The Rectal Complex in The Larvae of Lepidoptera

J. A. Ramsay

Email alerting service	Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

[203]

THE RECTAL COMPLEX IN THE LARVAE OF LEPIDOPTERA

By J. A. RAMSAY, F.R.S.

Department of Zoology, University of Cambridge

CONTENTS

In the so-called 'cryptonephric' condition of the excretory system in insects the distal ends of the Malpighian tubules are closely applied to the rectum and enclosed with it in a special chamber, the perinephric space, separated from the rest of the body cavity by the perinephric membrane. The term 'rectal complex' refers to this association of tubules and rectum, which is found in the larvae (but not in the adults) of most Lepidoptera.

In the mealworm (Coleoptera) the rectal complex has notable ability to remove water from the faeces, but this ability is not conspicuously developed in the larvae of the two species of Lepidoptera here studied: *Pieris brassicae* and *Manduca sexta*. On the other hand these larvae have notable ability to maintain salt balance under heavy dietary loading, and in this the rectal complex plays an important part.

A study of salt balance in more detail has shown that more sodium can be eliminated in the faeces than enters the rectal complex from the intestine. Consideration of other possible routes of entry points strongly to the Malpighian tubules. Superimposed upon a net flow of tubule fluid out of the rectal complex there is a tidal flow, brought about by the rectal musculature and amplified by dilatations of the cryptonephric tubules, which could bring in fluid from the free tubules and afford opportunity for the uptake of salts. Evidence is presented in support of this view.

This tidal flow of tubule fluid and uptake of salts could be the basis of the build-up of high osmolarity in the perinephric fluid and could contribute to the removal of water from the faeces. It could also be the basic mechanism in the mealworm, the leptophragmal mechanism being superimposed upon it.

1. Introduction

In the so-called 'cryptonephric' condition of the excretory system in insects the distal ends of the Malpighian tubules are closely applied to the rectum and enclosed with it in a special chamber, the perinephric space, separated from the rest of the body cavity by the perinephric membrane. The term 'rectal complex' refers to this association of tubules and rectum. It is found in the larvae and adults of some Coleoptera (such as the mealworm, *Tenebrio molitor*) and in the larvae (but not in the adults) of most Lepidoptera. In some of the Coleoptera, but never in any of the Lepidoptera, the cryptonephric tubules make contact with the inner side of the perinephric membrane, which is partially interrupted at the point of contact, and only a small window, known as a leptophragma, separates the lumen of the tubule from the haemocoel. The leptophragmata may be closely spaced along the tubule, or they may be present in some tubules and absent from others in the same animal. A full account of these relationships has been given by Saini (1964).

Two papers have been published on the rectal complex of the mealworm to which the present author has contributed (Ramsay 1964; Grimstone, Mullinger & Ramsay 1968). In the second of these papers a model was proposed for the mechanism of the rectal complex according to which the high osmolarity of the fluids within the rectal complex is brought about by the inward secretion of potassium chloride, unaccompanied by water, at the leptophragmata. The inward secretion of potassium chloride against a concentration gradient was well supported by experiment, but the corollary decrease in the osmolarity of the fluid surrounding the rectal complex could only be detected on occasion; loss of relative impermeability of the leptophragmata to water in a deteriorating preparation was the explanation offered for this failure. Be that as it may, the model is applicable only to a rectal complex which incorporates leptophragmata. It affords no explanation of the mechanism of the rectal complex in insects not provided with leptophragmata, and may not give a complete account of the mechanism in those insects which are so provided. These considerations point clearly to the importance of investigating the rectal complex in the larvae of Lepidoptera, from which leptophragmata are uniformly absent.

Two species were used in this investigation: *Pieris brassicae* (Pieridae) and *Manduca sexta* (Sphingidae). Larvae of the final instar were taken from cultures of these species maintained at the Entomological Field Station of the Department of Zoology. *Pieris* was generally available in unlimited numbers, but the culture of *Manduca* was of uncertain strength. Most of the work was done on *Pieris*, but *Manduca*, being larger, was preferred for most purposes and was used when available. I am most grateful to Dr B. O. C. Gardner for his efforts to maintain supplies of material.

2. The structure of the rectal complex

In essentials the anatomical relations of the Malpighian tubules conform to the same pattern in most families of *Lepidoptera*, as first described by Cholodkowsky (1887). In a more recent physiological study of the Malpighian tuoules of *Calpodes ethlius* (Hesperiidae) Irvine (1969)

found it useful to distinguish four regions in the free tubule (i.e. that part of the tubule not enclosed within the rectal complex), and his terminology will be followed here (figure 1).

There are six tubules, and each leaves the rectal complex separately from the others. The first portion is the relatively short and straight *rectal lead*, giving way to the highly convoluted *iliac plexus* bordering the intestine. From the iliac plexus the straight *yellow region* runs anteriorly over the surface of the midgut well beyond halfway and then turns and runs posteriorly as the *white region*. Near the junction of midgut and intestine the white regions of the three tubules on each side unite into a small pulsating vesicle from which a very short common duct leads into the lumen of the gut.
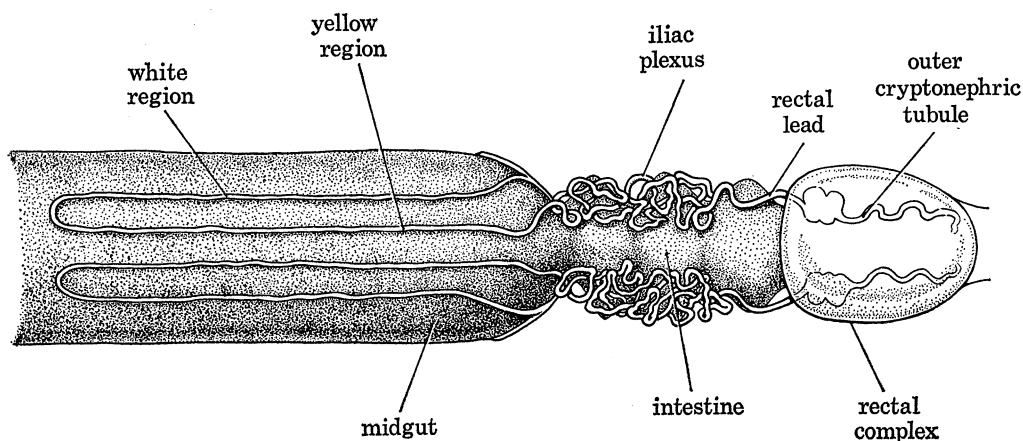

