

THE EXCRETORY SYSTEM OF THE STICK INSECT,
DIXIPPUS MOROSUS (ORTHOPTERA, PHASMIDAE)

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INTRODUCTION

The work which is to be described in this paper is in a sense a by-product of an investigation which is now being made of the process of urine formation in the Malpighian tubules of insects. The stick insect has been chosen as a suitable animal upon which to begin this investigation largely because of the relative ease with which its tubules can be studied as isolated preparations. Some of this work has already been published (Ramsay, 1953*b*, 1954, 1955). The lack of information upon the more general aspects of excretion in this insect has made itself felt, and for this reason it was decided that a survey of the whole excretory system would have to be undertaken.

Although the Malpighian tubules are commonly regarded as the excretory organs of insects it is becoming increasingly clear that this conception is misleading. Many of the substances which are excreted in the urine—by which is meant the fluid issuing from the tubules—are reabsorbed in other parts of the gut, in particular in the rectum. The excretory system is here understood to be the Malpighian tubules, together with the whole of the hindgut; but even this wider definition may not always be adequate since it does not include regions of the body in which excretory matter may be stored (e.g. uric acid in the fat-body of the cockroach) and takes no account of other routes (e.g. the genital system) by which, as so happens in the stick insect, the bulk of the calcium leaves the body.

It is necessary to correct any impression that all possible technical resources, even the limited technical resources available for physiological studies upon small animals, have been brought to bear upon this problem. Rather, it must be emphasized that this work is in the nature of a preliminary study and aims no further than to establish the broad outlines, leaving much detail still to be filled in.

MATERIAL AND METHODS

Except where otherwise stated, the results reported in this paper refer to the adult female stick insect, bred in the laboratory on the leaves of privet.

The methods used for the collection of fluids from various parts of the body cannot conveniently be summarized here, but will be described briefly in the appropriate context.

The methods of analysis used were as follows. Sodium and potassium; flame photometry (Ramsay, Brown & Falloon, 1953). Calcium; precipitation (twice) as

oxalate and titration with ceric sulphate (Kirk, 1950). Magnesium; titan yellow method (Orange & Rhein, 1951). Chloride; potentiometric titration with silver nitrate using apparatus devised by Mr P. C. Croghan, whom I wish to thank for instruction in the method. Phosphate; ammonium molybdate method as described by Delory (1949), adapted for 1 ml. cells. Uric acid; Benedict's arsenophosphotungstic method as described by Delory, adapted for 1 ml. cells. Osmotic pressure; cryoscopic method (Ramsay, 1949). pH; micro glass electrode (Hartree, 1952); I am indebted to Dr E. W. McConnachie for this measurement. Paper chromatography has been used as a qualitative method.

ANATOMY AND HISTOLOGY

A general account of the Phasmidae has been given by de Sinéty (1901). His description goes into great detail of some regions of the gut and its appendages while others, e.g. the whole of the hindgut, receive no mention at all.

The general arrangement of the excretory system is illustrated diagrammatically in Fig. 1. As is commonly the case in primitive insects the Malpighian tubules arise from an annulus which marks the division between midgut and hindgut.

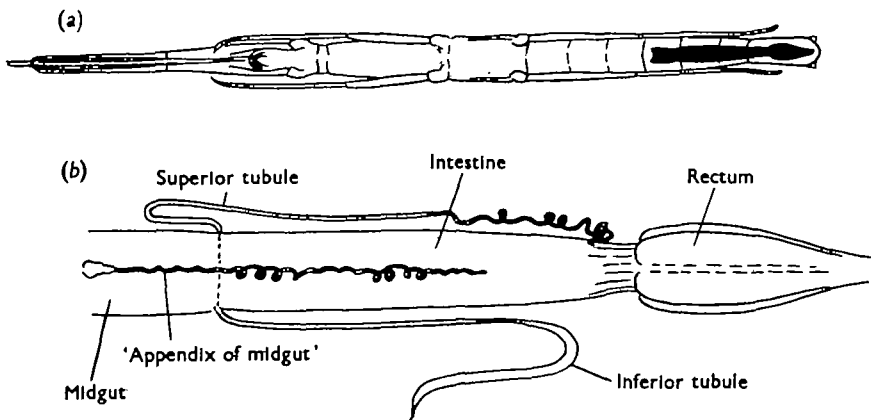


Fig. 1. (a) To show position of region illustrated in (b). (b) Malpighian tubules and posterior region of gut.

There is no sphincter or constriction in this region. The anterior region of the hindgut, which will be called the intestine, is separated from the short rectum by a sphincter which is normally closed. The three types of Malpighian tubule recognized by de Sinéty are distinguished most readily by their anatomical relations with the gut and other structures.

(a) The superior tubules arise from the annulus, run forward a short distance and then turn back following the intestine to which each tubule is attached by several short tracheae. These arise from the tracheal supply which invests the hindgut and on reaching the tubule each trachea divides into ascending and descending branches which supply a short length of tubule. Towards the distal

(blind) end the tubule is more contorted and ends at about the level of the rectal sphincter. The tubules of the nymph correspond to the superior tubules of the adult.

