J. exp. Biol. (1977), 69, 53–85
With 11 *figures*
Printed in Great Britain

THE CONTROL OF THE DIURESIS FOLLOWING A BLOOD MEAL IN FEMALES OF THE YELLOW FEVER MOSQUITO *AËDES AEGYPTI* (L.)

BY R. H. STOBBART

Department of Zoology, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU

(*Received* 24 *November* 1977)

SUMMARY

Control of post-feeding diuresis in females of the mosquito *Aëdes aegypti* has been studied by means of a weighing technique and simple surgical procedures. The primary controlling factor is (as in the larva) a nervous feedback mechanism and not an increased production of diuretic hormone. As the mosquito ingests blood, sensory information from the distending abdomen reaches the mid gut via the nerve cord, brain and stomatogastric system. This information probably inhibits retroperistaltic movements of the mid gut allowing fluid from the Malpighian tubules (which seems to be produced continually) to be moved back to the rectum for subsequent excretion, instead of being re-cycled to the haemolymph via the mid gut. Such a control does not preclude a role for the diuretic hormone, continual secretion of which may cause the continual production of fluid from the Malpighian tubules.

INTRODUCTION

Although we now have much detailed knowledge about the physiology of insect excretory systems at the tissue level (Maddrell, 1971; Stobbart & Shaw, 1974), relatively little attention has been directed to the question of the water balance of the whole insect (Maddrell, 1963, 1964*a*, *b*; Shaw & Stobbart, 1972).

As Ramsay (1958) pointed out, and as subsequent studies have confirmed (Phillips, 1964*a*, *b*, *c*; Balshin & Phillips, 1971), the insect excretory system has clear functional analogies with the vertebrate nephron. The non-regulatory fluid which is produced by the Malpighian tubules as a result of prior active transport of ions, and which contains waste products, is modified appropriately before discharge by the secretory activities of the rectal wall. The rectal wall can transport actively ions and amino acids (Phillips, 1964*b*; Balshin & Phillips, 1971) and apparently water too (Phillips, 1964*a*, 1969, 1970; Stobbart, 1968; Ramsay, 1971). The rectum is clearly the main regulatory element in the insect excretory system and as one would expect there is evidence that it is subject to hormonal control (Wall, 1967; Cazal & Girardie, 1968; Mordue, 1969, 1972; Vietinghoff, 1967). This evidence, however, is less precise than that for the hormonal control of secretion of fluid by the Malpighian tubules (Maddrell, 1971).

In the terrestrial insect it is the interaction between (i) water uptake through the gut (and in some cases, cuticle), (ii) production of metabolic water, (iii) transpiratory water loss, (iv) fluid production by the Malpighian tubules, (v) water resorption by

the rectum, which determines whether or not water balance is maintained at any given time (cf. Shaw & Stobbart, 1972). The control of the excretion of fluid in the whole terrestrial insect is best known in the blood sucking nymphs of *Rhodnius* (Maddrell, 1963, 1964*a*, *b*, 1966) which show a very rapid diuresis following a blood meal, a diuresis simplified by the fact that the rectum probably plays in it a minimal role. The diuresis starts when the distension of the mid gut by the blood meal stimulates abdominal stretch receptors. This stimulation causes the release of a diuretic hormone from the ends of neurosecretory axons on the surfaces of the abdominal nerves near the mesothoracic ganglionic mass. A similar situation exists in the subterranean larva of the beetle *Anisotarsus cupripennis* (Núñez, 1956) though here the hormone is produced in the brain, water is probably absorbed through the cuticle – perhaps as water vapour – and the role of the rectum is likely to be more important.

Like *Rhodnius* nymphs the adult females of the mosquito *Aëdes aegypti* perform a rapid diuresis after a blood meal. In the stock used here, diuresis generally starts before feeding has finished and some ten droplets of urine are discharged before the end of the meal. The act of feeding (described in detail by Christophers (1960) and Clements (1963)) normally takes 5 min or less, and diuresis starts a few seconds after the pleural membranes of the abdomen have become greatly extended. I have found that normal animals ingest about 2·63 mg (more than twice their body weight) but relatively smaller meals have been reported (Christophers, 1960). The engorged animal flies with considerable difficulty, so a rapid diuresis is likly to be of considerable selective value, and in fact some 20% of the weight of the meal is excreted as fluid within the first $\frac{1}{2}$ h after feeding. The first droplet of urine is quite often discharged at the start of the meal before the mid gut becomes distended with blood. This first droplet may be cloudy with the excretory sediment from a previous meal, and the first few droplets in a different stock have been shown to contain uric acid (Boorman, 1960). Subsequent droplets (discharged initially at a rate of several per minute) are, however, quite clear and contrast markedly with the ruby-red fluid in the mid gut, which, when distended, is clearly visible through the pleural membranes. The discharged fluid must be derived mainly from the Malpighian tubules since trypan blue and ^{144}Ce if present in the ingested blood do not appear in the excreted fluid (Boorman, 1960). As in *Rhodnius* the speed of the diuresis suggests a minimal role for the rectum.

Despite the general similarities in the post-feed diuresis in these two insects there are indications (Stobbart, 1971) that in *Aëdes* the control of fluid excretion differs markedly from that found in *Rhodnius* and *Anisotarsus*. This is certainly the case in *Aëdes* larvae which inhabit very dilute fresh waters and which are subjected to a continuous osmotic inflow of water. Here the control of fluid excretion is achieved primarily by a nervous control acting upon the gut and probably involving the stomatogastric system and stretch receptors in the body wall, and the output of fluid from the Malpighian tubules seems to be more or less constant (Ramsay, 1953; Stobbart, 1971). The question therefore arises as to whether the larval type of control persists in *Aëdes* adults, or is supplanted in them by one similar to that of *Rhodnius*. This paper attempts to resolve the question.

To avoid ambiguity we define certain terms as follows (cf. Stobbart, 1971): *diuresis*, the rapid production of urine; *tubular fluid*, the unmodified secretion of the Mal

pighian tubules; *rectal fluid*, the tubular fluid accumulating in the rectum prior to its discharge (and probably subject to minimal modification by the rectal epithelium); *urine*, the completed excretory fluid as discharged to the exterior.

MATERIALS AND METHODS

Techniques of culture

The stock ('L') of *Aëdes aegypti* used here was obtained in 1960 from the London School of Hygiene and Tropical Medicine. The larvae are fed on an infusion of ground dog-biscuit and stabilized wheat germ, and the adults on human blood. The larvae are reared in Newcastle tap-water and the adults are kept in a cage of dimensions 60 × 30 × 30 cm (both at a temperature of 25–30 °C). Between blood meals the adults are maintained on sucrose solutions and/or raisins soaked in water. Water is always available. Under these conditions the adult females live 4–6 weeks and yield 3–4 good crops of eggs. Egg-laying occurs only after a blood meal, and so is readily synchronized by means of these meals. The eggs may be stored dry for up to 6 months at room temperature and a R.H. of 75%.

Measurement of transpiration, diuresis, and size of blood meal

Although the mosquitoes will ingest rather small meals of sucrose solution and subsequently show a diuresis, I have confined my attention in this work to the more rapid and extensive diuresis which follows the ingestion of a blood meal by the females. No attempt was made to use animals at a standard time after emergence. Senile insects were avoided as in these diuresis sometimes failed to occur, or more rarely blood was passed directly back to the rectum and discharged to the exterior. Individuals containing developed eggs were also avoided (*a*) because a load of eggs reduces the amount of blood which can be ingested, (*b*) because decapitation (one of the operations used here) removes an inhibition for egg-laying (as transpiration and diuresis were measured as weight loss, any egg-laying clearly would have been an unnecessary complication). Apart from avoiding senile and gravid animals, the application of the various experimental treatments was randomized as far as possible, and the vast majority of animals used were reared from the stored eggs of one generation. Because the insects fed on sugary solutions between blood meals the ventral oesophageal diverticulum (Fig. 1) almost always contained fluid and air bubbles (Christophers, 1960). This does not affect the process of engorgement with blood and the subsequent diuresis and egg-laying. It was in fact advantageous for the present work as during engorgement the diverticulum is pushed to the anterior and ventral region of the abdomen where it is visible as a clear translucent pouch lying between the ventral nerve cord and the ruby-red distended mid gut (Figs. 1, 2) This facilitates the interruption of the nerve cord by pinching without any involvement of the mid gut (see below).

The mosquitoes were allowed to feed through the thin skin of the underside of the wrist. Feeding was generally completed within 5 min by unoperated and dummy-operated animals. The size of the meal was determined by weighing, and the rates of diuresis and transpiration were measured by following, in insects suspended by small wire hooks from the beam of a 5 mg torsion balance, the weight losses (to the nearest ±0·003 mg) during successive 2–5 min periods (for diuresis) or the losses during a few

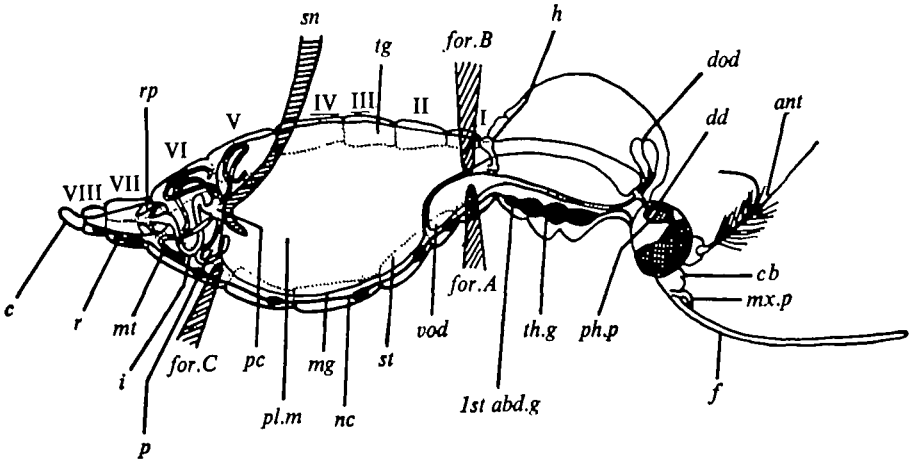

Fig. 1. Diagram of engorged mosquito showing some of the operations performed, and the disposition of the gut and Malpighian tubules. *1st abd.g*, 1st abdominal ganglion fused to metathoracic ganglion; ant, antennae; *c*, anal cerci; *cb*, cibarium; *dd*, dorsal dilator muscle of pharyngeal pump (lateral dilators not shown); *dod*, dorsal oesophageal diverticula; *f*, fascicle; *for.A*, pinch with fine forceps (position A); *for.B*, ditto (position B); *for.C*, ditto (position C); *h*, haltere; *i*, intestine; *mg*, distended midgut; *mt*, 5 Malpighian tubules discharging into pyloric chamber; *mx.p*, maxillary palp; *nc*, nerve cord; *p*, 'pocket' containing most of the haemolymph in the abdomen; *pc*, pyloric chamber; *ph.p*, pharyngeal pump; *pl.m*, extended pleural membrane; *r*, rectum; *rp*, rectal papillae (2 omitted); *sn*, bent steel needle clamping junction between mid gut and pyloric chamber; *st*, abdominal sternite; *tg*, abdominal tergite; *th.g*, thoracic ganglia; *vod*, ventral oesophageal diverticulum; I-VIII, abdominal segments. Part original, part after Clements (1963).