FIGURE 1. Diagram to show the anatomical relations of the alimentary canal and Malpighian tubules in lepidopteran larvae. The inner cryptonephric tubules are not shown.
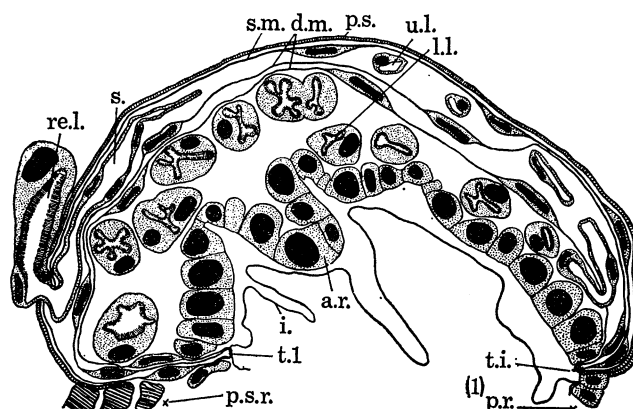

FIGURE 2. Coronal section through one side of the rectal complex (after Henson 1937). a.r., 'cryptonephric' rectal epithelium; d.m., inner perinephric membranes; i., detached cuticle; l.l., inner tubule; p.r., anal canal; p.s., muscular sheet over outer perinephric membrane; p.s.r., sphincter between intestine and rectum; re.l., rectal lead; s., dilatation of outer tubule; s.m., outer perinephric membrane; t.i., thickened cuticle at attachment of perinephric membranes; u.l., outer tubule.

The most detailed study of the rectal complex in Lepidoptera is that of Henson (1937) on *Vanessa urticae* (Nymphalidae); figure 2 is taken from his paper. Immediately after entering the rectal complex the tubule is dilated to form a large thin-walled sac of irregular shape. From this dilatation a thin-walled transparent tubule, which will be called the outer tubule, follows a sinuous course to the posterior margin of the rectal complex. Here it turns forward as the inner

tubule, readily distinguishable from the outer tubule by its thick opaque wall and restricted lumen. The inner tubule is highly convoluted and may be branched in some species. An account of some of the variations in the arrangement of the cryptonephric tubules in Lepidoptera has been given by Ishimori (1924).

Henson also described the membranes, of which there are three, enclosing the tubules and rectum. The outer membrane is a delicate transparent membrane on which the extrinsic musculature of the rectal complex is inserted. Within the outer membrane are two further membranes which are even thinner and more delicate than the outer membrane and are so transparent as not to be directly visible in dissections; but their presence can be verified indirectly by their retention of coloured liquids injected between them. Henson's figure suggests that the middle and inner membranes are formed out of greatly flattened cells, and this is confirmed in electron micrographs (from *Pieris*) kindly provided to me by Dr James Oschman.
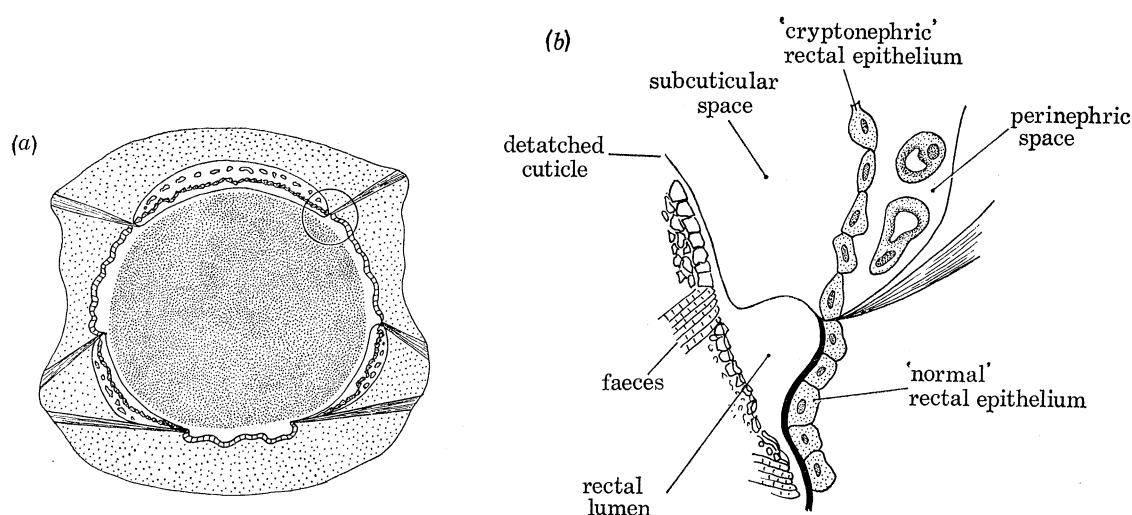

FIGURE 3. (a) Diagram of the rectal complex of a lepidopteran larva as seen in transverse section through the posterior end. (b) Detail of the encircled region of (a).

At the anterior and posterior margins of the rectal complex the three membranes come together and are inserted on the cuticle of the rectum. The perinephric space thus comprises three compartments: outer, middle and inner. It has not been possible in experimental work to differentiate between the outer and middle compartments, and for this reason the term *outer perinephric space* will be used to refer to the outer and middle compartments together, as distinct from the *inner perinephric space* which refers to the inner compartment alone. This has the convenience that one may now say that the outer tubule lies in the outer perinephric space (actually it lies mainly in the outer compartment) while the inner tubule lies in the inner perinephric space.

The inner perinephric space is bounded on its inner side by the rectal epithelium. It is now necessary to describe a feature of the rectal epithelium which, if it has not been overlooked by earlier workers, at least has not received the comment which it appears to deserve. The epithelium which lies within the rectal complex, as defined by the insertions of the perinephric membranes, is distinctly different from the epithelium which lies outside. The latter is of normal appearance; that is to say, the cells are roughly cubical and are in contact with the cuticle. Within the rectal complex the cells of the 'cryptonephric' epithelium may be described as bi-convex;

and, more importantly, they are separated from the cuticle by a space which will be called the *subcuticular space*. The separation of cuticle from cells is not an artefact of fixation; it can be observed in dissection of the fresh animal, and the subcuticular space can be injected and shown to be isolated from the inner perinephric space and from the rectal lumen.