(b) The inferior tubules arise from the annulus in pairs having a very short common trunk and run at first directly backwards. The distal region of the inferior tubule, which may represent one-third or more of its total length, is in the form of a dilatation containing white granules; it is not closely applied to the gut but lies free in the posterior body cavity being attached at its blind end by short tracheal branches to the connective tissue of the fat-body. The blind end itself is provided with a cap of cells of vesicular appearance, the cells of Sirodot, between which the tracheal branches pass to spread out over the distal dilatation. The only other tracheal supply is a single branch which applies itself to the tubule at the proximal end and runs in a loose spiral over the proximal and middle regions. There are no inferior tubules in the nymph and they are poorly developed in the male (de Sinéty).

(c) The tubules of the third kind ('appendices of the midgut') open separately into small pyriform dilatations of the midgut wall anterior to the annulus. They are thinner than the superior and inferior tubules and more contorted. They run directly back to end close to the intestine about one-third of the distance from the annulus to the sphincter.

All three types of tubule are provided with muscular elements running in loose spirals over their walls and can be seen to undergo gentle writhing movements.

In each of two insects an accurate count was made of the tubules of the three types, which were found to be present in the following numbers: superior tubules 24, 23; inferior tubules 134, 134; appendices of the midgut 32, 25.

The appearance of the epithelium in the different regions of the gut has been studied in sections fixed in Susa, cut at 10μ and stained in iron haematoxylin. At the region of the annulus the columnar epithelium of the midgut gives way to the cubical epithelium of the intestine and this in turn to the tall columnar epithelium of the six rectal glands. These epithelia are illustrated in Fig. 2.

The Malpighian tubules have been examined alive in haemolymph and are illustrated in Fig. 3.

(a) *Superior tubules.* When observed under the low power the cells of the healthy tubule appear as a bright transparent colourless wall whose inner margin is made discernible by the presence of granules of various kinds and in varying amount. In the middle and proximal regions the granules have a greenish yellow colour, in the distal region they are white and the tip is generally devoid of granules. When the insect has been feeding regularly very few granules are present, but when the insect has fasted for 3 or 4 days the tubule appears to be packed with granules which, however, are not free in the lumen but are attached in masses to the inner margin of the wall. These granules are soluble in dilute alkali but not in dilute acid. An alkaline extract of the tubules of fasting insects contains more than 20 times as much uric acid as a similar extract of the tubules of fed insects. This suggests that these granules contain uric acid; they do not, however, have the appearance of the

uratic spheres known from the urine of *Rhodnius* nor do they appear to be enmeshed by the filaments of the brush border as in that insect (Wigglesworth, 1931 *a, b*).

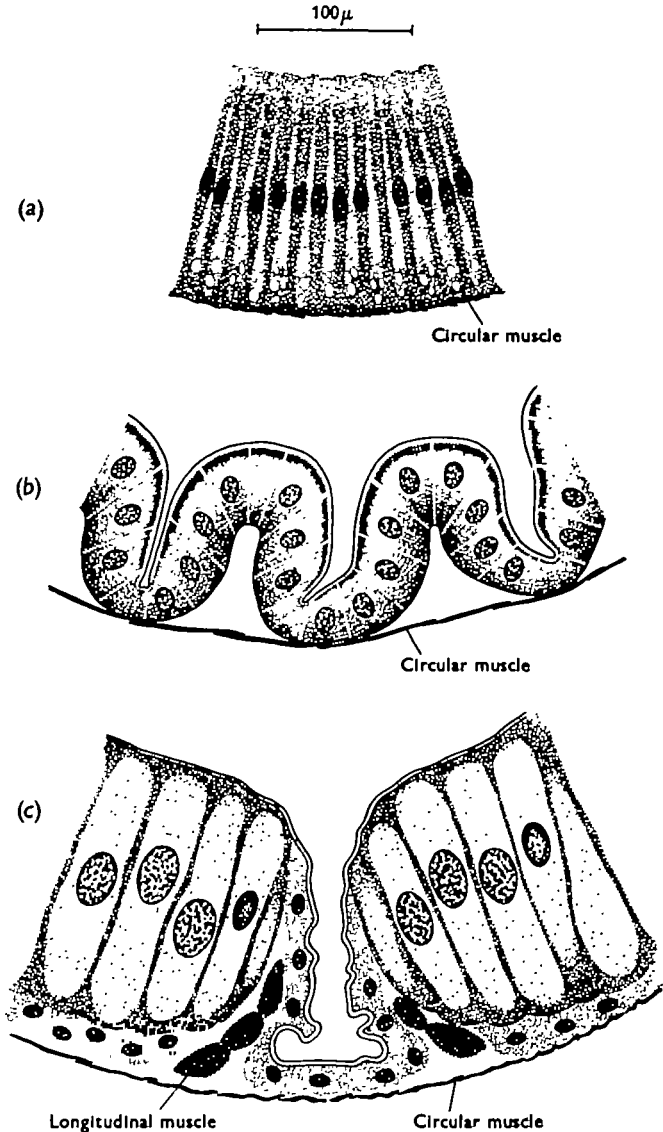


Fig. 2. Wall of gut in transverse section. From material fixed in Susa and stained in iron haematoxylin. (a) Midgut. (b) Intestine. (c) Rectum.