15–30 min periods (in the case of transpiration). The balance was checked for accuracy against a Mettler model M5 microbalance. A perspex shroud (5 × 5 × 7 cm) shielded the mosquito from air currents. It was open at the bottom and was unlikely to have caused any significant build-up of humidity gradient around the insect due to its transpiration. A thermometer was inserted through a hole in the lid of the shroud so that its bulb was near the mosquito. The minute droplets of urine (each weighing on average 0·007 mg) fell directly to the laboratory bench. Measurements of transpiration rate were made (i) before the animal was fed; (ii) when the rate of diuresis had fallen (*ca.* 30 min after feeding) and the abdomen was still partly distended (for these measurements the anus was sealed with a minute drop of beeswax/resin to prevent further excretion); (iii) during the process of diuresis when the rate of excretion was low (in certain operated animals). The rate of transpiration was assumed to be roughly constant during the diuresis, and its rate was subtracted from the rates of diuretic weight loss to give the corrected rates of diuresis. There was definite evidence that transpiration through a *grossly* distended abdomen was faster then normal (e.g. in animals which had fed after interruption of the nerve cord in the first abdominal segment), so for such animals transpiration rates as determined in (ii) and (iii) were used in estimating the rates of diuresis.

Attachment of wire hooks to the mosquitoes

This attachment was necessary for weighing the insects and also for holding them while operating (see below). The hooks were made from 40-gauge copper wire each with a small drop of beeswax/resin on the straight end. After being weighed (weigh

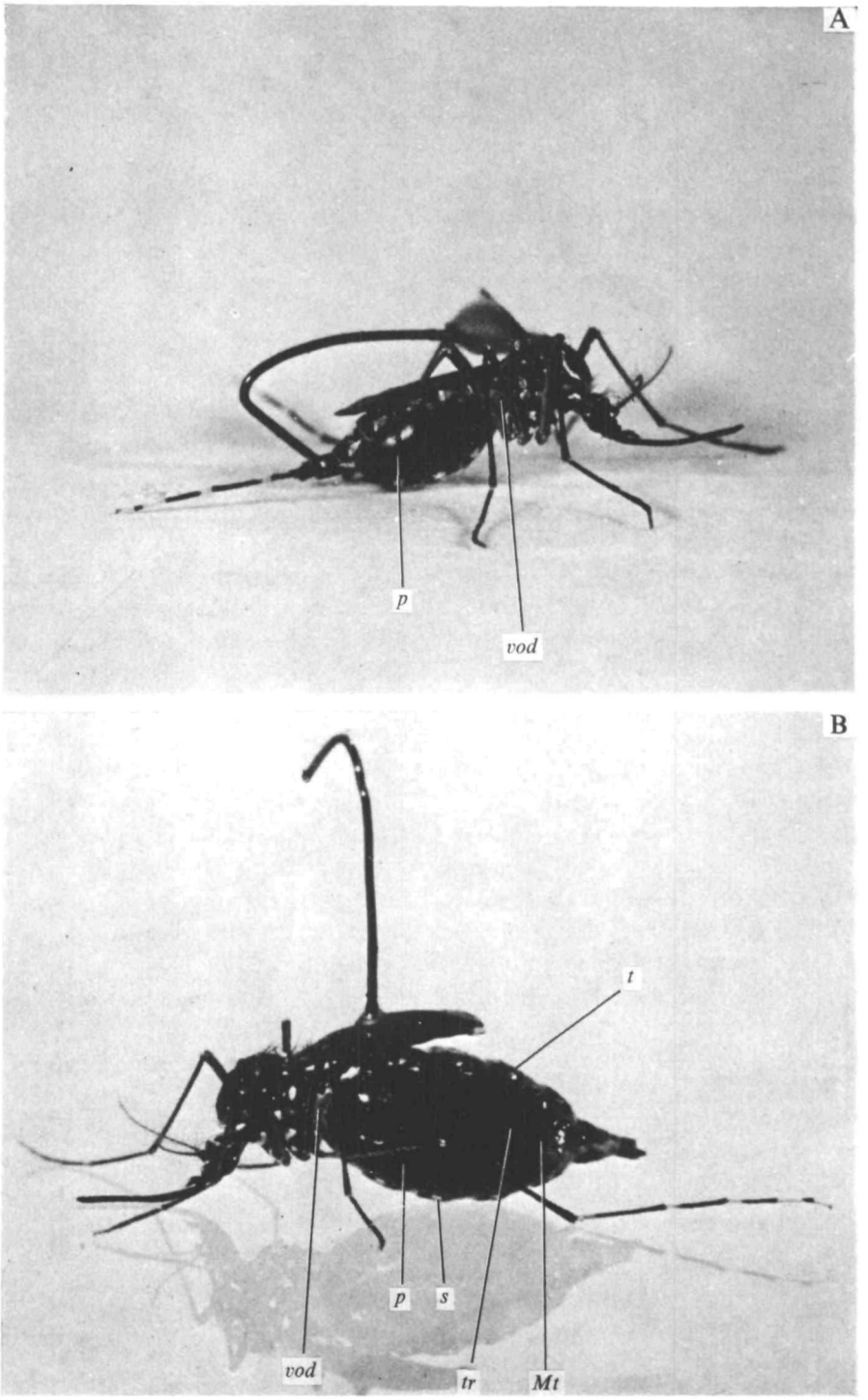

Fig. 2. (A) Normal engorged mosquito (hook attached to left wing only). *p*, Stretched pleural membrane; *vod* = oesophageal diverticulum. (B) Mosquito grossly engorged as a result of feeding after the nerve cord in abdominal segment I had been pinched. *Mt*, Malpighian tubules; *tr*, trachea; *t*, *s*, tergites and sternites well separated due to stretching of intersegmental membranes. The length of both animals was approximately 4 mm before feeding.

ca. 1 mg) hooks were attached as follows. A mosquito was observed through watchmaker's glasses as it probed at the skin. As soon as the proboscis started to penetrate the skin the beeswax/resin on the hook was melted with a microburner and the molten wax applied to the end of the wings. The wax usually solidified before the mosquito could withdraw its proboscis. Often the mosquito was not disturbed by the process and would have continued to feed. The mosquito was then gently removed to the torsion balance for weighing. The aim was to attach the hook to both wings, and with practice a success rate of about 70% could be achieved. Many mosquitoes attached to the hook by one wing were usable, but others were not, due to their flapping persistently when suspended.

The mosquitoes could easily carry the weight of the hook+wax and the extra weight seemed to have no effects at all upon feeding and diuresis. When returned to the wrist mosquitoes with hooks attached probed and fed promptly, and diuresing mosquitoes continued to produce droplets of urine without interruption when they were re-suspended on the torsion balance.

Operations performed on the mosquitoes

The following operations (see Fig. 1) were carried out beneath a binocular microscope, and the animals then resuspended on the balance within *ca.* 20 s.

(i) *Decapitation.* The head was grasped with fine forceps and was pulled off with a twisting motion, the neck was then sealed with a small drop of beeswax/resin.

(ii) *Crushing of the head.* The head was crushed with the forceps and then covered with beeswax/resin.

As these two operations gave the same results they will from now on both be designated by the term *decapitation.*

(iii) *Interruption of the abdominal nerve cord.* This was achieved by pinching a fold of integument which included the nerve cord; interruptions were made either in abdominal segment I or VI, in front of abdominal ganglia II or VI respectively (Fig. 1). In the former case the ventral oesophageal diverticulum was involved in the pinch, in the latter case the operation was only performed on unfed animals. After use the animals were preserved in Bouin's fixative for subsequent verification of the interruption.

(iv) *Pinching the mid gut in abdominal segment I.* This was performed only on engorged animals; the pinch is illustrated in Fig. 1, and involved the integument, the heart, and a relatively undistended region of the mid gut. Occasionally the mid gut was ruptured by the pinch. Any rupture was betrayed by the leakage of blood into the insect's haemolymph and the consequent colouring of the clear pocket of haemolymph which accumulates in engorged insects between the mid gut and rectum.

(v) *Dummy pinching.* This was performed only on unfed animals; the position of the pinch was as for (iv) but only the integument and heart were involved.

(vi) *Clamping of the junction between the mid gut and the pyloric chamber in animals with the nerve cord pinched in abdominal setgment I.* This was done (on engorged animals only) by means of a fine stainless-steel needle which was mounted on a Singer micromanipulator and bent at the tip. An engorged mosquito was laid on its side on a bed of beeswax/resin prepared on one surface of a microscope slide. The insect was immobilized beneath the binocular microscope by pressing the wire hook down into

the bed, and was further secured with another piece of fine copper wire which arched over and exerted a light pressure on the metathorax or abdominal segment I, and which was anchored by its ends having been pressed into the bed. The insect was then illuminated strongly from below by means of a high-intensity lamp (the light from which was passed through two pieces of heat absorbing glass), and the needle was manoeuvred so that its 'elbow' was just in front of the pyloric chamber and its shaft and end overlay the mid gut (see Fig. 1). It was then pressed gently downwards until the junction between the mid gut and pyloric chamber was judged to have been occluded. The needle was held in position by the inertia of the micromanipulator; the pressure exerted by the needle was usually sufficient to deform slightly the beeswax/resin beneath it, but was insufficient to rupture the mid gut. Successful occlusions were difficult to achieve and sometimes one or more Malpighian tubules were involved in the clamp. However, by choosing animals in which the Malpighian tubules had come to lie mainly posterior to the mid gut in the pocket of haemolymph between mid gut and rectum, and by manipulating the needle carefully, a number of successes were obtained.

(vii) *Dummy clamping in animals with the nerve cord pinched in abdominal segment I (engorged animals only).* The technique was the same as in (vi) but only the tergite of abdominal segment VI was clamped onto the wax bed.

(viii) *Sealing of anus.* This was usually undertaken when the insect was suspended from the torsion balance. The balance was clamped, and, while observing with watchmaker's glasses, a very small amount of molten beeswax/resin was applied to the anal cerci and abdominal segment VIII by means of a fine wire loop.

Induction of feeding in experimental animals (hooks attached)

Hungry animals were always used in the experiments; consequently normal and dummy-pinched animals started to feed within a few seconds of being placed on the underside of the wrist. Animals with the nerve cord interrupted in abdominal segment VI fed almost as promptly, but animals with the nerve cord interrupted in abdominal segment I were much more variable in their feeding behaviour, some feeding readily, others showing various degrees of recalcitrance. All recalcitrant animals were eventually induced to feed (though sometimes only after periods as long as an hour) by various combinations of the following stimuli: (i) animal waved through air as though flying and then dropped onto skin from a height of *ca.* 1 cm; (ii) animal stimulated repeatedly to walk over skin; (iii) end of fascicle pressed gently onto skin with a fine needle, and head moved from side to side by pressure on fascicle (i.e. rough imitation of probing movements); (iv) abdomen lifted with fine needle so that body pivoted about legs and end of fascicle made contact with skin (i.e. rough imitation of feeding posture).

General comments on design of experiments

Determination of the time-course of diuresis was quite straightforward in normal animals and in those operated on before feeding (dummy-pinched, or nerve cord pinched in abdominal segment I or VI). The animals were fed after being weighed (and in some cases after measurement of the transpiration rate) and were then transferred to the balance for re-weighing and subsequent measurement of weight

loss. Then in most cases the anus was sealed after some 30 min (i.e. when the diuresis was largely finished) and measurements of the transpiration rate were made. The animals were observed frequently with watchmakers' glasses during their stay on the balance.