The posterior margin of the rectal complex does not lie exactly in a transverse plane. The perinephric space extends posteriorly in three wide bays, and a section taken through this region (figure 3a) is well placed to illustrate the existence of the subcuticular space and its relations with adjacent spaces. The cuticle detached from the 'cryptonephric' epithelium is seen to be continuous with the cuticle adhering to the 'normal' epithelium outside the rectal complex. The inner surface of the 'cryptonephric' epithelium (as seen with the light microscope) is entire as though it were covered by a thin but distinct cuticle of its own; the appearance is not that of a ragged edge from which the cuticle has been torn off.

A few small tracheae, not in any regular arrangement, reach the sides of the rectal complex and spread over the surface of the outer perinephric membrane towards the mid-lines. Two large tracheae, one on each side, enter the rectal complex ventro-laterally and immediately break up into branches supplying the inner tubules. There does not appear to be any tracheal supply to the outer tubules or to the 'cryptonephric' epithelium of the rectum.

There is nothing in previous studies to suggest that the spaces within the rectal complex are anything other than completely isolated from one another and from the haemocoel and rectal lumen. In the present study, however, it has been found that colouring matter injected into the haemocoel can be detected in the inner perinephric space within half an hour. For this purpose a solution of haemoglobin (laked slaughterhouse blood) was used in preference to dyes, which are liable to suffer appreciable decolorization after injection. It is difficult to tell by simple inspection whether or not the haemoglobin penetrates the outer perinephric space since the outer space is ordinarily very restricted compared with the inner. But if the outer perinephric membrane is carefully removed under liquid paraffin it is often possible to detect the colour along the margins of the outer tubules where, under surface tension, the outer perinephric fluid collects in crevices.

If a suspension of Indian ink is injected into the inner perinephric space the rectal complex becomes moderately distended and then the ink is seen escaping into the haemocoel from the regions where the two main tracheal trunks enter the rectal complex. An examination, admittedly perfunctory, of this region in light microscope sections did not reveal any opening with a defined margin, and in view of the extreme thinness of the membranes it is difficult to decide whether apparent gaps are real or artefactual.

The subcuticular space appears to be completely isolated from the inner perinephric space and from the rectal lumen.

There remain a few structural details which deserve mention since they are related to the experimental techniques by which the rectal complex may be investigated. (1) The outer perinephric membrane is attached to the hypodermis dorsolaterally on each side and it has not been found possible to dissect it away without damage. This means that it has not been possible to make a preparation of the rectal complex isolated from the rest of the body, as is possible in the case of the mealworm. (2) The rectal complex is best approached by an incision along the mid-dorsal line of the body wall; approaching from this direction the number of tracheae which have to be cut is a minimum. When the larva has been opened in this way the rectal leads of the dorsolateral tubules may be found, but with difficulty since they are almost completely

transparent and not easy to pick out among the muscles and tracheae which traverse the haemocoel. (3) The yellow region and the white region are readily accessible, and collection of fluid from them presents no problems.

3. The ability to withdraw water from the faeces

In view of the suggestion by Wigglesworth (1934) that the cryptonephric system in those insects which possess it contributes to the ability of the rectal complex to withdraw water from the faeces, and in view of the extent of drying of the faeces achieved by the mealworm (Ramsay 1964), it is perhaps understandable that this aspect of the physiology of the rectal complex in Lepidoptera was the first to receive attention in the present study. It has to be borne in mind, however, that in respect of the availability of water the dietary regime of the mealworm is very different from the dietary regime of most lepidopteran larvae. The mealworm living on dry bran takes in food having a water content of about 12 % and passes faecal pellets which have much the same water content and which are, on average, in equilibrium with an atmosphere of 90 % r.h.; the mealworm is obliged to remove most of the water from its faeces if it is to remain in water balance. Lepidopteran larvae living on fresh leaves take in food having a water content of 80–90 % and like the mealworm pass faecal pellets which have the same water content as the food. Any ability to remove water from the faeces which these lepidopteran larvae may possess is not brought into use under their natural dietary regime and will be manifested only under artificially applied physiological stress.

In order to subject them to the stress of water deprivation larvae of *Pieris* were kept without food in a current of dry air for 48 h and were then provided with dried, or partially dried, cabbage leaves. The water content of these leaves had been reduced from its normal value of 5.7 g water per g dry mass to 2.3, to 0.87 and to zero (dried in an oven at 110 °C). The partially dried leaves were eaten, though less avidly than fresh leaves, but oven-dried leaves were left untouched. The leaves, with the larvae feeding on them, were suspended over a funnel leading into a small plastic vial in which the faecal pellets were collected for later determination of their water content (table 1).

Table 1. Water content (ml water per g dry mass) of faeces in relation to water content of leaves: *Pieris*

leaves	faeces
2.3	1.25
0.87	0.72

In these experiments collection was continued for an hour or more. The faecal pellets were retained within a very small space with a restricted opening, but they could well have lost some water by evaporation. The figures for water content of faeces in table 1 may therefore be regarded as minimum figures. The problem of establishing the water content of faecal pellets without opportunity for loss of water by evaporation is much more serious in the case of the mealworm, and for this insect a more sophisticated method had to be devised (Ramsay 1964) according to which single faecal pellets were caught and weighed on a quartz-fibre balance immediately after being dropped. This method was modified for use with *Pieris*. The lowest water content recorded by this method for a faecal pellet passed by a maximally stressed larva was 0.61 g water per g dry mass.

In order to relate the water content of the faecal pellets to the 'force' which must be overcome in removing further water from them faecal pellets were allowed to come into equilibrium with atmospheres of known humidity, controlled by saturated solutions of salts. The highest humidity at which this was practicable was 97.5 % r.h. (saturated K_2SO_4 at 24 °C; Winston & Bates (1960). In this atmosphere the water content was 0.575 g water per g dry mass. The larva of *Pieris*, under maximum stress, is therefore unable to reduce the activity of water in its faeces even to the level corresponding to 97.5 % r.h. This figure is to be compared with the minimum recorded figure of 75 % r.h. for the mealworm (Ramsay 1964).

In *Manduca* the faeces are obviously moist to the touch, and though it has not been possible to test the effect of water deprivation on *Manduca* there is no reason to believe that its ability to remove water from the faeces is any greater than that of *Pieris*. One may conclude that in respect of water conservation the possession of a cryptonephric rectal complex does not seem to place these lepidopteran larvae at any advantage over other insects not thus endowed.