Over most of the length of the tubule the cells (which are bi-nucleate, de Sinéty) are provided with a well-developed brush border which has no clearly defined inner margin and is therefore of the 'bürstensaum' type (Wigglesworth, 1931 *b*). At the proximal end the diameter is greatest, about 120 μ, and the brush border is

deep, but with very fine striations and a very indefinite inner margin. Towards the middle region the diameter decreases to about 100μ , the brush border becomes a little less deep and the striations (filaments) are clearer and all of the same length which defines the inner margin, although no surface can be brought to focus (Fig. 3*b*). The diameter of the distal region is about 80μ . Over the last 2 or 3 mm. at the tip the character of the border changes. The filaments are shorter and gathered into clumps, rather in the way that the hairs of an animal's fur are held together by surface tension when wet, and the inner margin can be brought to focus (Fig. 3*a*).

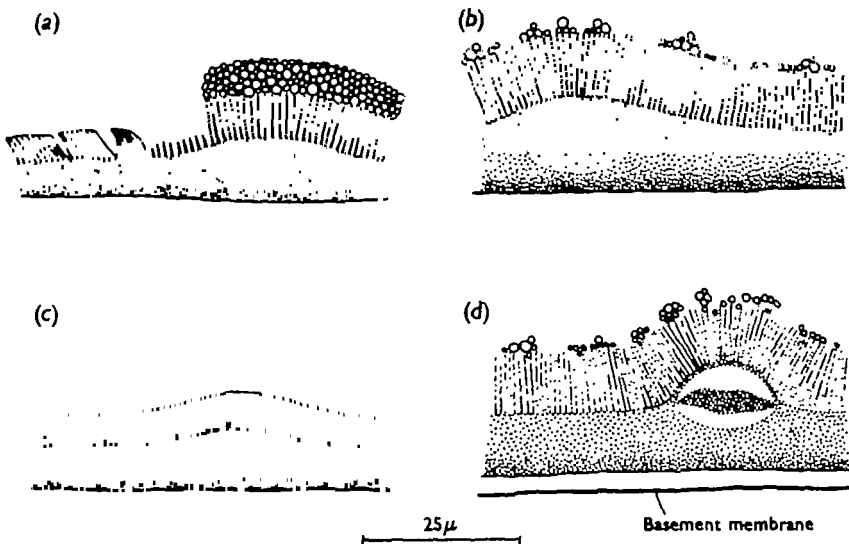


Fig. 3. Walls of Malpighian tubules. From living material. (a) Superior tubule, distal region, showing change in brush border near the tip (left); in serum. (b) Superior tubule, middle region; in serum. (c) 'Appendix of midgut'; in serum. (d) Superior tubule, middle region, after 2 hr. in Ringer solution, showing changes in nucleus and brush border.

(b) *Inferior tubules.* Over their middle and proximal regions the inferior tubules present the same appearance as the superior tubules, apart from the fact that they contain rather fewer granules. The distal dilatations, $250-300\mu$ in diameter, are provided with a brush border of what appears to be the same type as found in the proximal and middle regions; this can be established in favourable cases where the dilatation is not completely packed with granules. The cells of Sirodot have not been studied further.

The contents of the distal dilatation were analysed qualitatively in the related phasmid *Leptynia attenuata* by de Sinéty. He demonstrated the presence of carbonate by microdiffusion of CO_2 into baryta. He claimed to have identified uric acid, oxalate and leucine upon crystal form but without any more specific tests. In the course of the present work carbonate has been confirmed but not oxalate or leucine. Uric acid is present in small amounts. There is a great deal of calcium and only a trace of magnesium. It is concluded that the principal constituent of

these granules is calcium carbonate. The egg shells of phasmids are hardened with calcium oxalate (Moscona, 1950). Since the inferior tubules with their dilatations and granules are absent in the nymph and poorly developed in the male, and since the granules appear to be reduced in quantity during fasting (egg production continuing), it seems likely that they are a calcium reserve which can be drawn upon to provide material for the egg shells.

(c) *Appendices of the midgut.* These tubules are narrower (*c.* 50μ) than the others and are uniform throughout their length. There is a well-developed brush border and the inner margin can be brought to focus (Fig. 3*c*). It is perhaps less sharply defined than the scalloped edge of the 'wabensaum' in *Rhodnius* (Wigglesworth, 1931*b*), but more definite than the edge of the border as seen at the tip of the superior tubule (Fig. 3*a*). No granules have been seen in these tubules.