In the case of animals operated on after feeding (decapitated, or nerve cord or mid gut in abdominal segment I pinched) the procedure was modified slightly as follows. After feeding they were transferred to the balance for re-weighing and for measurement, during a 2 min period, of the initial rate of diuresis. They were then removed from the balance, operated on beneath a binocular microscope, and returned to the balance – this took on average a further 2 min. The measurements of weight loss were then re-started, and the above procedure followed through to the end. As 4 min were spent in measuring the initial diuresis rate and in performing the operation, and as the diuresis is largely finished by 30 min, the amount of fluid excreted over the period 4–30 min was compared in all the categories in which weight losses were used to estimate excretion.

In the case of animals with the gut clamped between pyloric chamber and mid gut, the transpiration rate, the size of the blood meal, and the rate of any excretion occurring before the application of the clamp were determined by weighing. The rate of excretion occurring after the application of the clamp was estimated roughly by observing, with the binocular microscope, the rate of extrusion of the droplets of urine.

Miscellaneous points

Since the specific gravity of insect urine is about 1·007 (Barbour & Hamilton, 1926) 1 mg of urine may for all practical purposes be taken as equivalent to 1 μl. Nevertheless the data for diuresis are presented throughout this paper in terms of weight.

As mentioned earlier in unoperated animals an average of ten droplets of urine (each *ca.* 0·007 mg in weight) were extruded before feeding was completed. This amounts to only *ca.* 2·6% of the total fluid ingested, and so this early extrusion has been ignored. The experiments were all carried out at ambient room temperature which, in the places where the mosquitoes were situated, varied between about 20 and 25 °C. About 20 animals were used in each experimental category and the usual convention of significance was employed in the statistical tests.

RESULTS

The environmental temperature was not controlled in this work, but had no apparent effect upon the results (Table 1).

Normal animals

The normal course of diuresis is shown in Fig. 3 for two animals in which diuresis was allowed to run practically to completion. About 74% of the diuresis is accomplished within 30 min of feeding, and from 60–90 min onwards the weight losses are due mainly to transpiration. In order to economize on time and effort diuresis was not followed to completion in the vast majority of cases. Instead diuresis was observed only for some 30–50 minutes in both normal and experimental animals. Some results typical of normal animals are shown in Fig. 4A. The transpiration rate before feedin

Table 1. *Regression data: for weight of fluid excreted during the period 4–30 min after feeding against temperature; and for initial rate of excretion against temperature*

(Correlation coefficient (r) ± standard deviation (no. of observations) and significance (P) of r.)

Treatment	(i) r for wt. of fluid excreted during period 4–30 min after feeding	P	(ii) r for initial rate of excretion	P	r for combined data of (ii)	P
Normal	$-0{\cdot}146 \pm 0{\cdot}23$ (21)*	0·7–0·5	$0{\cdot}207 \pm 0{\cdot}23$ (20*)	0·5–0·3		
Dummy pinched before feed (1)	$-0{\cdot}050 \pm 0{\cdot}24$ (19)	0·9–0·8	$0{\cdot}012 \pm 0{\cdot}25$ (18)	> 0·9		
Decapitated after feed (2)	$0{\cdot}208 \pm 0{\cdot}26$ (16)	0·5–0·3	$0{\cdot}258 \pm 0{\cdot}26$ (16)*	0·5–0·3		
Nerve cord in abdominal segment I pinched after feed (3)	$-0{\cdot}005 \pm 0{\cdot}24$ (20)	> 0·9	$-0{\cdot}083 \pm 0{\cdot}24$ (20)*	0·8–0·7		
Mid gut in abdominal segment I pinched after feed (4)	$0{\cdot}261 \pm 0{\cdot}22$ (22)	0·2–0·1	$0{\cdot}229 \pm 0{\cdot}22$ (22)*	0·5–0·3	$0{\cdot}106 \pm 0{\cdot}10$ (96)	0·4–0·3
Nerve cord in abdominal segment I pinched before feed (5)	$-0{\cdot}307 \pm 0{\cdot}23$ (19)	0·2	—	—		
Nerve cord in abdominal segment VI pinched before feed (6)	$-0{\cdot}332 \pm 0{\cdot}22$ (20)	0·2–0·1	—	—		

The standard deviation of $r = \sqrt{[(1-r^2)/(n-2)]}$ where n = number of observations. The probability values were obtained from tables of t and c.

* Animals unoperated when observations were made.

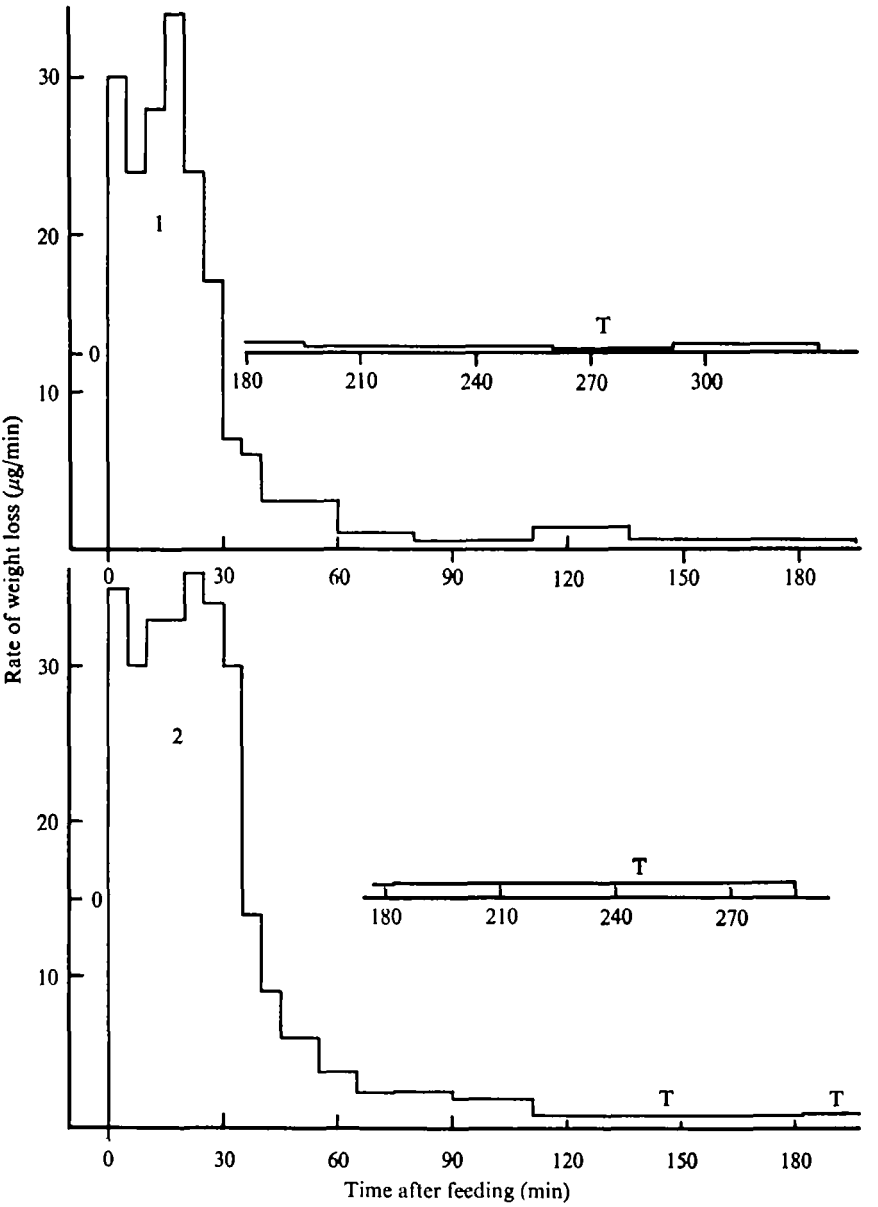

Fig. 3. The time-course of normal diuresis in two examples. In this and subsequent figures the numbers associated with the graphs are the serial numbers of the mosquitoes used. *T* = weight losses for the time intervals due only to transpiration.

is fairly similar to the rate measured after feeding and partial diuresis when the anus had been sealed (Table 2).

Animals dummy-pinched before feed (*treatment* 1)

These served as controls to all the experimental animals except those with the nerve cord pinched in abdominal segment I, and the junction between the mid gut and the pyloric chamber clamped. Results were very similar to those for normal animal

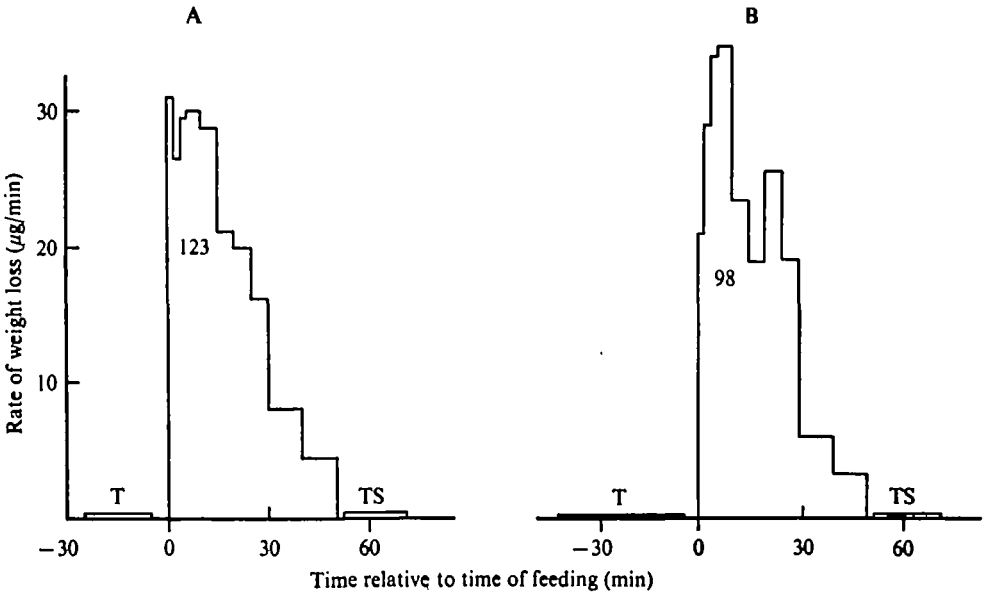

Fig. 4. Comparison of diuresis in (A) a normal and (B) a dummy-pinched mosquito. In this and subsequent figures TS = transpirational weight loss, anus sealed. A variable time (usually between 4 and 6 min) elapsed between the pre-feeding measurement of transpiration and feeding. For reasons of simplicity this time is indicated on the graphs of this and subsequent figures as a 5 min period; in the case of Figs. 9 and 10 the time was often appreciably longer (see text).

(Fig. 4B and Tables 4–8). Pinching did not affect the transpiration rate in this and other experimental categories (Table 2).

Animals decapitated after feeding and shortly after the start of diuresis (treatment 2)

Decapitation always resulted in a prompt and marked reduction in the rate of diuresis, which generally dropped to some 12–15% of the initial value (Fig. 5). In a few cases (e.g. serial 96, Fig. 5) there was a partial recovery of the rate some 30 minutes or so after the operation, but this was never marked.

Animals with the nerve cord in abdominal segment I pinched after feeding and shortly after the start of diuresis (treatment 3)

The results clearly show that integrity of the nerve cord is essential for the maintenance and control of diuresis (Table 4, Fig. 6). The effect of pinching the nerve cord was almost identical to that of decapitation, namely an 'immediate' and extensive reduction in the rate of diuresis. In a few cases there was a partial recovery (e.g. serial 73, Fig. 6) as with decapitated animals.