4. The ability to maintain salt balance under salt loading

A technique has been developed by David & Gardner (1965) for the rearing of *Pieris* larvae on a semi-synthetic diet. The precise composition of the diet, which is complex since the diet contains a variety of growth-promoting additives, need not be reproduced here; the importance of the technique for the present work is that it makes it possible to subject the larvae to the physiological stress of dietary salt loading, which cannot be achieved if the larva will not accept any food other than the leaves of the normal host plant. A semi-synthetic diet of the same type is also acceptable to the larvae of *Manduca*. Variants of the standard diet were prepared in which the normal quantity of sodium (or potassium) was replaced by potassium (or sodium) and in addition large quantities of potassium (or sodium) were added. These variants will be referred to as 'high K' (or 'high Na') diets, the actual amounts of the two ions, based on analyses of the diets, being given in table 2.

The initial aim of the experiments on salt loading was no more than to place the insect under physiological stress in the hope that some clue to the mechanism of the rectal complex would be picked up. An obvious line of approach was to begin by examining the effect of salt loading on the concentrations of sodium and potassium in the haemolymph. The larvae were hatched on normal artificial diet and transferred to the special diet at the beginning of the second instar. On none of the diets tested did the larvae fail to pupate, but growth was a little slower on high Na diet than on normal or high K diet. Samples of haemolymph were collected from the final instar and the concentrations of sodium and potassium were determined with a modified† Niham flame photometer. For analyses of samples of diet (and, in other experiments, of faeces) an E.E.L. flame photometer was used. Osmolarity was measured as freezing-point depression by the method of Ramsay & Brown (1955).

The results of one such experiment, for which *Manduca* was used, are presented in table 3. The amounts of sodium and potassium present in the diets (high Na compared with high K)

---

† The modifications affect the flame and the arrangement for introducing the platinum wire into it. I gratefully acknowledge the technical assistance of Mr R. Holder. The photocells and the display unit were as supplied by the makers. The modifications were developed during the course of the physiological study, and some of the earlier results were less accurate than those obtained later. Figures for concentrations of sodium and potassium may be taken as accurate to ± 10 % standard error.

differed by a factor of about 13 (table 2) whereas the concentrations of these ions in the haemo-lymph differed only by a factor of about 2. The actual differences are 7 mmol/l for sodium and 14 mmol/l for potassium. These figures are to be compared with the much greater variations of haemolymph composition in other insects under much less exacting dietary regimes, as first reported for the cockroach by Tobias (1948), and also with the considerable change in the composition of the haemolymph of the locust when fed or starved (Hoyle 1954).

TABLE 2. ANALYSES OF ARTIFICIAL DIETS

(Na, K: mmol per g dry mass; water: ml per g dry mass.)

	Na	K	water
normal	0.25	0.31	3.8
high Na	1.64	0.12	3.5
high K	0.12	1.57	3.4

TABLE 3. ANALYSES OF HAEMOLYMPH IN RELATION TO DIETARY REGIME: *Manduca*

(Na, K: mmol/l; Δ in °C.)

	normal			high Na			high K		
	Na	K	Δ	Na	K	Δ	Na	K	Δ
	11	32	0.550	13	23	0.695	10	32	0.685
	10	35	0.575	21	22	0.605	6.5	35	0.635
	7	25	0.575	11.5	12	0.665	5.5	27	0.725
	9	28	0.555	11.5	18	0.625	5.5	39	0.730
av.	9.2	30	0.564	14	19	0.647	6.9	33	0.694

Before accepting these figures for *Manduca* as evidence for exceptional ability to maintain homeostasis under salt loading one requires to be assured that a substantial proportion of the salt load which is ingested is also assimilated; that is to say, that it is taken up from the alimentary canal into the haemolymph and then removed by the excretory system – not simply passed direct from mouth to anus. Sodium and potassium were therefore determined in different parts of the alimentary canal. For sampling, ligatures were placed around the larva so as to constrict the body between mid gut and intestine and between intestine and rectum; the larva was then opened, haemolymph was removed with absorbent paper, and the contents of the midgut (posterior half), intestine and rectum were taken for analysis. Each sample was weighed, dried, weighed again, digested with concentrated nitric acid and diluted appropriately for flame photometry. The amounts of sodium, potassium and water were expressed in terms of the dry mass of the material. The results are presented in figure 4.

On normal diet potassium and water increase in the midgut and thereafter decrease, sodium remaining at about the same level throughout; this inward secretion of potassium in the midgut is found in many lepidopteran larvae and has been much used in biophysical studies following its initial exploration by Harvey & Nedergaard (1964). On a high K diet, potassium further increases in the midgut, decreases in the intestine and increases thereafter. On a high Na diet, sodium decreases in the midgut and increases thereafter. The important fact brought to light in this experiment is the almost complete replacement of sodium by potassium in the midgut of larvae on a high Na diet; nearly all the sodium leaves the alimentary canal in the midgut and returns to it in the intestine and rectum. Sodium is therefore fully assimilated on a high Na diet.