Wigglesworth has reported that in *Rhodnius* the normal histological appearance of the tubules is not preserved in artificial media. The same is true of the tubules of the stick insect in all artificial media as yet tested. A Ringer solution approximating to haemolymph in composition has been prepared (Ramsay, 1955). Within an hour or two after being placed in Ringer the walls of the tubules begin to lose their characteristic bright appearance. The striations of the brush border become fainter and the border eventually disintegrates. The appearance of the nuclei changes with what appears to be the separation of a coagulum from a clear fluid within the nuclear membrane (Fig. 3*d*). A space appears between the cells and the basement membrane which is clearly visible only under these conditions, and after 5 or 6 hr. a disintegrating mass of cells fills the lumen. The contraction of the muscular elements in the walls continues for some time longer.

Table 1. *Relative quantities of mineral bases as percentage of total mineral base expressed in equivalents*

	Privet leaves (1)	Nymph faeces (2)	Adult faeces (3)	Eggs (4)
Na	13	9	11	10
K	35	33	66	5
Ca	46	51	8	83
Mg	6	7	15	2
	100	100	100	100

EXCHANGE OF MINERAL BASES

The insect takes in sodium, potassium, calcium and magnesium in the proportions in which they are present in privet leaves (Table 1, col. 1), and should presumably eliminate them in the same proportions. In the case of the nymph elimination is entirely by way of the faeces, and it is found that in the faeces of the nymph the four bases are present in much the same proportions as in the leaves (Table 1, col. 2). In the case of the adult female the eggs constitute a second channel of elimination. The rate of egg production is considerable and in terms of ash a somewhat greater weight is eliminated as eggs than as faeces. It is difficult, however, to

make out a balance sheet since there is at any time a large number of eggs in the body at various stages of development, and a large and variable store of calcium in the inferior tubules. Cols. 3 and 4, Table 1, show that most of the potassium and magnesium is eliminated in the faeces while most of the calcium is eliminated in the eggs.

THE HAEMOLYMPH

No extensive analysis of stick-insect haemolymph, comparable with Levenbook's (1950) analysis for *Gastrophilus*, has yet been made, but a certain amount of information from various sources is assembled in Table 2, col. 1. The stick insect conforms to the usual pattern of the herbivorous insect in that its haemolymph contains more potassium than sodium and more magnesium than calcium. It also contains a great deal of phosphate and appears to be supersaturated with respect to magnesium phosphate.

Table 2

	Haemolymph (1)	Serum (2)	Urine (3)
pH	—	6.6	6.8-7.5
Δ	160 mM./l. NaCl*	171 mM./l. NaCl	171 mM./l. NaCl
Na	8.7 m.equiv./l.†	11 m.equiv./l.	5 m.equiv./l.
K	27.5 m.equiv./l.†	18 m.equiv./l.	145 m.equiv./l.
Ca	16.2 m.equiv./l.†	7 m.equiv./l.	2 m.equiv./l.
Mg	145 m.equiv./l.†	108 m.equiv./l.	18 m.equiv./l.
Cl	93 m.equiv./l.‡	87 m.equiv./l.	65 m.equiv./l.
PO ₄ ³⁻	120 m.equiv./l.	39 m.equiv./l.	51 m.equiv./l.
Uric acid	10.4 mg./100 ml.§	4.5 mg./100 ml.	43 mg./100 ml.

* Rouschal (1940). † Duchâteau *et al.* (1953). ‡ May (1935). § Florkin (1936).

The fresh haemolymph of the stick insect is not a very convenient medium for physiological work since it almost invariably coagulates during the course of an experiment. It has been found that if the fresh haemolymph is heated to 100° C. for about 5 min. and then centrifuged, a fluid, which will here be called serum, is separated from a compacted clot. This fluid still preserves the characteristic blue-green colour of fresh haemolymph which, according to Abeloos & Toumanoff (1926) is due to 'carotinalbumines'. As a physiological medium for Malpighian tubules it does not appear to be in any way inferior to fresh haemolymph, and has been extensively used in an investigation which is reported elsewhere (Ramsay, 1955). An analysis of serum is given in Table 2, col. 2. The concentrations of calcium, magnesium and phosphate are reduced as compared with haemolymph, and it is probable that earthy phosphates have been precipitated and removed with the clot. Even if all the phosphate is present as PO₄³⁻, which is unlikely, there is an anion deficit of about 20 m.equiv./l. in serum.

A word of comment is needed on the subject of sodium and potassium concentrations. The figures given by Duchâteau, Florkin & Leclercq (1953) show a lower concentration of sodium and a higher concentration of potassium than do the figures for serum (Table 2, cf. cols. 1 and 2). This difference is not due to the heat-treatment of serum since the same concentrations are found in the haemolymph

from which serum is prepared. Later in this paper (Table 5) further figures are given for haemolymph, showing higher sodium and lower potassium than in serum. When this inconsistency was discovered investigation was made into its cause. The collections of haemolymph from which serum was prepared were made by cutting off a leg and drawing up the haemolymph into a tube, the exudation of haemolymph being assisted by light pressure applied to the insect's body with the fingers. A number of insects were subjected to this treatment and then dissected, and in some of them it was found that the midgut was perforated near the annulus. The effect of this would be to contaminate the haemolymph with urine having a low sodium and high potassium concentration. It is possible that similar contamination affected the collections made by Duchâteau *et al.* (1953) in greater degree.