Animals with the mid gut in abdominal segment I pinched after feeding and shortly after the start of diuresis (treatment 4)

In the larva excretion of fluid may be inhibited by ligaturing the neck or by pinching or freezing the nerve cord in the thorax or in abdominal segment I (Stobbart, 1971). The effect is due to the removal of an inhibition of the spontaneous retroperistaltic movements of the mid gut. In operated larvae these movements can be seen to

Table 2. *Transpiration rates and the effects upon them of operative procedures and partial distension of the abdomen*

(The measurements of transpiration made after the feed were obtained from animals with the anus sealed, and in the case of decapitated animals with the neck wound sealed as well.)

Treatment	Transpiration rate (μg/min) Mean ± standard error (no. of observations)	Factor(s) under test	Significance (P) of difference between measurements (t test)
Normal	Before feed 0·620 ± 0·07 (5) After feed 0·419 ± 0·06 (15)	Partial distention of abdomen	0·05–0·02
Dummy-pinched before feed (1)	Before feed 0·053 (1) After feed 0·570 ± 0·38 *(18)	Pinching, partial distension of abdomen	0·2†
Decapitated after feed (2)	Before feed 0·744 ± 0·18 (5) After feed 1·576 ± 0·67 (14)	Decapitation, partial distension of abdomen	0·3–0·2
Nerve cord in abdominal segment I pinched after feed (3)	Before feed 0·379 ± 0·08 (6) After feed 0·740 ± 0·15 (20)	Pinching of nerve cord, partial distension of abdomen	0·05
Mid gut in abdominal segment I pinched after feed (4)	Before feed 0·580 ± 0·11 (7) After feed 0·700 ± 0·27 (21)	Pinching of midgut, partial distention of abdomen	0·7–0·5
Nerve cord in abdominal segment VI pinched before feed (6)	After feed 0·944 ± 0·11 (20)		

* Standard deviation, not standard error.
† t test for individual reading.

carry tubular fluid to the mid gut from which it is then resorbed into the haemolymph. The inhibition is presumably controlled in normal larvae by the stomatogastric ('autonomic') nervous system after it has received sensory information from the body by way of the nerve cord and brain. In view of these results I thought it worthwhile to investigate the possibility that activity of the mid gut might play a role in the post-feeding diuresis. The stomatogastric system is not very well developed in *Aëdes aegypti* (Christophers, 1960), and stomachic ganglia and recurrent nerves posterior to the hypocerebral (recurrent) ganglion have not been found. Nevertheless, pinching the relatively undistended mid gut in abdominal segment I (see Fig. 1) may reasonably be expected to prevent 'autonomic' stimulation of the distended mid gut if the nerves and ganglia do exist, or to prevent intercellular transmission of excitability in the gut if the 'autonomic' nerve supply ends anterior to the abdomen.

Examples of the effects of this operation are given in Fig. 7. The effects are similar to, but somewhat more variable than those of the preceding two operations. In the majority of cases the results were similar to those obtained from serials 66 and 74 (Fig. 7), i.e. an 'immediate' and extensive reduction in the rate of excretion. In other cases the reduction was less pronounced and was occasionally (e.g. serial 83) relativ

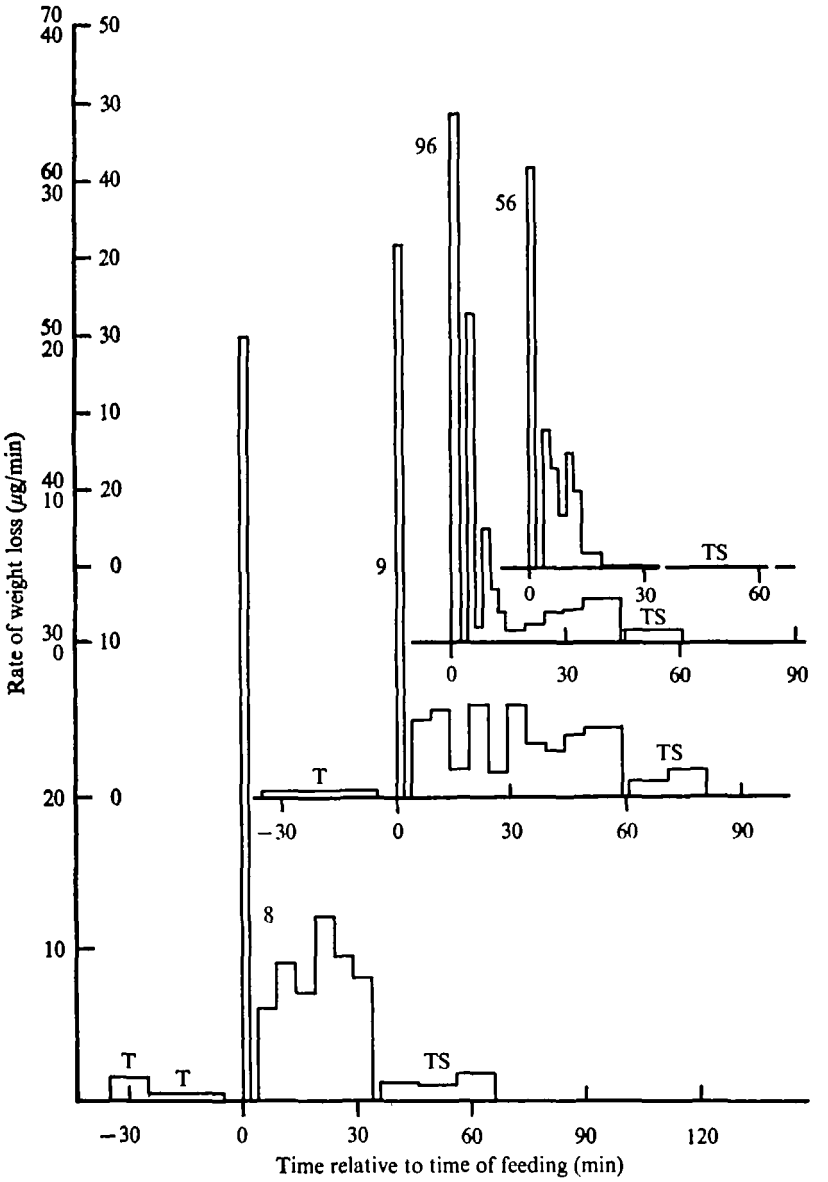

Fig. 5. The effect upon diuresis of decapitation in four examples. The operation was performed within the period of 2–4 min after feeding.

slight. In eight animals the mid gut was ruptured by the pinch, and blood from the gut escaped into the haemocoel. In these animals the excretion stopped 'immediately' and completely. The results from these animals with ruptured guts have been excluded from the statistical analyses, and are considered separately in the Discussion.

Animals with the nerve cord in abdominal segment I pinched before feeding (treatment 5)

The aim of this operation was to discover whether sensory information from the domen (which is known to be necessary for the proper termination of feeding –

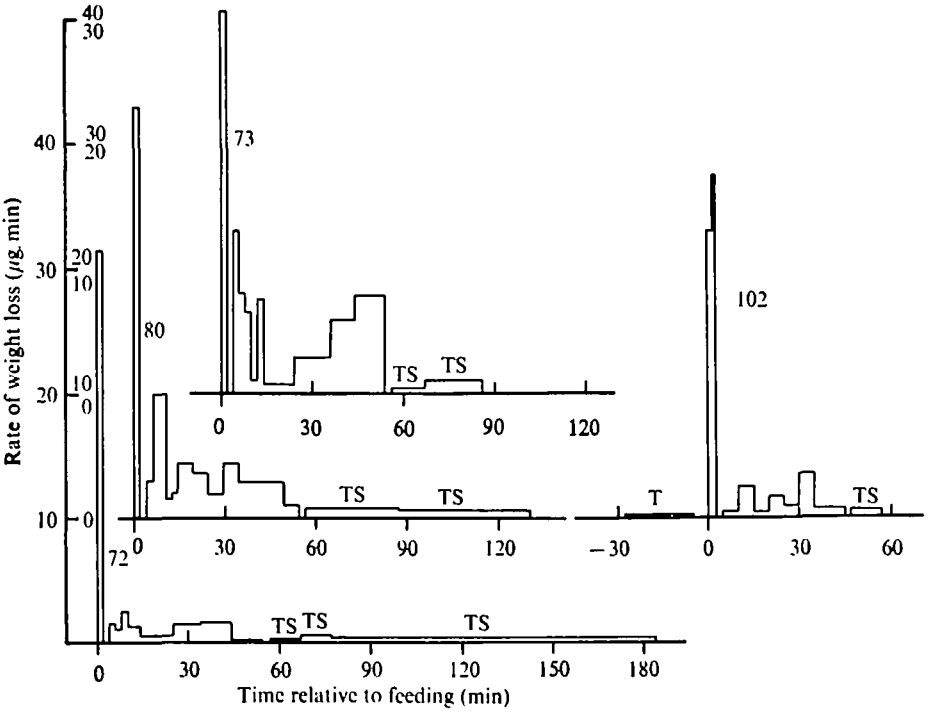

Fig. 6. The effect upon diuresis of pinching the nerve cord in abdominal segment I in four examples. The operation was performed within the period of 2–4 min after feeding.

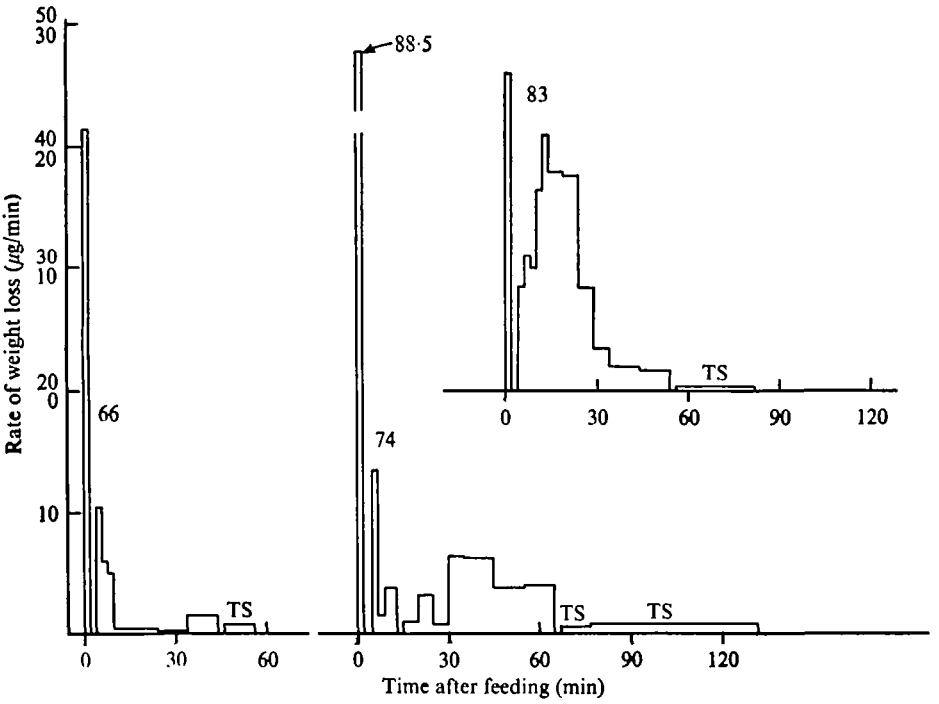

Fig. 7. The effect upon diuresis of pinching the mid gut in abdominal segment I in three examples. The operation was performed within the period of 2–4 min after feeding.