In order fully to appreciate the performance of *Manduca* it is necessary to know the extent of salt loading in terms of salt turnover. From the experiment recorded in figure 4 *Manduca* larvae on a high Na diet pass faeces which contain 2.37 mmol Na per g dry mass. In another experiment 10 *Manduca* larvae produced 8.36 g dry faeces in 48 h; the average body mass of a single larva over the period of this experiment was 3.56 g. From these figures the rate of elimination of sodium (as sodium chloride) in the faeces is 16.2 mg per g larva per day; this may be taken
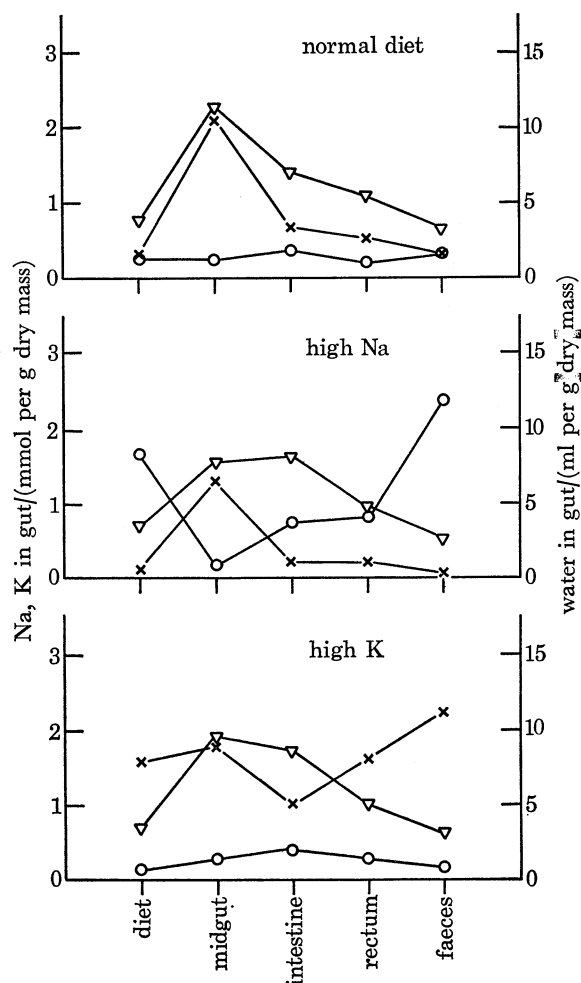
FIGURE 4. Sodium, potassium and water in the gut contents, in relation to dietary regime. Sodium (○) and potassium (×), unit: mmol per g dry mass of gut contents. Water (▽), unit: ml per g dry mass of gut contents. *Manduca*.

as the daily turnover of sodium chloride. By simple proportion this would be a daily turnover of 1134 g of sodium chloride for a man weighing 70 kg – far beyond the capability of the human kidney. Professor K. Schmidt-Nielsen (personal communication) informs me that the highest salt turnover known to him in mammals is that for the sand rat *Psammomys obesus*, and he has kindly allowed me to quote his own unpublished figure for this animal, namely 13 mg per g rat per day. This remarkable ability of *Manduca* to maintain homeostasis under heavy salt loading will be taken up again in the Discussion.

5. The response of the excretory system to salt loading

The ability of the insect to maintain homeostasis of the haemolymph under dietary salt loading having been established, it is an obvious next step to examine the role of the excretory system, and in particular of the rectal complex, in this process. But before various parts are studied separately and in detail it is of some interest to assess the response of the excretory system as a whole to salt loads applied by injection into the haemocoel rather than through the diet.
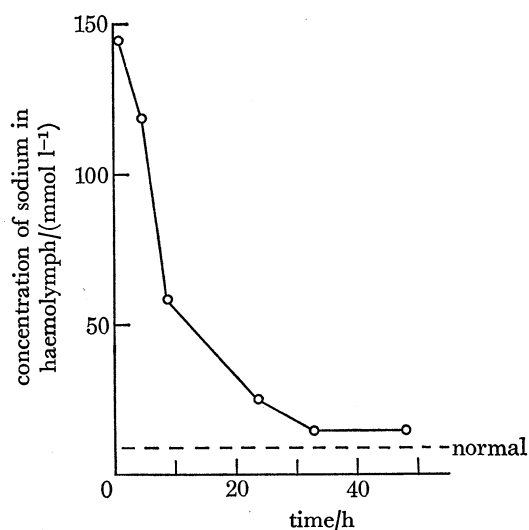

Figure 5. Elimination of sodium injected into the haemocoel. Concentration of sodium in the haemolymph plotted against time.

(a) The elimination of salt injected into the haemocoel

Larvae of *Manduca* reared on normal artificial diet were used in these experiments. Each larva was injected with an approximately measured volume of sodium chloride solution at molar strength, weighed, and placed by itself in a covered dish. The dish was inspected about an hour later, and at intervals of several hours from then on. At each inspection the faecal pellets were collected and a small (1 µl) sample of haemolymph was taken. The concentration of sodium in the haemolymph and the amount of sodium in each collection of faecal pellets were determined in the usual way; from the faecal pellets the rate of elimination of sodium, averaged over the period between one collection and the next, was calculated. The data were plotted against time, as in figure 5, and from this plot the half-time for the return of the sodium concentration to its normal value of 9 mmol/l was read off. The results for four larvae are assembled in table 4. It will be observed that none of these larvae attained the high rate of elimination of sodium chloride (16.2 mg per g larva per day) recorded under dietary salt loading; but it is to be remembered that the diet-loaded larvae had been reared on high Na diet from the second instar onwards and had ample time to become adapted to high sodium turnover, whereas the injection-loaded larvae had been reared on normal artificial diet throughout their lives.

It is convenient to use table 4 for the presentation of comparable figures for the locust *Schistocerca gregaria* and for the stick insect *Carausius (Dixippus) morosus*. The figures for the stick insect are derived from data presented in an earlier paper (Ramsay 1955a); the figures for the

locust were obtained from experiments carried out during the course of the present work. *Manduca* is clearly superior to both the locust and the stick insect in the ability of its excretory system to dispose of injected salt loads. These insects have in common that they ingest large quantities of plant leaves, but only *Manduca* has a cryptonephric system.

TABLE 4. ELIMINATION OF SODIUM INJECTED INTO THE HAEMOCOEL

	approximate amount of injection	peak sodium concentration	half-time to normal sodium concentration	maximum rate of elimination
	μm NaCl per g larva	mmol/l	h	mg NaCl per g larva per day
(a) *Manduca*	100†	145	8	5.4
	100	105	6	7.1
	50	90	6	9.8
	50	85	6	5.9
			av. 6.5	7.05
(b) *Schistocerca*	125	250	30	1.95
(c) *Carausius*	10	50	> 70	1.93

† The full data for this larva are plotted in figure 5.

(b) Collection of fluids from the excretory system

Attempts were made to collect fluids from the following parts of the excretory system: white region, rectal lead, outer tubule, inner tubule, outer perinephric space, inner perinephric space, subcuticular space, rectal lumen. In making such collections one may simply aim to remove all or part of the fluid present in the cavity at the moment of collection, or one may aim to collect the fluid continuously as it accumulates, so that information on rate of flow as well as material for analysis can be obtained.