Duchâteau, Sarlet & Florkin (1952), using methods of biological assay, have given an extensive list of amino-acids, free or combined in a non-protein form, found in stick-insect haemolymph. In the present work a brief investigation using paper chromatography was made of haemolymph de-proteinized with trichloroacetic acid. Only two ninhydrin-positive compounds which withstood hydrolysis with concentrated hydrochloric acid were found in substantial concentration; one of these was glycine, the other could not be identified by R_F values. An imposing array of spots appeared after hydrolysis, but these were not further studied. It is concluded that most of the amino-acids recoverable from de-proteinized haemolymph are present as peptides.

The total volume of the haemolymph in a stick insect can be found approximately in the following way. A leg is cut off, as much haemolymph as possible is collected and its volume measured in a capillary pipette. An equal volume of Ringer solution is then injected, and after about half an hour has been allowed for it to become uniformly distributed a second sample of haemolymph is taken. Both first and second samples are then heat-coagulated and centrifuged. The dilution of the second sample is found by adding Ringer to the first sample until the same depth of blue-green colour is obtained. A simple calculation then gives the total volume of haemolymph in the insect. Figures thus obtained ranged from 76 to 186 mm.³ with an average value of 132 mm.³ for six insects. The average weight of an adult female stick insect is 0.8 g., so that the haemolymph represents about 15% of the insect's weight.

THE URINE

Sufficient urine to make possible the analyses in Table 2, col. 3, was collected in the following way. The insect was fastened down on its back with plasticine, and the abdomen was opened along the mid-ventral line. A cotton-thread ligature was tied around the intestine just posterior to the annulus, passing between the intestine and the Malpighian tubules so as to leave the latter free. A slit was then made into the midgut wall about 2 mm. anterior to the annulus, the gut contents were removed and a cannula was tied into place, the ligature passing just anterior to the annulus. In this way it was possible to collect the fluid, presumably urine, accumulating in the short stretch of gut between the ligatures. Urine was produced at rates of

4.0–6.5 mm.³/hr. The urine rose in the cannula as a pale yellow fluid soon becoming dark brown on exposure to air.

As was already known, the potassium concentration is higher and the sodium concentration is lower in the urine than in the haemolymph. Calcium, magnesium and chloride are lower in the urine, but phosphate is higher. There is an apparent anion deficit of some 50 m.equiv./l. Uric acid is in higher concentration in the urine as might be expected.

CHANGES IN THE URINE DURING ITS PASSAGE THROUGH INTESTINE AND RECTUM

In this and in the next section consideration is restricted to water, sodium and potassium. Methods of analysis suitable for the small volumes available have not yet been developed for other constituents of the urine.

It is immediately obvious that with urine being produced at a rate of about 6 mm.³/hr. all the water would be removed from the haemolymph in less than 24 hr. if it were not reabsorbed in the hindgut. It is also obvious that all the potassium in the haemolymph would be removed in less than 3 hr. and therefore potassium, like water, must presumably be reabsorbed. To demonstrate such reabsorption is not altogether a straightforward problem since besides the urine semi-digested food containing potassium is being passed into the hindgut at an unknown rate. This complication can be avoided by using fasting insects.

For these experiments the insects were kept without food in glass jars lined with moist filter-paper, in which they could survive for more than 10 days. The production of faeces fell off rapidly from about 20 mg. to about 2 mg. dry weight per insect per day. 20 mg. of 'fasting' faeces were ashed and found to contain 0.3 μ equiv. of sodium and 18 μ equiv. of potassium; this indicates a loss of 0.03 μ equiv. of sodium and 1.8 μ equiv. of potassium per insect per day. From figures already quoted it can be calculated that the Malpighian tubules will excrete 0.7 μ equiv. of sodium and 21 μ equiv. of potassium in one day, so that considerable reabsorption must take place.

This of course presupposes that the rate of flow and composition of the urine are maintained at the same levels in the fasting insect. The conspicuous accumulation of granules in the Malpighian tubules of the fasting insect suggest at first sight that the flow of urine must be reduced almost to zero, but in fact this is not so. Collections of urine made in parallel experiments on fed and fasting insects showed only a small decline in rate of flow, the figures being 5.4 and 6.4 mm.³/hr. for fed insects and 4.2 and 2.9 mm.³/hr. for fasting insects.

Sodium and potassium concentrations in the urine of fasting insects are given in Table 3, col. 3, and when these are compared with the figures in Table 2, col. 3, it can be seen that the sodium concentration is slightly greater and the potassium concentration is slightly less in 'fasting' urine as compared with 'fed' urine. Taking rate of flow as 3.5 mm.³/hr., sodium concentration as 6.5 m.equiv./l. and potassium concentration as 125 m.equiv./l. the quantities excreted by the Malpighian tubules of a fasting insect per day are 0.54 μ equiv. of sodium and 10.5 μ equiv.

of potassium, so that about 95% of the sodium and about 80% of the potassium must be reabsorbed.