Table 3. *Comparison of weights of blood ingested by normal animals, and those with the nerve cord in abdominal segment I pinched before feed*

Treatment	Measurement: Wt of blood ingested (mg) mean ± standard error (no. of observations)	Measurement: Significance (*P*) of difference between the means (*c* test)
All animals unoperated at time of feeding*	2.635 ± 0·08 (75)	< 0·001
Nerve cord in abdominal segment I pinched before feed (5)	4.828 ± 0·38 (19)	

* Normal animals plus those of treatments 2–4 (see text).

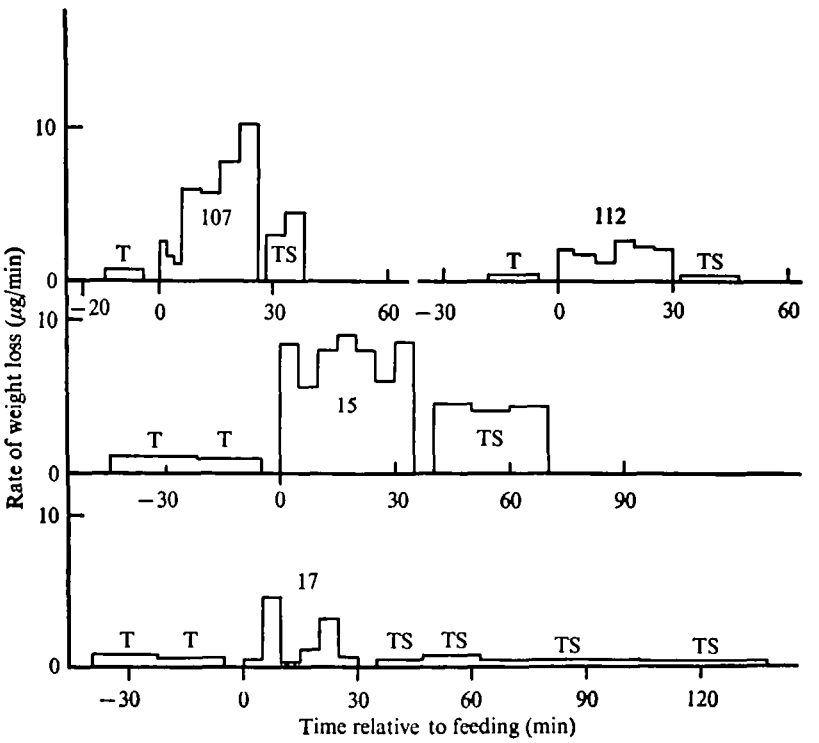

Fig. 8. The effect upon diuresis of pinching the nerve cord in abdominal segment I before feeding in four examples.

Gwadz, 1969) is also necessary for the initiation and maintenance of diuresis. Some of the operated animals fed readily; all but a few of the rest were induced to feed eventually using the methods described earlier. All fed animals became grossly engorged (Fig. 2A, B) and perhaps fed until the cibarial and pharyngeal pumps could no longer work against the hydrostatic pressure of the blood in the distended mid gut. In a few extremely distended specimens a small amount of blood leaked out from the anus. The act of feeding in some cases lasted up to 20 min, and always took longer than 3–5 min required by normal animals. The pleural membranes of the abdomen

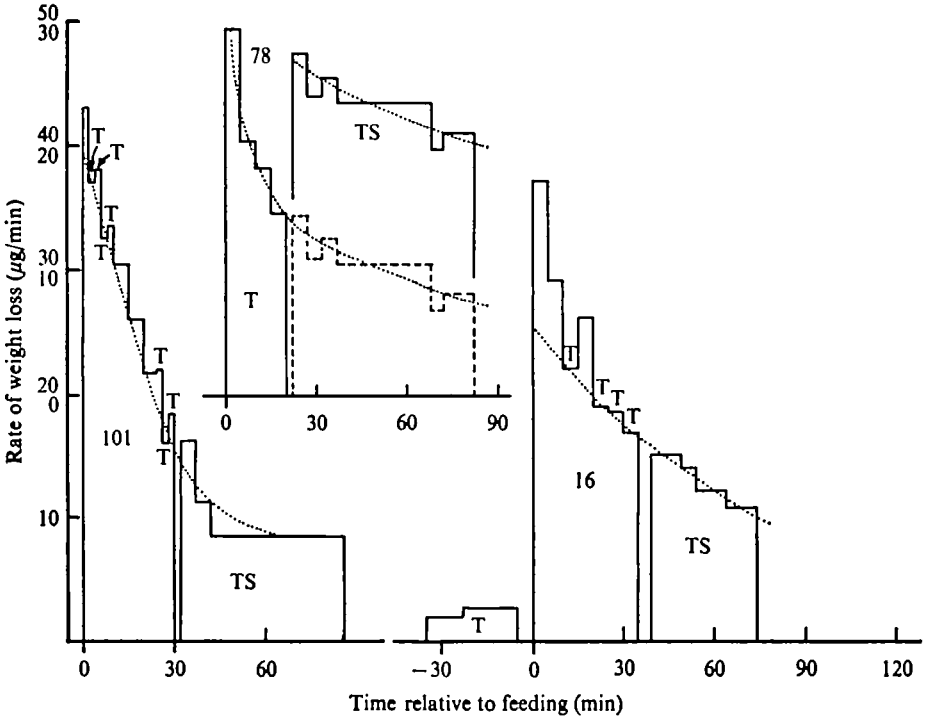

Fig. 9. The effect upon transpiration rates of extreme distension of the abdomen in four mosquitoes with the nerve cord in abdominal segment I pinched before feeding. The transpiration rates are indicated by the dotted lines. In the case of serial 78 the abdominal cuticle was slightly damaged by hot beeswax/resin when the anus was sealed (see text).

became excessively stretched and the abdominal tracheae became plainly visible beneath them; in addition the abdominal tergites and sternites became widely separated due to the stretching of the intersegmental membranes, and the haemolymph in the abdomen was forced backwards to form a pocket of clear fluid surrounding the rectum, intestine and most of the Malpighian tubules (Figs. 2B and 1). As Tables 3–6 show, the operated animals ingested nearly twice as much blood as did the controls, and also as did the animals which had not been operated on at the time of feeding. The differences between the operated animals and the others are highly significant. These results are very similar to those obtained by Gwadz (1969) after transection of the nerve cord in abdominal segment I of *Aëdes aegypti* (Rockefeller Institute stock) and five other species of mosquito. Gwadz' *A. aegypti*, however, ingested a much larger quantity (11·99 mg as compared with 4·83 mg) and tended to rupture due to excessive feeding. This difference may be due to an incomplete functional interruption of the nerve cord by pinching in my work, but it may equally well be due to differences between the stocks in the extensibility of the abdominal cuticle and/or the power of the suctorial pumps (an explanation supported by the occasional leakage of blood from the anus in my animals).

The excretion which follows the feed is much less extensive in the operated animals than in the controls (Fig. 8 and Table 4). Typical results are shown in Fig. 8. In three individuals (serials 16, 78 and 101) which ingested blood meals amounting to, respectively, 556%, 386% and 596% of their body weights, greatly increased rates

transpiration were observed (Fig. 9). Presumably this was a mechanical effect due to extreme extension of the pleural and intersegmental membranes of the abdomen. This interpretation is supported by the observation that as transpiration proceeded and abdominal distension diminished, the rate of transpiration fell roughly exponentially in relation to time. In serial 78 the act of sealing the anus increased the transpiration rate. In this case the beeswax/resin used was too hot; some of it ran forward onto the distended pleural membrane of one side and presumably damaged it slightly and increased its permeability. In spite of the high rates of transpiration in these three individuals the rates of excretion were very low (serials 16 and 101) or absent (serial 78).

In the above four experimental categories (treatments 2–5) it is very clear that the operations reduce the rate of diuresis drastically and promptly. It is nevertheless also evident that the production of tubular fluid does not stop completely; there was a slow rate of excretion, and tubular fluid collected in the recta which became noticeably distended. It was possible to press tubular fluid out of such a rectum using a fine bent needle, but because of the fragility of the rectal tissue and the efficacy of the closure of the anus it was difficult to do this without damaging the rectum (see later). These observations together with the effects of pinching the mid gut in abdominal segment I after feeding (an operation which leaves possible sites of hormone production intact) show that the activity of the gut is at least as important in controlling the rate of diuresis as any hormone-dependent variations in the rate of production of tubular fluid. It follows from this that changes in the stimulation of the rectum alone could also alter the rate of diuresis.

I have attempted to investigate this point by studying diuresis in the following category of animals.

Animals with the nerve cord in abdominal segment VI pinched before feeding (treatment 6)

Although innervation of the rectum has apparently not been demonstrated, there would seem to be two routes by which it may received stimulation. The first is by way of the stomatogastric nervous system; the second is by way of nerves arising from the last abdominal ganglion. If this second route is operative a pinch of the nerve cord in front of the ganglion in abdominal segment VI (see Fig. 1) should isolate the rectum from higher nervous centres. Unfortunately it is not practicable to perform this operation on animals which have fed because of the danger of including other organs (mid gut, intestine, Malpighian tubules) in the pinch; these organs are more crowded together in the engorged animal than Fig. 1 indicates. I therefore pinched the animals before they were fed. The results from such animals were somewhat equivocal. The pattern of ingestion and diuresis was in some cases fairly normal (e.g. serials 162 and 163, Fig. 10); in others (e.g. 174, 179, Fig. 10) less blood was ingested and less fluid excreted than in normal and control animals (see Tables 4–8 and below). In general the results do not support the contention that information routed through the posterior end of the nerve cord is involved in maintaining the activity of the rectum.

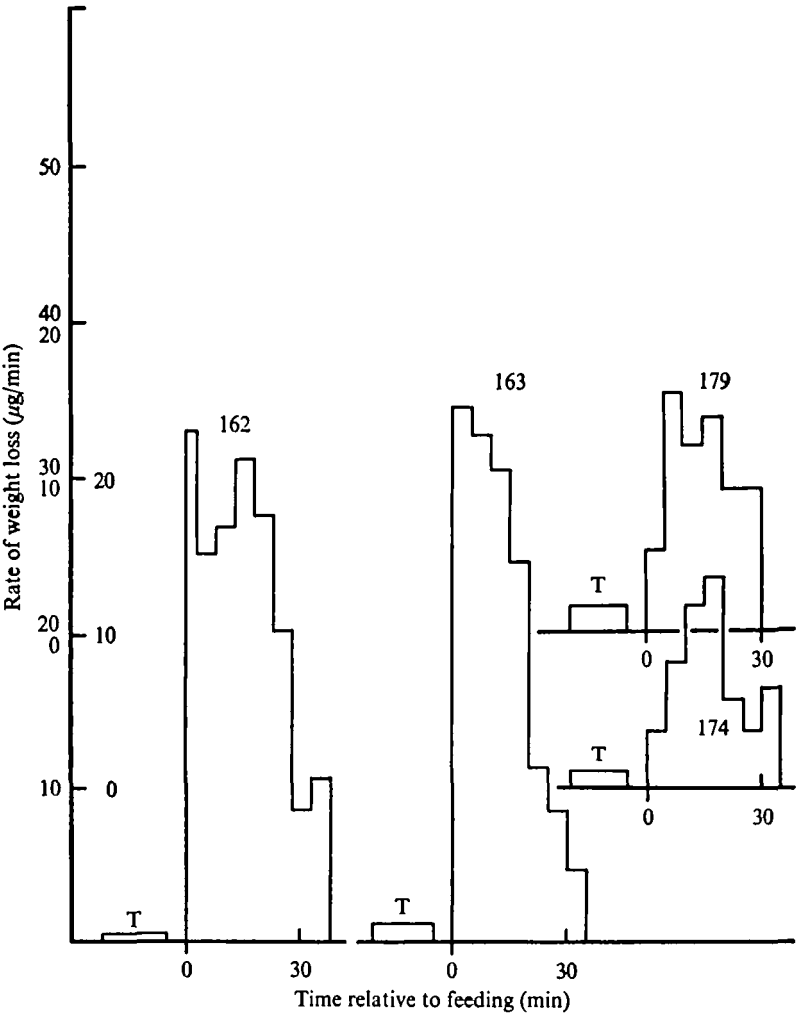

Fig. 10. The effect upon diuresis of pinching the nerve cord in abdominal segment VI before feeding in four examples.