Simple collection was attempted on all the parts listed above, but on the outer and inner tubules this was unsuccessful. For purposes of simple collection it was usual to open the larva in a dissecting dish under liquid paraffin and to use a micro-manipulator of a type described elsewhere (Ramsay 1953, figure 1) which carries a pair of tungsten-tipped forceps to grasp the organ and a silica pipette filled with liquid paraffin to penetrate the cavity. Where there was uncertainty about the identification of the cavity it was usual to add a red dye, Sudan III, to the liquid paraffin in the pipette and to eject a droplet of liquid paraffin prior to collection of fluid; subsequent dissection then identified the cavity containing the droplet.

For purposes of continuous collection micropuncture suffers from the disadvantage that the silica pipette is liable to become blocked; and in experiments of long duration it is desirable to allow access of air to the spiracles. Continuous collection was attempted only on the white region and rectal lead, and for this an arrangement was devised as follows. Ligatures were tied around the neck and around the last pair of legs, and the larva was stretched dorsal side uppermost upon a table of Perspex. Petroleum jelly (vaseline) was applied from a hypodermic syringe and spread with a hot wire over the dorsal surface. The larva was then opened along the mid dorsal line and the flaps of body wall were held apart by wire hooks (steel, *Manduca*; tungsten, *Pieris*). A wall of jelly was then built up upon the flaps to make a trough (figure 6). Liquid paraffin was poured into the trough to cover the haemolymph and viscera. On either side of the larva, above the level of the hooks, a Perspex platform provided a handrest in dissection. When the required region of tubule had been isolated it was picked up on a fine tungsten hook. The

hook was mounted upon a base of Perspex on which it could be raised or lowered by means of a screw. A layer of heavy grease between the base and the platform made it possible to position the hook above the tubule; the hook was then lowered and used to grapple the tubule in the manner of a fisherman's gaff. The tubule was raised out of the haemolymph into the liquid paraffin and was cut through close to the hook. Fluid collected as a droplet at the gaffed end of the tubule and could be removed by means of a fine Pyrex pipette.
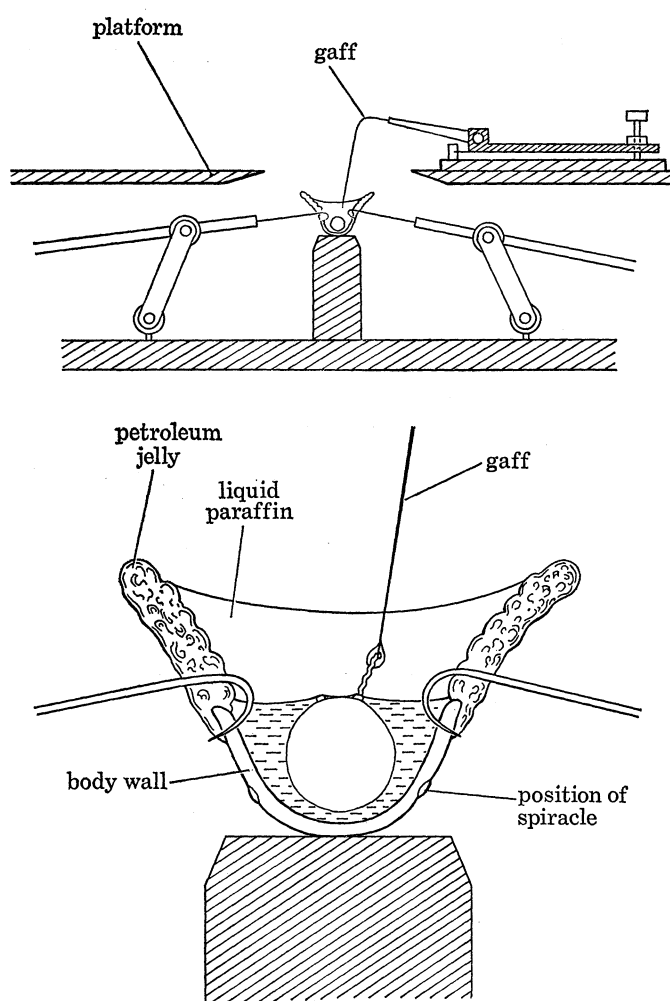
FIGURE 6. Arrangement for the continuous collection of fluid from Malpighian tubules.
For further explanation see text.

With the larva prepared in this way the spiracles were unobstructed. But, aided by the residual movement of the incompletely restrained larva, the liquid paraffin would work its way through the jelly in the course of an hour or two and then flow down over the spiracles and into the tracheal system. Continuous collections were therefore of relatively short duration.

Fluids collected in these ways were analysed for sodium and potassium, and freezing-point depression was determined. In continuous collection a record was usually kept of the volume collected (diameter of droplet under liquid paraffin; Ramsay 1955 b) and the times of collection, from which the average rate of flow over the interval could be calculated.

(c) The composition of the fluid from the proximal end of the Malpighian tubule

In his important paper on sodium and potassium secretion by the isolated Malpighian tubules of *Calpodes* Irvine (1969) has shown, among other things, that the composition of the fluid secreted by the free tubule, over all its regions, is sensitive to the composition of the bathing medium, so that when the sodium concentration exceeds the potassium concentration in the medium sodium can almost completely replace potassium in the fluid which is discharged into the gut. This finding strongly suggests that in *Calpodes* (in contrast to *Carausius*; Ramsay 1955 *a*) the Malpighian tubules could be of importance in regulating the composition of the haemolymph.

TABLE 5. CONCENTRATIONS OF SODIUM AND POTASSIUM (mmol/l) AND FREEZING-POINT DEPRESSION (Δ, °C) OF FLUID COLLECTED FROM THE PROXIMAL END (WHITE REGION) OF THE MALPIGHIAN TUBULE, IN RELATION TO DIETARY REGIME: *PIERIS*

	normal			high Na			high K		
	Na	K	Δ	Na	K	Δ	Na	K	Δ
	5	156	0.725	175	6	0.875	—	—	—
	5	146	0.765	170	33	0.865	5	225	0.895
	12	124	0.765	205	30	0.905	2	235	0.845
	7	136	0.760	160	34	0.825	3.5	175	0.765
av.	7	140	0.754	177	26	0.867	3.5	212	0.835

To test this suggestion continuous collections were made of the fluid issuing from the proximal end of the white region in *Pieris* (*Manduca* not being available) reared on normal artificial diet and on high Na and high K diets. The results of determinations of sodium and potassium in these collections are presented in table 5, and they clearly support Irvine's findings. The Malpighian tubules of *Pieris* respond to sodium loading in the diet by increasing the concentration of sodium in the tubule fluid above its normal level by a factor of ×25; the increase in potassium concentration under potassium loading is lower, ×1.5; this is perhaps understandable in view of the high concentration of potassium in the tubule fluid on normal artificial diet.

