an osmotic gradient must be partly responsible for the high rate of urine excretion (Fig. 3*a*).

DISCUSSION

The general arrangement of the gut in *Dysdercus fasciatus* is similar to that already described for *D. cuturellus* (Glasgow, 1914) and *D. koenigii* (Saxena, 1955), although a discontinuous intestine was not reported for either of the latter two species. The gut of *Oncopeltus fasciatus*, however, has a discontinuity in a similar position to that found in *D. fasciatus* (Miles, 1958). The caeca in *D. fasciatus* are also similar in position and appearance to those in *D. cuturellus* and *D. koenigii*; there is no indication that they operate as water excretory organs as proposed for the caeca of other Heteroptera (Goodchild, 1963*a*, *b*). The connexion of the Malpighian tubules at their distal ends to form a continuous loop has previously been described for *D. koenigii* (Srivastava & Bahadur, 1961). The significance of such an arrangement is by no means clear.

In view of the widespread discontinuities in the intestines of insects (Imms, 1957; Miles, 1958; Goodchild, 1963*a*, *b*) it is remarkable that advantage has not been taken of this anatomical feature in previous studies of insect excretion. Much of the literature on the composition of insect excreta is confusing, because no precautions were taken to exclude contamination of the urine by gut residues. In *Dysdercus* discontinuity of the intestine during the larval stages provides an opportunity to study inorganic excretion (Berridge, 1965*a*) and nitrogen excretion (Berridge, 1965*b*) in an animal where contamination from the gut can definitely be excluded. The urine appearing in the rectum is derived solely from the Malpighian tubules. When the rate of excretion is studied throughout the instar two distinct phases of excretion are apparent. During the excretory phase, while the animal is feeding, osmoregulation is presumably achieved by balancing water loss *via* the excretory system with drinking. This oversimplifies the problem, because in this phase there is a very rapid output of urine, which is considerably more concentrated than that stored in the rectum. The composition of this urine, and ionic regulation, will be discussed elsewhere (Berridge 1965*a*, *b*). The second phase of excretion is characterized by retention of dilute urine in the rectum, evaporative water loss being balanced by drawing water from this reservoir. The effectiveness of this mechanism is dependent on the amount of water available in the rectum. The volume of fluid reabsorbed from the rectum before urine and haemolymph become isosmotic is 5·8 μl.; on the fifth day the haemolymph volume is 13·6 μl. (Berridge, 1965*a*), which means that the animal is potentially capable of replacing 42·6% of its haemolymph volume. Clearly, water stored in the rectum during the post-excretory phase does play an important part in osmoregulation.

The role of the excretory system in the water regulation of terrestrial insects has not received much attention. In the tsetse fly *Glossina morsitans* loss of water through the excretory system is largely dependent on relative humidity, 'full powers of conservation are exercised only when water reserves are threatened' (Bursell, 1960). If flies are kept at a high relative humidity the water content of the faeces is about 75%, whereas in dry air it is only 35%. This retention of water has resulted in a saving of more than 30% of the total water reserve (Bursell, 1960). The ability to store water in the excretory system, however, has not been described before in insects, although the phenomenon is known in amphibia.

In *Dysdercus* the inability of the rectal epithelium to reabsorb water against an osmotic gradient is fundamentally different from that which has been recorded for

other insects, in which the rectal fluid can become considerably hypertonic (Wigglesworth, 1931*a*; Ramsay, 1952, 1955*a*; Phillips, 1961, 1964*a*). The rectum of the locust *Schistocerca gregaria*, and of the blowfly *Calliphora erythrocephala*, has been shown to take up water by active processes (Phillips, 1961, 1964*a*). No such active transport of water is evident in the rectal epithelium of *Dysdercus*; the reabsorption of water by the rectum probably depends solely on the transfer of water by passive diffusion.

SUMMARY

1. The intestine of *Dysdercus* is discontinuous in the larval instars, and urine from the Malpighian tubules is therefore uncontaminated by gut contents.

2. Excretion has been studied during the fifth instar, which lasts 8 days. Feeding only occurs in the first 4 days, during which there is a marked fall in water content. In the last part of the instar, when the animal only drinks, the water content returns to its original level.

3. The urine of *Dysdercus* is always liquid. There is a rapid rate of excretion during the first half of the instar, but when feeding ceases there is no further micturition and urine is retained in the rectum. Therefore there are two phases of excretion, designated excretory and post-excretory phases respectively.

4. The rectal epithelium is incapable of reabsorbing water against an osmotic gradient.

5. The urine which is retained in the rectum during the post-excretory phase acts as a water store; evaporative water loss is balanced by withdrawing water from this reservoir. During the excretory phase, a large volume of liquid is lost *via* the excretory system, but the loss is made good by drinking.

This work is part of a thesis submitted to the University of Cambridge for the degree of Ph.D. I am grateful to Prof. Sir Vincent Wigglesworth for his advice and supervision and to Dr E. Bursell for reading and criticizing the manuscript. I thank the Commonwealth Scholarship Commission for generous financial support.

REFERENCES

BERRIDGE, M. J. (1965*a*). The physiology of excretion in the cotton stainer, *Dysdercus fasciatus* Signoret. II. Inorganic excretion and ionic regulation. *J. Exp. Biol.* (in the Press).

BERRIDGE, M. J. (1965*b*). The physiology of excretion in the cotton stainer, *Dysdercus fasciatus* Signoret. III. Nitrogen excretion and excretory metabolism. *J. Exp. Biol.* (in the Press).

BROWN, A. W. A. (1936). The excretion of ammonia and uric acid during the larval life of certain muscoid flies. *J. Exp. Biol.* **13**, 131–9.