General comparison of results obtained from normal animals, controls, and those used in treatments 1–6

The amounts of blood ingested and of fluid excreted are shown in Table 4 for the various categories of animals. The significances of the differences between the categories are shown in Tables 5–9. From these tables the following major points emerge.

(i) The normal and control (dummy-pinched) animals do not differ significantly ($P > 0{\cdot}05$).

(ii) The differences between treatments 2, 3 and 4 are small, though some of them are significant ($P < 0{\cdot}05$). These treatments cause the amount of fluid excreted to drop on average to *ca.* 30% of that excreted by control animals, and the differences between the experimental and the control animals are highly significant (Tables 7 and 8).

Table 4. *The ingestion of blood and the subsequent diuresis in normal and experimental animals*

(Each set of figures gives: mean ± standard error (no. of observations.))

Treatment	Wt of blood ingested (mg)	Wt of blood ingested (% body wt.)	Wt of fluid excreted between 4 and 30 min after feeding (mg)	Wt of fluid excreted between 4 and 30 min after feeding (% of wt of blood ingested)	Initial excretory rate (mg/min)
Normal	2·527 ± 0·13 (19)§	218·84 ± 15·21 (19)	0·595 ± 0·03 (20)	23·82 ± 1·02 (19)	0·0350 ± 0·003 (20)•
Dummy-pinched before feed (1)	2·632 ± 0·14 (18)	232·59 ± 13·39 (18)	0·573 ± 0·05 (19)	21·14 ± 1·29 (18)	0·0306 ± 0·004 (19)†
Decapitated after feed (2)	2·747 ± 0·17 (14)§	272·35 ± 18·17 (13)	0·125 ± 0·02 (16)	4·61 ± 0·66 (14)	0·0260 ± 0·002 (16)‡
Nerve cord in abdominal segment I pinched after feed (3)	2·490 ± 0·21 (20)§	231·45 ± 12·19 (20)	0·166 ± 0·03 (20)	6·09 ± 1·13 (20)	0·0338 ± 0·003 (20)‡
Midgut in abdominal segment I pinched after feed (4)	2·527 ± 0·14 (22)§	217·35 ± 12·47 (22)	0·213 ± 0·04 (22)	8·25 ± 1·30 (22)	0·0370 ± 0·004 (22)‡
Nerve cord in abdominal segment I pinched before feed (5)	4·828 ± 0·38 (19)	408·37 ± 24·72 (19)	0·103 ± 0·02 (19)	2·47 ± 0·56 (19)	0·00393 ± 0·0011 (19)†
Nerve cord in abdominal segment VI pinched before feed (6)	1·860 ± 0·10 (20)	150·89 ± 7·46 (20)	0·411 ± 0·04 (20)	21·31 ± 1·87 (20)	0·0190 ± 0·003 (20)†
	See Table 5 for significance of differences	See Table 6 for significance of differences	See Table 7 for significance of differences	See Table 8 for significance of differences	See Table 9 for significance of differences

• Rate immediately after end of feeding. † Rate after operation and immediately after end of feeding.
‡ Rate immediately after feeding and before operation.
Mean initial excretory rate (mg/min) for all animals unoperated at time of measurement (•, ‡): 0·03350 ± 0·001580 (78).
Mean wt (mg) of blood ingested for all animals unoperated at time of feeding (§): 2.6337 ± 0·0794 (75).

Table 5. *The significances (P values, estimated by 't' or 'c' tests) of the differences between the values for weight of blood ingested (cf. Table 4)*

Treatment	Normal					
Dummy-pinched before feed (1)	0·6–0·5	Dummy-pinched before feed (1)				
Decapitated after feed (2)	0·3–0·2	0·6–0·5	Decapitated after feed (2)			
Nerve cord in abdominal segment I pinched after feed (3)	0·9–0·8	0·6–0·5	0·4–0·3	Nerve cord in abdominal segment I pinched after feed (3)		
Mid gut in abdominal segment I pinched after feed (4)	> 0·95	0·6–0·5	0·3	0·9–0·8	Mid gut in abdominal segment I pinched after feed (4)	
Nerve cord in abdominal segment I pinched before feed (5)	< 0·001	< 0·001	< 0·001	< 0·001	< 0·001	Nerve cord in abdominal segment I pinched before feed (5)
Nerve cord in abdominal segment VI pinched before feed (6)	< 0·001	< 0·001	< 0·001	0·01–0·001	< 0·001	< 0·001

Table 6. *The significances (P values, estimated by 't' or 'c' tests) of the differences between the values for weight of blood ingested expressed as % of body weight (cf. Table 4)*

Treatment	Normal	Dummy-pinched before feed (1)	Decapitated after feed (2)	Nerve cord in abdominal segment I pinched after feed (3)	Mid gut in abdominal segment I pinched after feed (4)	Nerve cord in abdominal segment I pinched before feed (5)
Dummy-pinched before feed (1)	0·5–0·4					
Decapitated after feed (2)	0·05–0·02	0·1–0·05				
Nerve cord in abdominal segment I pinched after feed (3)	0·5–0·4	0·95	0·1–0·05			
Mid gut in abdominal segment I pinched after feed (4)	0·95–0·90	0·4–0·3	0·02–0·01	0·5–0·4		
Nerve cord in abdominal segment I pinched before feed (5)	< 0·001	< 0·001	< 0·001	< 0·001	< 0·001	
Nerve cord in abdominal segment VI pinched before feed (6)	< 0·001	< 0·001	< 0·001	< 0·001	< 0·001	0·001

Table 7. *The significances (P values, estimated by 't' or 'c' tests) of the differences between the weights of fluid excreted between 4 and 30 min after feeding (cf. Table 4)*

Treatment	Normal					
Dummy-pinched before feed (1)	0·7–0·6	Dummy-pinched before feed (1)				
Decapitated after feed (2)	< 0·001	< 0·001	Decapitated after feed (2)			
Nerve cord in abdominal segment I pinched after feed (3)	< 0·001	< 0·001	0·3–0·2	Nerve cord in abdominal segment I pinched after feed (3)		
Mid gut in abdominal segment I pinched after feed (4)	< 0·001	< 0·001	0·05–0·02	0·4–0·3	Mid gut in abdominal segment I pinched after feed (4)	
Nerve cord in abdominal segment I pinched before feed (5)	< 0·001	< 0·001	0·4–0·3	0·2–0·1	0·02–0·01	Nerve cord in abdominal segment I pinched before feed (5)
Nerve cord in abdominal segment VI pinched before feed (6)	< 0·001	0·02–0·01	< 0·001	< 0·001	< 0·001	< 0·001

Table 8. *The significances (P values, estimated by 't' or 'c' tests) of the differences between the weights of fluid excreted between 4 and 30 minutes after feeding expressed as % of weight of blood ingested (cf. Table 4)*

Treatment	Normal					
Dummy-pinched before feed (1)	0·1	Dummy-pinched before feed (1)				
Decapitated after feed (2)	< 0·001	< 0·001	Decapitated after feed (2)			
Nerve cord in abdominal segment I pinched after feed (3)	< 0·001	< 0·001	0·3–0·2	Nerve cord in abdominal segment I pinched after feed (3)		
Mid gut in abdominal segment I pinched after feed (4)	< 0·001	< 0·001	0·02–0·01	0·3–0·2	Mid gut in abdominal segment I pinched after feed (4)	
Nerve cord in abdominal segment I pinched before feed (5)	< 0·001	< 0·001	0·02–0·01	0·01–0·001	< 0·001	Nerve cord in abdominal segment I pinched before feed (5)
Nerve cord in abdominal segment VI pinched before feed (6)	0·3–0·2	0·95–0·90	< 0·001	< 0·001	< 0·001	< 0·001

Table 9. *The significances (P values, estimated by 't' or 'c' tests) of the differences between the values for initial rates of excretion (cf. Table 4)*

Treatment	Normal					
Dummy-pinched before feed (1)	0·4–0·3	Dummy-pinched before feed (1)				
Decapitated after feed (2)	0·02–0·01	0·3–0·2	Decapitated after feed (2)			
Nerve cord in abdominal segment I pinched after feed (3)	0·8–0·7	0·5–0·4	0·05–0·02	Nerve cord in abdominal segment feed (3)		
Mid gut in abdominal segment I pinched after feed (4)	0·7–0·6	0·3–0·2	0·02–0·01	0·5–0·4	Mid gut in abdominal segment I pinched after feed (4)	
Nerve cord in abdominal segment I pinched before feed (5)	< 0·001	< 0·001	< 0·001	< 0·001	< 0·001	Nerve cord in abdominal segment I pinched before feed (5)
Nerve cord in abdominal segment VI pinched before feed (6)	< 0·001	0·02–0·01	0·1–0·05	< 0·001	< 0·001	< 0·001

(iii) Treatment 5 results in the greatest ingestion of blood, the smallest initial excretory rate, and the smallest subsequent excretion of fluid (the latter not however significantly smaller than in treatments 2 and 3). Consequently the excretion is the lowest of all when expressed on a percentage basis.

(iv) Treatment 6 results overall in a reduced intake of blood and a proportionately reduced diuresis and initial excretory rate, with the result that the weight of fluid excreted, when expressed on a percentage basis, does not differ significantly from that of normal and control animals.

Animals with the nerve cord in abdominal segment I pinched before feeding, engorged, and with the junction between mid gut and pyloric chamber clamped (treatment 7)

Treatments 2–5 show that pinching the mid gut after the feed and interrupting the nerve cord either before or after the feed reduce the diuresis drastically. The operations do not, however, stop excretion completely, nor do they stop tubular fluid from accumulating in the rectum. In larvae in which the inhibition of the retroperistalses of the mid gut has been removed by interrupting the nerve cord, and the excretion of urine consequently reduced, the excretion can be increased again by ligaturing the animals anterior to the pyloric chamber. These results, together with visual observations, suggest that adequate hydrostatic pressure in the pyloric chamber is needed before tubular fluid is conveyed to the rectum by intestinal peristalsis, and that discharge of urine from the rectum is probably triggered off by hydrostatic pressure as well (Stobbart, 1971). In an attempt to discover whether a similar situation exists in the adult, I undertook the experiments described below. Before dealing with these experiments however, a brief description is required of the process of extrusion of the droplets of urine. In the normal animal a slight twitching of abdominal segments VIII and VII (which are held roughly horizontal) indicates that extrusion of a droplet is imminent. The rectal peristalsis develops very soon (usually less than a second) after this and is accompanied by a tilting upwards of segments VIII and VII. At this point the anus opens and segments VIII and VII move closer to each other and to the rest of the abdomen as the peristaltic wave passes to the end of the rectum. A droplet of urine now appears at the end of the abdomen and at the same time the anal cerci splay apart. The anus now shuts quite suddenly upon completion of the peristalsis, and segments VIII and VII separate slightly from each other and the rest of the abdomen and return to their original position; these movements take only a fraction of a second and almost always cause the droplet to be dislodged cleanly from the end of the abdomen. In animals in which diuresis has been reduced by surgery, segments VIII and VII become extended due to accumulation of fluid in the rectum and droop downwards, often quite markedly. In such animals the same sequence of events occurs but now the preparatory twitchings of segments VIII and VII are much more evident and in some cases may be almost continual, starting again immediately after the discharge of a droplet in spite of the fact that the interval between successive discharges is longer than in normal animals. Also a sequence of events which I have termed 'incipient peristalsis' is quite common. In this sequence segments VIII and VII move upwards and the anal cerci may splay apart, segments VIII and VII move as before, but no droplet appears at the end of the abdomen. The sequence is completed by segments VIII and VII taking up their original positions as before. It is difficult to

decide whether the retention of the fluid is due to the non-occurrence of the peristalsis, or the non-opening of the rectum, or to both of these.