The next question to be asked is whether this response materially contributes to the elimination of sodium on a high Na diet, and for this to be answered it is necessary to know the rate of flow. Unfortunately this was not recorded in the experiments to which table 5 refers, but in similar experiments carried out under precisely the same conditions an average rate of flow of 25 nl/min was recorded. This information, together with the average concentration of sodium in the tubule fluid, 177 mmol/l, and the average mass of the *Pieris* larvae used in these experiments, 0.37 g, makes it possible to calculate the amount of sodium chloride removed from the haemolymph by all six tubules, per day, on a high Na diet. The figure so calculated is 6 mg per g larva per day. This should be compared with a figure for the total turnover of sodium chloride which has already been found for *Manduca* to be 16.2. A preliminary experiment on *Pieris*, comparable with that on *Manduca* but lasting 240 h (too long, because the larvae more than doubled in mass) gave a figure of 9.4 for total turnover. There being no requirement at this stage to establish the exact proportion of the total salt turnover which passes directly from the Malpighian tubules into the intestine, this preliminary experiment on *Pieris* has not been repeated. All that is here claimed to have been demonstrated is that *in vivo* the Malpighian tubules

216 J. A. RAMSAY

of *Pieris* respond to dietary salt loading in such a way as to maintain homeostasis of the haemo-lymph and that they can carry a substantial proportion, but probably not the whole, of the sodium load.

(d) The composition of fluid from the rectal lead of the Malpighian tubule

The rectal lead is relatively shorter in *Pieris* and *Manduca* than in *Calpodes*, and the most accessible region is close to the rectal complex. Simple collections (*Manduca*) were made with the micropipette inserted towards the rectal complex, and the greater proportion of the fluid collected must have come from the outer tubule. In continuous collections (*Pieris*) nearly all the fluid collected must have originated within the rectal complex with very little opportunity for its composition to be modified in passing through the short length of rectal lead. It is reasonable to regard these collections as representative of the fluid produced by the cryptonephric tubules under different dietary regimes.

The results are presented in table 6, and it is at once apparent that in both genera the fluid collected from the rectal lead has the low-sodium, high-potassium composition characteristic of Malpighian tubule fluid of most insects, and that (in contrast to the fluid collected from the proximal end of the free tubule) it is remarkably constant in composition, being only slightly responsive to the dietary regime.

TABLE 6. CONCENTRATIONS OF SODIUM AND POTASSIUM (mmol/l), FREEZING-POINT DEPRESSION (Δ, °C) AND RATE OF FLOW (r, nl/min) OF FLUID COLLECTED FROM THE RECTAL LEAD OF THE MALPIGHIAN TUBULE, IN RELATION TO DIETARY REGIME

(a) *Manduca*

	normal			high Na			high K		
	Na	K	Δ	Na	K	Δ	Na	K	Δ
	—	—	—	23	212	0.835	9	200	0.725
	10	192	0.670	17	192	0.585	8	200	0.745
	9	208	0.650	30	175	0.715	9	210	0.735
	18	188	0.580	16	215	0.670	11	220	0.835
av.	12.3	196	0.633	22.5	198	0.701	9.25	207	0.760

(b) *Pieris*

	cabbage				high Na				high K			
	Na	K	Δ	r	Na	K	Δ	r	Na	K	Δ	r
	—	—	—	—	—	—	—	—	10	173	0.740	2.4
	—	—	—	—	7.	174	0.725	3.9	5	230	0.755	9.7
	5	170	0.755	2.5	10	140	0.555	6.5	6	216	0.740	11.5
	8	243	0.785	10.8	8	265	0.770	6.2	4	212	0.740	17.0
av.	6.5	206	0.770	6.6	8.3	193	0.683	5.5	6.25	208	0.743	10.25

(e) The composition of fluid from the inner perinephric space

The discovery of the extent of the subcuticular space came at a late stage in the course of this work, and it seems likely that some of the collections from the inner perinephric space of *Pieris*, made earlier, may have been contaminated with fluid from the subcuticular space. The results presented in table 7 are for *Manduca* only.

Saini (1962) collected fluids from the rectal complex in four genera of insects: *Tenebrio* and *Dermestes* (Coleoptera), *Galleria* and *Pieris* (Lepidoptera). In all cases he found that the freezing-point depression of the perinephric fluid was intermediate between that of the haemolymph and that of the rectal fluid. His figures for *Pieris* are haemolymph, 0.60; perinephric fluid, 0.86; rectal fluid, 1.3. The corresponding figures for *Manduca* on normal artificial diet, haemolymph 0.564 (table 3); inner perinephric fluid, 0.773 (table 7), are not out of line with Saini's figures for *Pieris*.

The inner perinephric fluid has a potassium/sodium ratio higher than the haemolymph but lower than the fluid from the rectal lead; and, as with both of these, its composition shows only slight response to dietary regime.

TABLE 7. CONCENTRATIONS OF SODIUM AND POTASSIUM (mmol/l) AND FREEZING-POINT DEPRESSION (Δ, °C) OF FLUID COLLECTED FROM THE INNER PERINEPHRIC SPACE, IN RELATION TO DIETARY REGIME: *MANDUCA*

	normal			high Na			high K		
	Na	K	Δ	Na	K	Δ	Na	K	Δ
	15	65	0.705	31	85	1.700	18	290	1.090
	32	128	0.635	7	85	0.690	12	75	1.385
	21	100	0.980	8	130	0.935	24	58	1.365
av.	23	98	0.773	15	100	1.108	18	141	1.280

(f) The composition of fluid from the subcuticular space

The analyses of collections of subcuticular fluid, presented in table 8, show a marked response to dietary regime. The sodium concentration under sodium loading is increased above its normal level by a factor of × 14; the corresponding increase in potassium concentration is × 1.6, the concentration of potassium on normal diet being already high. In fact, in their general trend these figures for the subcuticular fluid of *Manduca* closely parallel the figures for the proximal tubule fluid of *Pieris* (table 5). The rectal epithelium must be active in maintaining the difference in composition between inner perinephric fluid and subcuticular fluid; but there is nothing new in this, the activity of the rectal epithelium (including rectal glands) in ion transport having been well documented for a variety of insects.