The fact of reabsorption being established, the next question to be asked is whether this takes place exclusively in the hindgut or whether there is a forward movement of urine into the midgut as may occur in the mosquito larva (Ramsay, 1953*a*). This was tested by injecting phenol red into the body cavity. The dye is quickly excreted by the Malpighian tubules, and when the insect is opened it is found to be present in the intestine and rectum. On one occasion only was the dye found in the midgut and then only a few millimetres anterior to the annulus. It is therefore in the intestine and rectum that we may expect to find reabsorption of urinary constituents.

Table 3. *Haemolymph, intestinal fluid and urine from three fasting insects*

Haemolymph (1)			Intestinal fluid (2)			Urine (3)		
Na	K	O.P.	Na	K	O.P.	Na	K	O.P.
15	11	160	7	102	162	5	120	150
11	10	173	5	101	172	7	113	162
15	10	155	6	133	166	7	135	158

O.P. = osmotic pressure.

Table 4. *Rectal fluid from six feeding insects*

Na	K	O.P.	Na	K	O.P.
21	530	551	22	320	449
8	225	255	29	415	520
11	160	199	21	310	364

It is well known from the work of Wigglesworth (1932) that the rectal glands of insects are concerned in the reabsorption of water. It is sometimes possible, by gentle pressure upon the abdomen, to force a drop of fluid out of the stick insect's anus. Analyses of such drops from six insects are given in Table 4. The high values of osmotic pressure indicate the withdrawal of water which is to be expected. The sodium/potassium ratios are very variable, and since these measurements were made on fed insects they are difficult to interpret.

It is also possible that reabsorption takes place in the intestine. Analyses of intestinal fluid from fasting insects are given in Table 3, together with analyses of urine collected in the usual way after the samples of intestinal fluid had been taken. The composition of intestinal fluid is seen to be substantially the same as that of urine, and there is no indication of increased osmotic pressure as in rectal fluid.

The possibility still remains, however, that there is reabsorption of urine in the intestine without substantial change in its composition. This possibility was studied in two ways. According to the first method the insect was opened and

ligatures were tied around the rectal sphincter and around the midgut just anterior to the annulus. After some hours the intestine was seen to be abnormally distended. A third ligature was then tied just posterior to the annulus, and a few hours later the distension of the intestine was noticeably less. According to the second method a cannula was filled with urine from another insect and was tied into the intestine by a ligature just posterior to the annulus, the rectal sphincter being ligatured as before. A small pressure (1.5 cm. of water) applied to the cannula kept the intestine slightly distended. The urine was gradually absorbed at rates of 1.5 and 0.9 mm.³/hr. in the two experiments performed. The urine remaining in the intestine and cannula was analysed after the experiment and was not found to have changed appreciably in composition.

It is therefore concluded that the main site of reabsorption of sodium, potassium and water is the rectum, and that while some reabsorption can take place in the intestine this is probably inconsiderable under normal conditions (but see next section).

THE ROLE OF THE EXCRETORY SYSTEM IN REGULATING THE COMPOSITION OF THE HAEMOLYMPH

The figures presented in the early part of the previous section show that the excretory system is responsible for a rapid turnover of sodium, potassium and water, and that this is not incompatible with a haemolymph of reasonably constant composition. As Boné (1944) first showed, the sodium/potassium ratio in the haemolymph of insects is correlated with the diet, being high in carnivorous and low in herbivorous insects. Boné did not go so far as to suggest that this was a *direct* result of the high potassium intake of herbivorous insects, but a simple relationship of this kind is not excluded as a possibility. Tobias (1948) was able to lower the sodium/potassium ratio in the haemolymph of the cockroach by feeding the insect on lettuce, but not to the level characteristic of truly herbivorous insects. Hoyle (1954) has recently shown that in the locust the potassium concentration in the haemolymph falls by about 50% during starvation; it appears that in the locust the normal composition of the haemolymph represents the balance struck between processes of assimilation and excretion.

It was therefore natural to begin the investigation of this matter in the stick insect by following the changes in the composition of the haemolymph during starvation. Six insects were isolated without food in glass jars lined with moist filter-paper. Samples of haemolymph, about 1 mm.³ in volume, were collected by thrusting a pipette through the arthro-dial membrane at the base of a leg. The first sample was taken immediately after the insect had been removed from a cage containing privet leaves, the second sample after 48 hr. and the third sample after 96 hr. starvation. The analyses of these samples are given in Table 5. In all cases except one there is a slight (<20%) decrease in the potassium concentration while the sodium concentration and osmotic pressure remain relatively constant. Quite clearly the effect of starvation is much less in the stick insect than in the locust.

The next step was to inject solutions into the haemolymph so as to alter the concentrations and then to follow the process of return to normal. For these experiments the same six insects were used. Immediately after the last sample of haemolymph had been taken (at 96 hr.) the insects were injected, nos. 1-3 with 50 mm.³ of 170 mM./l. NaCl and nos. 4-6 with 50 mm.³ of 170 mM./l. KCl. After 1 hr. had been allowed for the injected solution to become uniformly distributed about the body the first sample was taken, and subsequent samples at 6, 24, 72 and 96 hr. reckoned from the time of injection. As soon as the last sample had been taken the insect was opened, a sample of intestinal fluid was taken and a small sample of urine was collected in the usual way.