The clamping experiments were carried out as follows. After pinching its nerve cord in abdominal segment I and then measuring its transpiration rate, an animal was allowed to feed. The feed was interrupted after the animal had ingested a roughly normal amount of blood as it is difficult to apply clamps successfully to grossly distended animals. The animal was then replaced on the balance for 15 to 20 min for measurement of the rate of weight loss (transpiration + excretion) and for observation of the rate of production of urine droplets. The clamp was then applied and the production of urine droplets and the general activity of the rectum were observed with the binocular microscope for at least 20 min. Knowing the rate of transpiration and the rate of droplet production before the application of the clamp one can estimate the average droplet size, and also (assuming that this remains constant) the excretion rate after the application of the clamp.

Dummy (control) clamps were applied to 12 animals. These clamps did not alter the rate of droplet production. Twenty-nine experimentally clamped preparations were made and in 7 (i.e. 24 %) of these the application of the clamp caused an increase in droplet production. The results from these preparations are presented in Table 10. The application of the clamp causes on average a significant fourfold increase in the rate of droplet production and also in the rate of excretion. In all these animals the recta were obviously distended at the time of clamping, and in serial 173 the rectum subsequently became very distended in spite of the extrusion of droplets. The estimated excretion rate after clamping was in all cases except serial 173 greater than that of the initial excretory rate of the similar but unclamped animals of treatment 5 (Table 4) even though the animals of treatment 5 had ingested more than twice as much blood. However, the mean post-clamping excretory rate of Table 10 is abnormally low, being about 25 % of the initial excretory rate of unoperated animals (Table 4).

Of the 22 experimentally clamped preparations which did not show an increase in excretion rate following the clamp, all had the rectum obviously distended at the time of clamping. In 3 clamping caused a reduction in excretion rate probably because of involvement of one or more Malpighian tubules in the clamp. In 13 others there was no discernible effect due to clamping. In a further 3 the rectum became extremely distended after clamping and one of these animals showed continual 'incipient peristalsis'. In a further 2 fluid could be easily pressed out of the rectum, or the rectum could be induced to discharge by stimulating the end of the abdomen, and in the final case the end of the abdomen twitched continually and the rectum was observed to distend and then shrink (presumably due to reabsorption of fluid) and in addition the animal showed the 'incipient peristalsis' on several occasions.

Taken together these results suggest strongly that a build-up of fluid in the hind gut is an important factor in triggering off rectal discharge. However, by no means can it be the only one. Rectal discharge must be meaningfully coordinated (at any rate in the normal non-diuretic animal) with rectal reabsorption, and external sensory information also seems to be involved in its initiation.

Table 10. *The effect of clamping the junction between mid gut and pyloric chamber in engorged animals which had had the nerve cord in abdominal segment I pinched before feeding: means ± standard errors*

Serial	Wt blood ingested (mg)	Transpiration rate (μg/min)	Droplet size, μg (before clamp)	Droplet production/min before clamp	Droplet production/min after clamp	Excretory rate before clamp (μg/min)	Excretory rate after clamp (μg/min)*	
132	1·450	0·60	10·67	0·05	0·46	0·533	4·91	Rectum distended at time of clamping
173	2·283	1·60	11·50	0·133	0·267	1·53	3·07	Rectum distended at time of clamping, became very distended after clamping
156	2·036	1·20	—	0	0·45	0	5·66†	
155	2·837	1·27	12·00	0·20	0·68	2·40	8·16	
153	2·584	0·75	11·13	0·40	1·20	4·46	13·40	
142	1·721	1·50	20·83	0·20	0·733	4·17	15·30	Rectum distended at time of clamping
136	2·096	1·58	9·43	0·15	0·912	1·42	8·60	
Means	2·144 ± 0·18	1·214 ± 0·15	12·593 ± 1·69	0·162 ± 0·05	0·672 ± 0·12	2·073 ± 0·65	8·443 ± 1·70	
				0·01–0·001		0·01–0·001		
				Significance of difference between means (*P* value, *t* test)		Significance of difference between means (*P* value, *t* test)		

Mean wt of blood ingested for all 29 clamped animals (mg) 1·6131 ± 0·0974.

* Calculated assuming that droplet size remains constant. † Calculated using the mean droplet size for these data of 12·59 μg.

Observations on absorption of fluid from the gut

In normally diuresing animals fluid must be absorbed from the contents of the gut at a rate roughly equal to the one at which it is excreted, since there seems to be no drastic increase in haemolymph volume.

As far as can be judged from the volume and viscosity of the mid gut contents at about 35 min after feeding, absorption from the gut occurs at roughly the normal rate in all animals in which diuresis has been stopped or prevented from occurring by surgery. In such animals the absorbed fluid accumulates in the haemocoel of the abdomen, and this accumulation is of course most marked in engorged non-diuretic animals (treatments 5 and 7). Thirty-five minutes after feeding there is in these animals a thick layer of clear fluid between the gut and the abdominal wall. Volumes greater than 1 μl of this fluid can easily be collected after puncturing the abdominal wall. In view of the small amount of haemolymph initially present in the abdomen, analyses of this fluid from these animals could be used to show whether the absorption of ions from the gut is a selective process or not.

DISCUSSION

To make consideration of the data easier, the main findings of this work have been summarized briefly in Table 11. The following conclusions can be drawn.

(1) Integrity of the nerve cord at the level of abdominal segment I is necessary for (*a*) the normal termination of feeding (as shown by Gwadz, 1969), (*b*) the initiation of the diuresis, (*c*) the maintenance of the diuresis.

(2) The presence of the head is necessary at least for the maintenance of diuresis and probably also for its initiation.

(3) Hormonal effects are not the main way in which control of diuresis is achieved. This conclusion follows from the observation that pinching the relatively undistended mid gut in abdominal segment I stops diuresis. The operation leaves the hormone-producing tissues (thoracic ganglia, brain, retrocerebral complex) intact, but it presumably interrupts the stomatogastric stimulation of the mid gut. It can of course be argued that molecules liberated from damaged tissues inhibit hormone production, but this interpretation is rendered most unlikely by the fact that the dummy pinch has no effect. The speed with which pinching the nerve cord, decapitation, and pinching the mid gut stop diuresis (response complete in 20 s or less) also argues against hormonal control.

(4) Tubular fluid is probably produced continually, perhaps in response to a continual production of diuretic hormone (cf. the larva, Stobbart, 1971) and most of it is then (except during periods of diuresis) moved forwards into the mid gut for subsequent recycling to the haemolymph because:

(i) clamping the junction between Malpighian tubules and mid gut causes an increase in excretion in animals which fed after the nerve cord was pinched, and in which diuresis was consequently never started;

(ii) in animals in which diuresis has been stopped (or prevented from occurring) by surgery, excretion of fluid seldom stops completely, and in addition the rectum becomes distended with accumulated tubular fluid; this accumulation in the rectum

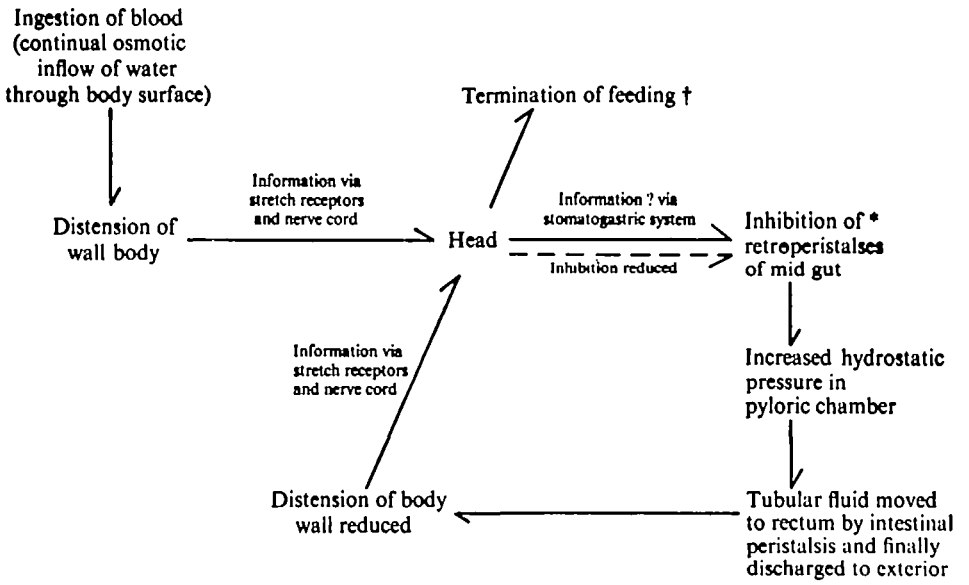

Fig. 11. Proposed scheme for regulation of excretion in *Aëdes aegypti* (cf. Stobbart, 1971). Conveyance of information or the result of a process are indicated by solid arrows, negative feedback is indicated by the broken arrow; ( ) = for larvae only; * = event established for larvae, but almost certainly occurring in adult; † = for adult only.

Table 11. *Summary of the effects of the various treatments upon ingestion of blood, and the subsequent diuresis during the period 4–30 min after feeding*

Treatment	Effects
Normal animals Dummy-pinched (1) (controls)	~ 2·7* mg blood ingested, ~ 20% of wt of blood excreted as fluid during period 4–30 min after feeding
Decapitated after feed (2)	Normal ingestion, ~ 4·6% excreted
Nerve cord in abdominal segment I pinched after feed (3)	Normal ingestion, ~ 6·1% excreted
Mid gut in abdominal segment I pinched after feed (4)	Normal ingestion, ~ 8·2% excreted
Nerve cord in abdominal segment I pinched before feed (5)	Often reluctant to feed. ~ 4·8 mg blood ingested, ~ 2·5% excreted
Nerve cord in abdominal segment VI pinched before feed (6)	~ 1·9 mg blood ingested, ~ 20% excreted
Nerve cord in abdominal segment I pinched before feeding; engorged to roughly normal degree and with junction between mid gut and pyloric chamber clamped (7)	~ normal amount blood ingested, in 7 of 29 preparations (24%) clamping causes a 2- to 9-fold increase in excretion rate

* See last paragraph of Discussion.

(which also seems to occur in *Anopheles quadrimaculatus* – Gwadz, 1969, fig. 1 B) is probably due to interruption of the supply of information via the stomatogastric system to the gut (see below) and a consequent drop in the peristaltic activity of the rectum.

These observations suggest strongly that the diuretic control of the adult is similar to that of the larva, and they are compatible with the following interpretation (Fig. 11).