BROWN, A. W. A. (1938*a*). The nitrogen metabolism of an insect (*Lucilia sericata* Mg.). I. Uric acid, allantoin, and uricase. *Biochem. J.* **32**, 895–902.

BROWN, A. W. A. (1938*b*). The nitrogen metabolism of an insect (*Lucilia sericata* Mg.). II. Ammonia and other metabolites. *Biochem. J.* **32**, 903–12.

BURSELL, E. (1960). Loss of water by excretion and defaecation in the Tsetse fly. *J. Exp. Biol.* **37**, 689–697.

GLASGOW, H. (1914). The gastric caeca and the caecal bacteria of the Heteroptera. *Biol. Bull., Woods Hole*, **26**, 101–70.

GOODCHILD, A. J. P. (1963*a*). Studies on the functional anatomy of the intestines of Heteroptera. *Proc. Zool. Soc. Lond.* **141**, 851–910.

GOODCHILD, A. J. P. (1963*b*). Some new observations on the intestinal structures concerned with water disposal in sap-sucking Hemiptera. *Trans. R. Ent. Soc. Lond.* **115**, 217–37.

HARINGTON, J. S. (1961). Studies of the amino acids of *Rhodnius prolixus*. II. Analysis of the excretory material. *Parasitology*, **51**, 319–26.

IMMS, A. D. (1957). *A General Textbook of Entomology*, 9th ed. London: Methuen.

IRREVERRE, F. & TERZIAN, L. A. (1959). Nitrogen partition in excreta of three species of mosquitoes. *Science*, **129**, 1358–9.

MILES, P. W. (1958). Retention of food residues in the midgut by nymphs of the milkweed bug, *Oncopeltus fasciatus* Dall. *Nature, Lond.*, **182**, 959.

MITLIN, N., VICKERS, D. H. & HEDIN, P. A. (1964). End products of metabolism in the boll weevil, *Anthonomus grandis* Boheman: non-protein amino acids in the faeces. *J. Insect Physiol.* **10**, 393–7.

NATION, J. L. & PATTON, R. L. (1961). A study of nitrogen excretion in insects. *J. Insect Physiol.* **6**, 299–308.

NELSON, W. A. (1958). Purine excretion by the sheep ked, *Melophagus ovinus* L. *Nature, Lond.*, **182**, 115.

PATTON, R. L. & CRAIG, R. (1939). The rates of excretion of certain substances by the larvae of the mealworm, *Tenebrio molitor* L. *J. Exp. Zool.* **81**, 437–57.

PHILLIPS, J. E. (1961). Rectal absorption of water and salts in the locust and blowfly. Ph.D. thesis, Cambridge.

PHILLIPS, J. E. (1964*a*). Rectal absorption in the desert locust, *Schistocerca gregaria* Forskål. I. Water. *J. Exp. Biol.* **41**, 15–38.

PHILLIPS, J. E. (1964*b*). Rectal absorption in the desert locust, *Schistocerca gregaria* Forskål. II. Sodium, potassium and chloride. *J. Exp. Biol.* **41**, 39–67.

PHILLIPS, J. E. (1964*c*). Rectal absorption in the desert locust, *Schistocerca gregaria* Forskål. III. The nature of the excretory process. *J. Exp. Biol.* **41**, 69–80.

POWNING, R. F. (1953). Studies on the digestion of wool by insects. VIII. The significance of certain excretory products of the clothes moth, *Tineola bisselliella*, and the carpet beetle, *Attagenus piceus*. *Austr. J. Biol. Sci.* **6**, 109–17.

RAMSAY, J. A. (1951). Osmotic regulation in mosquito larvae: the role of the Malpighian tubules. *J. Exp. Biol.* **28**, 62–73.

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. (1953). Active transport of potassium by the Malpighian tubules of insects. *J. Exp. Biol.* **30**, 358–69.

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*a*). The excretory system of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae). *J. Exp. Biol.* **32**, 183–99.

RAMSAY, J. A. (1955*b*). The excretion of sodium, potassium and water by the Malpighian tubules of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae). *J. Exp. Biol.* **32**, 200–16.

RAMSAY, J. A. (1956). Excretion by the Malpighian tubules of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae): calcium, magnesium, chloride, phosphate and hydrogen ions. *J. Exp. Biol.* **33**, 697–708.

RAMSAY, J. A. (1958). Excretion by the Malpighian tubules of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae): amino acids, sugars and urea. *J. Exp. Biol.* **35**, 871–91.

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.

SAXENA, K. N. (1955). Studies on the passage of food, hydrogen ion concentration and enzymes in the gut and salivary glands of *Dysdercus koenigii* F. *J. zool. Soc. India*, **7**, 145–54.

SRIVASTAVA, U. S. & BAHADUR, I. (1961). The development of the Malpighian tubules in *Dysdercus koenigii* (Hemiptera, Pyrrhocoridae). *Quart. J. Micro. Sci.* **102**, 347–60.

TERZIAN, L. A., IRREVERRE, F. & STAHLER, N. (1957). A study of nitrogen and uric acid patterns in the excreta and body tissues of adult *Aedes aegypti*. *J. Insect Physiol.* **1**, 221–8.

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

WIGGLESWORTH, V. B. (1931*b*). The physiology of excretion in a blood sucking insect, *Rhodnius prolixus* (Hemiptera, Reduviidae). II. Anatomy and histology of the excretory system. *J. Exp. Biol.* **8**, 428–42.

WIGGLESWORTH, V. B. (1931*c*). The physiology of excretion in a blood sucking insect, *Rhodnius prolixus* (Hemiptera, Reduviidae). III. The mechanism of uric acid excretion. *J. Exp. Biol.* **8**, 443–51.