The effects of injection of NaCl and KCl were unmistakably different. The NaCl-injected insects appeared to be normal in every way, whereas the KCl-injected insects quickly lost the ability to make co-ordinated movements. Of these no. 4 showed a considerable measure of recovery, but nos. 5 and 6 remained completely paralysed for 24 hr. and were judged to be dead, although in the case of no. 6 it seems that eventual recovery may still have been possible. Had this been realized at the time, the attempt would have been made to collect urine from no. 6.

The results of analyses are assembled in Table 6. Assuming that the normal concentration of sodium (and of potassium) is 15 m.equiv./l. and that the volume of the haemolymph is 135 mm.³ the injection of 50 mm.³ at 170 m.equiv./l. should raise the sodium (or potassium) concentration in the haemolymph to about 55 m.equiv./l. Concentrations of this order, though somewhat lower, are found 1 hr. after injection.

In insects nos. 1-3 the sodium concentration fell slowly towards the normal value, but this change had only gone about half way in 3 days. The sodium concentration in the urine is about 3 times its usual 'fasting' level, while the potassium concentration is not increased. It is to be noted that in all three cases there is a substantial rise in sodium concentration and fall in potassium concentration in the intestinal fluid as compared with the urine.

These three insects continued to produce faeces which were collected and analysed. The sodium content was found to be much greater than in 'fasting' faeces and the potassium content less. The rates of excretion per day worked out at 1.1 μ equiv. of sodium and 0.85 μ equiv. of potassium; that is to say, in the NaCl-injected insect the rate of sodium excretion is more than 30 times greater and the rate of potassium excretion is more than 50% less than in the fasting insect.

There is therefore clear evidence of an adaptive response by the excretory system to an increase of the sodium concentration in the haemolymph.

On the other hand, the adaptive response is not a very effective one when seen in relation to its corrective action upon the haemolymph. The amount of sodium injected was 8.5 μ equiv., so that at a rate of excretion of 1.1 μ equiv. per day it would take over a week for the haemolymph to return to normal. From the figures in Table 6 the average concentration of sodium in the urine is 22 m.equiv./l. Taking the average rate of flow as 3.5 mm.³/hr. it can be calculated that sodium is excreted by the Malpighian tubules at a rate of 1.8 μ equiv. per day. This means that

Table 5. Changes in haemolymph during fasting

Hr.	Insect no. 1			Insect no. 2			Insect no. 3			Insect no. 4			Insect no. 5			Insect no. 6		
	Na	K	O.P.	Na	K	O.P.	Na	K	O.P.	Na	K	O.P.	Na	K	O.P.	Na	K	O.P.
0	12	13	161	11	14	154	10	15	159	10	17	153	10	14	167	11	14	163
48	14	12	159	13	13	156	11	10	160	11	13	160	13	14	160	13	14	160
96	12	11	160	10	12	154	10	12	167	11	12	161	10	17	151	12	13	154

Na and K in m.equiv./l.; O.P. in mm./l. NaCl.

Table 6. Changes in haemolymph after injection, nos. 1-3 with NaCl, nos. 4-6 with KCl

Hr.	Insect no. 1			Insect no. 2			Insect no. 3			Insect no. 4			Insect no. 5			Insect no. 6		
	Na	K	O.P.	Na	K	O.P.	Na	K	O.P.	Na	K	O.P.	Na	K	O.P.	Na	K	O.P.
0	12	11	160	10	12	154	10	12	167	11	12	161	10	17	151	12	13	154
1	48	8	—	49	10	—	51	10	—	9	42	—	8	54	—	9	45	—
6	45	9	—	45	10	—	49	12	—	9	23	—	6	70	—	9	38	—
24	42	9	—	44	11	—	47	10	—	9	19	—	6	82	—	10	24	—
72	31	10	161	38	11	—	38	10	157	10	14	152	—	—	—	—	—	—
96	—	—	—	35	11	150	—	—	—	—	—	—	—	—	—	—	—	—
Urine	21	126	147	22	126	—	24	124	154	5	148	148	—	—	—	—	—	—
I.F.	47	107	159	35	99	162	42	99	157	3	149	180	—	—	—	—	—	—

Na and K in m.equiv./l.; O.P. in mm./l. NaCl. I.F. = intestinal fluid.

0.7 μ equiv. per day must be reabsorbed at a time when the excretory system could best serve the insect by eliminating the maximum possible quantity of sodium.

In the case of potassium the concentration falls very much more rapidly (except in no. 5, in which the opposite change suggests general breakdown of the cell membranes). The return towards the normal concentration has progressed half way in about one day. Unfortunately these insects passed no faeces so it is not possible to estimate how much of this progress can be attributed to excretion. The evidence of the urine and intestinal fluid of the single insect examined indicate an increase in the rate of potassium excretion and a decrease in the rate of sodium excretion. It is also possible that some regulation occurs by potassium being taken up by the tissues, as was found by Tobias (1948) in the cockroach. It is certainly true that potassium can be given out by the tissues since it is possible to continue collection of urine for 6 hr. or more, and to recover from the urine much more potassium than could have been present originally in the haemolymph.