As the animal feeds, sensory information from the distending abdominal wall is conveyed by the nerve cord to the brain (cf. Gwadz, 1969). The gut presumably provides no sensory information via this pathway as it receives no innervation from the nerve cord. When a certain degree of distension has been achieved information is conveyed from the brain by way of the stomatogastric nervous system to the mid gut. This information inhibits the retroperistaltic movements of the mid gut (which movements cannot be observed in the adult) and may increase the peristaltic activity of the intestine and rectum thus allowing the tubular fluid to be passed to the rectum and excreted. At about the same time (usually a little later) feeding is ended. In the absence of sensory information feeding continues until the animal becomes severely bloated, and diuresis is not started. In parentheses we may note that the rather small amount of ingestion observed in animals with the nerve cord pinched in abdominal segment VI (and hence with the three posterior abdominal segments denervated) is probably best interpreted as a result of abnormal sensory information coming from the pinched region. If the nerve cord is transected at this level and the animals are allowed to recover for 48 h (Gwadz, 1969) they show an ingestion significantly greater than normal. Although peristaltic movements of the intestine cannot be observed in the adult it is likely that (as in the larva) fluid is carried back along the intestine to the rectum by peristalses which are initiated by a build-up of hydrostatic pressure in the pyloric chamber. The peristaltic discharges of the rectum are also likely to be initiated by hydrostatic pressure.

This having been said some qualification needs to be made. The evidence for continual production of tubular fluid is by no means perfect. Although clamping the junction between Malpighian tubules and mid gut in engorged non-diuretic animals (4 (i) above, treatment 7) does cause a rise in the rate of excretion, the rate is still only about 25 % of the rate in normal diuretic animals. Although this discrepancy could have a mechanical cause (damage to some of the tubules, occlusion of the proximal regions of some of the tubules, incomplete closure of the mid gut in front of the pyloric chamber) it could also be due to a lower level of diuretic hormone in the non-diuretic animals. The results evidently do not rule out some increase in hormone production by normal animals in response to feeding.

Little has so far been said about the reabsorptive function of the rectum other than to point out that in the normal non-diuretic animal it must be properly coordinated with rectal discharge. In the normal diuretic animal reabsorption is presumably inhibited, unless it is so small relative to the diuretic excretion as to render any inhibition worthless.

The result obtained from fed animals in which the relatively undistended region of the mid gut in abdominal segment I was ruptured during pinching (treatment 4) is rather difficult to interpret. In all eight animals concerned excretion stoppe

'immediately' and completely. If rupturing the mid gut caused a reduction in the hydrostatic pressure there, presumably it would then have been easier for fluid to pass forwards from the Malpighian tubules and so cause a stoppage of excretion. A reduction of pressure is certainly suggested by the prompt appearance of the red colour of haemoglobin in the haemocoel. On the other hand the wall of the distended mid gut is very thin and fragile, and, as it bears on a layer of haemolymph which in turn bears on the abdominal wall, one gets the impression that most of the hydrostatic pressure must be borne by the abdominal wall. If rupturing involves no appreciable drop in hydrostatic pressure in the mid gut, a possible explanation of the result may be that the Malpighian tubules cannot secrete their fluid when bathed with a mixture of haemolymph and blood.

The type of diuretic control described here in which control is achieved mainly by the use of sensory information to modify the activity of the mid gut, and in which the production of tubular fluid is more or less continual, has so far been found only in *Aëdes aegypti*. This type of control (which can be conveniently called 'gut control') may have evolved to cope with the continual osmotic gain of water suffered by the larva (Stobbart, 1971) and its retention in the adult may be due to the advantage offered by its high speed of response, a factor likely to be of extreme importance to a flying animal which ingests relatively heavy fluid meals. Clearly it would be interesting to know whether this control occurs in all mosquitoes or only in those with freshwater larvae, and to know whether it occurs in other types of blood-sucking fly. The presence of a diuretic hormone has been demonstrated recently in the tsetse fly (Gee, 1975; Maddrell & Gee, 1974), and the isolated Malpighian tubules of both *Aëdes taeniorhyncus* and *A. aegypti* secrete more rapidly when treated with 5-hydroxytryptamine, which mimics the diuretic hormone, or with cyclic AMP which probably mediates the response of the tubules to the diuretic hormone and 5-hydroxytryptamine (Maddrell, unpublished; Maddrell, Pilcher & Gardiner, 1969, 1971). However, it must be stressed that gut control and the presence of diuretic hormone are not necessarily mutually exclusive; for example, gut control could occur in conjunction with a continual or constitutive synthesis of diuretic hormone, or conceivably both hormonal and gut controls could occur in the same insect. Gut controls may well be fairly common amongst the Insecta, but presumably they will be brought to light only by investigations which use a 'whole organism' approach. In this regard it is interesting to note that severance of the stomatogastric nerves reduces the rate of defecation in the grasshopper *Melanoplus sanguipes* (Dogra & Ewen, 1971).

In conclusion some comments need to be made about the size of the blood meal taken by unoperated animals. The mean weight of the meal has been found in this work to be about 2·63 mg (Table 4). This weight must be corrected for (*a*) the fluid excreted during feeding, (*b*) the transpiratory weight loss during the feeding period (5 min or less). The fluid excreted during feeding on average amounts to 0·07 mg (i.e. 10 droplets of urine) and the transpiratory weight loss to 0·003 mg (i.e. 5 min transpiration at a rate of 0·0006 mg/min). The corrected weight should therefore be about 2·70 mg. This agrees well with the generally reported figure of about 2·5 mg, but is appreciably smaller than the figure of 4·21 mm^3 (1 mm^3 blood $\cong$ 1·06 mg) given by Boorman (1960). Boorman's figure was obtained by feeding mosquitoes on blood labelled with ^{144}Ce (which is not excreted during the period of diuresis) and

comparing the radioactivity in the mosquitoes with that of a known volume of blood. Details of the techniques used in the measurement of radioactivity are not given by Boorman but as it is known (Stobbart, 1967) that tissue macerates increase the counting rates of samples of ^{22}Na and ^{36}Cl, it seems at least possible that Boorman's high figure is due to unsatisfactory sample geometry.

REFERENCES

BALSHIN, M. & PHILLIPS, J. E. (1971). Active absorption of amino acids in the rectum of the desert locust *Schistocerca gregaria*. *Nature, Lond.* **223**, 53–5.

BARBOUR, H. G. & HAMILTON, W. F. (1926). The falling drop method for determining specific gravity. *J. biol. Chem.* **69**, 625–40.

BOORMAN, J. P. T. (1960). Observations on the feeding habits of the mosquito *Aëdes* (*Stegomyia*) *aegypti* (Linnaeus): the loss of water after a blood-meal and the amount of blood taken during feeding. *Ann. trop. Med. Parasit.* **54**, 8–14.

CAZAL, M. & GIRARDIE, M. (1968). Contrôle humoral de l'équilibre hydrique chez *Locusta migratoria migratoriodes*. *J. Insect Physiol.* **14**, 655–68.

CHRISTOPHERS, S. R. (1960). Aëdes aegypti (*L.*) *the Yellow Fever Mosquito: Its Life History, Bionomics and Structure*. Cambridge University Press.

CLEMENTS, A. N. (1963). *The Physiology of Mosquitoes*. Oxford, London, New York & Paris: Pergamon Press.

DOGRA, G. S. & EWEN, A. B. (1971). Effects of severance of stomatogastric nerves on egg-laying, feeding and the neuroendocrine system in *Melanoplus sanguipes*. *J. Insect Physiol.* **17**, 483–9.

GEE, J. D. (1975). The control of diuresis in tsetse fly *Glossina austeni*: a preliminary investigation of the diuretic hormone. *J. exp. Biol.* **63**, 391–401.

GWADZ, R. W. (1969). Regulation of blood meal size in the mosquito. *J. Insect Physiol.* **15**, 2039–44.

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 diuretic hormone. *J. exp. Biol.* **41**, 459–72.

MADDRELL, S. H. P. (1966). The site of the release of the diuretic hormone in *Rhodnius* – a new neurohaemal system in insects. *J. exp. Biol.* **45**, 499–508.

MADDRELL, S. H. P. (1971). The mechanism of insect excretory systems. *Adv. Insect Physiol.* **8**, 199–331.

MADDRELL, S. H. P. & GEE, J. D. (1974). Potassium-induced release of the diuretic hormones of *Rhodnius prolixus* and *Glossina austeni*: Ca dependence, time course and localisation of neurohaemal areas. *J. exp. Biol.* **61**, 155–71.

MADDRELL, S. H. P., PILCHER, D. E. M. & GARDINER, B. O. C. (1969). Stimulatory effect of 5-hydroxytryptamine (serotonin) on secretion by the Malpighian tubules of insects. *Nature, Lond.* **222**, 784–5.

MADDRELL, S. H. P., PILCHER, D. E. M. & GARDINER, B. O. C. (1971). Pharmacology of the Malpighian tubules of *Rhodnius* and *Carausius*: the structure–activity relationship of tryptamine analogues and the role of cyclic AMP. *J. exp. Biol.* **54**, 779–804.

MORDUE, W. (1969). Hormonal control of malpighian tube and rectal function in the desert locust *Schistocerca gregaria*. *J. Insect Physiol.* **15**, 273–85.

MORDUE, W. (1972). Hormones and excretion in Locusts. *Gen. & comp. Endocrinol.*, suppl. 3, pp. 289–98.

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

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**, 39–69.

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.

PHILLIPS, J. E. (1969). Osmotic regulation and rectal absorption in the blowfly, *Calliphora erythrocephala*. *Can. J. Zool.* **47**, 851–63.

PHILLIPS, J. E. (1970). Apparent transport of water by insect excretory systems. *Amer. Zool.* **10**, 413–36.

RAMSAY, J. A. (1953). Exchanges of sodium and potassium in mosquito larvae. *J. exp. Biol.* **30**, 79–89.

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. (1971). Insect rectum. *Phil. Trans. R. Soc.* B **262**, 251–60.

SHAW, J. & STOBBART, R. H. (1972). The water balance and osmoregulatory physiology of the desert locust (*Schistocerca gregaria*) and other desert and xeric arthropods. *Symp. zool. Soc. Lond.* **31**, 15–38.

STOBBART, R. H. (1967). The effect of some anions and cations upon the fluxes and net uptake of chloride in the larva of *Aëdes aegypti* (L.), and the nature of the uptake mechanisms for sodium and chloride. *J. exp. Biol.* **47**, 35–57.

STOBBART, R. H. (1968). Ion movement and water transport in the rectum of the locust *Schistocerca gregaria*. *J. Insect Physiol.* **14**, 269–75.

STOBBART, R. H. (1971). Factors affecting the control of body volume in the larvae of the mosquitoes *Aëdes aegypti* (L.) and *Aëdes detritus* Edw. *J. exp. Biol.* **54**, 67–82.

STOBBART, R. H. & SHAW, J. (1974). Salt and water balance; excretion. In *Physiology of Insecta*, 2nd ed., (ed. M. Rockstein), chap. 8, pp. 361–446. New York, London: Academic Press.

VIETINGHOFF, V. (1967). Active transport in the rectal glands and the question of hormonal regulation of rectal reabsorption in *Carausius morosus* Br. *Věstník Československé Společnosti Zoologické* (*Acta Soc. zool. Bohemoslovacae*) **31**, 376–82.

WALL, B. J. (1967). Evidence for antidiuretic control of rectal water absorption in the cockroach *Periplaneta americana* L. *J. Insect Physiol.* **13**, 565–78.