DISCUSSION

Existing knowledge of excretion in insects has been adequately reviewed (Wigglesworth, 1953; Roeder, 1953), and discussion will therefore be limited to such new facts about the stick insect as are here presented. When the scope of the investigation involves several organs and covers processes in which several stages can be recognized, it is scarcely possible to be both brief and comprehensive without being superficial. Investigations of this type, seeking to provide a general outline, tend to leave behind them more problems than they set out to solve. It will be necessary throughout this discussion to draw attention to many questions which have been left open.

The analyses of serum broadly confirm the figures given by other workers for the haemolymph. They extend existing knowledge only in respect of phosphate which is present in rather unexpectedly high concentration. As stated earlier both haemolymph and serum appear to be supersaturated with magnesium phosphate—that is to say, it is not possible to make up an artificial solution containing these ions at the same concentrations and at the same pH as in serum—but what this means in physico-chemical terms is not easy to see. The apparent anion deficit is possibly met by amino-acids of the acidic type. This is not incompatible with the view that most of the non-protein amino-acids are present as peptides. It is a little surprising not to find evidence of more than two free amino-acids in substantial concentration, and it may be added that preliminary chromatograms in which different samples of serum were tested revealed a certain lack of uniformity. It seems likely that the relationship between free amino-acids, peptides and proteins in the haemolymph is a labile one, depending no doubt on the state of nutrition as well as on other factors.

The analyses of urine, on the other hand, provide more complete information about the composition of the fluid produced by the Malpighian tubules than is available for any other insect. In insects it is not usually possible to collect this fluid without admixture of fluid from the midgut or without exposing it to the

action of the rectal glands, and most of the 'urines' which have been analysed are in fact the fluids collected from the anus. The urine as collected in the present work does not include any contribution from the Malpighian tubules of the third type ('appendices of the midgut'), but this probably does not amount to more than 10% of the total. That the urine contains much potassium and little sodium was already known (Ramsay, 1953*b*), and the reabsorption of these ions in the rectum was not unexpected since this is known to occur in the mosquito larva (Ramsay, 1953*a*). The relation between the concentrations in the haemolymph and in the urine of these two ions has been more fully studied in another paper (Ramsay, 1955). It would be interesting to have similar information about the other inorganic ions; in particular it would be interesting to compare the excretion of calcium in the adult and in the nymph.

Very little can be said about nitrogenous excretion beyond that uric acid is present in the haemolymph and is definitely concentrated in the urine. It can be calculated that about 0.06 mg. of uric acid per day is eliminated by the Malpighian tubules, much less than in the smaller insect *Rhodnius* which excretes about 0.5 mg. of uric acid per day (Wigglesworth, 1931*a*). The deep brown colour of extracts of faeces makes it impossible to apply colorimetric methods directly, but chromatography shows the presence of uric acid in the faeces and gives no definite indication of allantoin or urea in faeces or in urine.

The action of the excretory system is modified so as to restore the normal composition of the haemolymph after this has been disturbed by injection of sodium or potassium, but its ability to do so is not great. It has been shown that 8.5 μ equiv. of sodium injected into the haemolymph is eliminated at a rate of 1.1 μ equiv. per day, with prospect of restoring the normal composition of the haemolymph in about a week. When one considers that *Rhodnius* takes in about 13 μ equiv. of sodium at a meal and gets rid of most of it in 3 hr. (calculated from Wigglesworth, 1931*a*) while maintaining the composition of its haemolymph remarkably constant (Ramsay, 1952), one cannot avoid the conclusion that the stick-insect's powers of regulation are relatively feeble.

SUMMARY

1. The excretory system (Malpighian tubules and hindgut) of the stick insect is described, in extension of the earlier description by de Sinéty.
2. Analyses have been made of the inorganic components of serum (haemolymph heat-coagulated and centrifuged), of urine (the fluid produced by the Malpighian tubules), of faeces, eggs and privet leaves.
3. In the adult female insect most of the calcium ingested leaves the body with the eggs.
4. The haemolymph has the characteristic mineral base pattern of the herbivorous insect; it appears to be supersaturated with magnesium phosphate. Its volume varies from 70 to 180 mm.³, with an average value of 130 mm.³, representing about 15% of the weight of the insect.

5. The urine has the usual high concentration of potassium; except for phosphate the other inorganic components are present in lower concentration than in the haemolymph. Urine is produced at a rate of about 6 mm.³/hr., implying complete turnover of the water of the haemolymph every 24 hr.

6. Except for a slight (20%) decrease in potassium concentration the composition of the haemolymph remains constant during fasting.

7. The regulatory powers of the excretory system have been tested by injecting NaCl and KCl into the haemolymph and are shown to be relatively feeble.

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