TABLE 8. CONCENTRATIONS OF SODIUM AND POTASSIUM (mmol/l) AND FREEZING-POINT DEPRESSION (Δ, °C) OF FLUID COLLECTED FROM THE SUBCUTICULAR SPACE, IN RELATION TO DIETARY REGIME: *MANDUCA*

	normal			high Na			high K		
	Na	K	Δ	Na	K	Δ	Na	K	Δ
	15	200	0.635	300	22	1.610	15	220	1.080
	22	57	0.700	270	28	1.610	18	230	1.260
	15	190	0.860	140	20	1.350	23	280	1.400
av.	17	149	0.732	237	23	1.523	19	243	1.247

It is most unlikely that the detached cuticle has any power of active transport. The view taken here is that there is probably passive exchange of water and solutes between the faeces and the subcuticular fluid and an approach towards equilibrium as far as time and the permeability of the cuticle allow.

6. The movements of salts and water into and out of the rectal complex

The response of the excretory system to salt loading is puzzling. On the one hand there is a clear and *prima facie* adaptive response by the free Malpighian tubules and by the rectal epithelium to the stresses of salt loading, whereas on the other hand the perinephric fluid and the fluid from the cryptonephric tubules respond less dramatically and not in such a way as to indicate that they play any important part in carrying the salt load out of the body. Yet it is difficult to believe that they are not involved in the process; for, as figure 4 suggests, the rectal complex of which they are a part is heavily engaged in eliminating the salt which is administered in excess.

The results presented in figure 4 are compromised by the use of dry mass of the gut contents as the standard to which quantities of sodium, potassium and water are referred; the fact that an appreciable fraction of the ingested material may be absorbed from the gut obviously makes dry mass of gut contents unsatisfactory as a standard of reference. What is called for is some marker substance which passes through the gut without being absorbed or metabolized. The dye amaranth has been successfully used as a marker by Treherne (1957); amaranth was tried with *Manduca* but had to be given up because the amount of dye recovered from the faeces was found to be less than the amount ingested. The marker finally selected was fine washed silica sand. 25 % of sand was added to the diet when it was prepared. After the digestion of the gut contents with nitric acid and the removal of the supernatant for flame photometry the sand was recovered, dried and weighed, and the quantities of sodium, potassium and water were expressed as millimoles or millilitres per gram of sand. Apart from this the experiment was carried out following the same procedure as described in § 4. The results are set out in table 9 and plotted in figure 7; they confirm the general trends brought to light in the earlier experiment.

Table 9. Sodium and potassium (mmol/g sand) and water (ml/g sand) in the gut contents, in relation to dietary regime: *Manduca*

high Na	Na	K	water
diet	0.82	0.06	1.8
midgut	0.06	0.80	2.95
intestine	0.41	0.13	2.35
rectum	0.53	0.04	1.6
faeces	0.84	0.02	1.2
high K			
diet	0.06	0.92	1.75
midgut	0.02	0.59	2.2
intestine	0.04	0.38	1.6
rectum	0.05	0.68	1.3
faeces	0.05	1.05	1.5

Although this later experiment was of better design than its predecessor there remain various matters of uncertainty which do not allow of any precise interpretation being placed upon the figures. The ingestion of food can be a steady continuous process over a period of an hour or two, but the passing of faeces is intermittent. The faeces are continuously accumulated in the rectum through the period between defaecations, so that some portions of the faecal pellet will have been acted upon by the rectal complex for longer than others. Faecal pellets are therefore

unlikely to be homogeneous in composition, and any one faecal pellet may have a different average composition from its predecessor or successor, depending upon the duration of stay in the rectum. The anomaly of the apparent input of water into the faeces in the rectum on high K diet seen in figure 7*b* (but not in figure 4*c*) may have this explanation. Even where the passage of the gut contents is to all intents and purposes continuous, uncertainties are still met with.
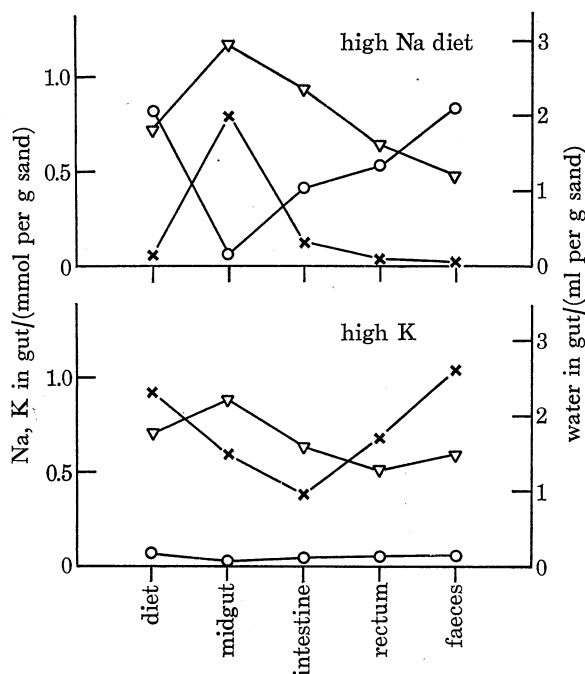

FIGURE 7. Sodium, potassium and water in the gut contents, in relation to dietary regime. Sodium (O) and potassium (×), unit: mmol per g sand. Water (▽), unit: ml per g sand. *Manduca*.

Take, for example, the case of sodium on high Na diet. The amount of sodium in the intestine is 0.41 mmol/g sand as compared with 0.06 in the midgut. All this additional sodium could come in with the Malpighian tubule fluid at the anterior end of the intestine and thereafter the amount of sodium could remain unchanged. Or, the amount of sodium could be steadily increased by secretion through the intestinal epithelium in which case the amount of sodium per gram of sand entering the rectum could be greater than 0.41, which is an average figure for the whole length of the intestine. It will be assumed that the amount of sodium entering the rectum from the intestine cannot be less than 0.41, the average for the whole intestine (case A), and cannot be greater than 0.53, the average for the rectum itself (case B). The differences between these amounts and the amount recorded for the faeces, 0.84, set upper and lower limits to the amount which must enter the rectal complex by some other route or routes.

It is convenient to have the figures of table 9 in terms of per gram larva per day rather than in terms of per gram of sand. The average amount of sand passed in the faeces was found to be 0.25 g sand per g larva per day; so the figures of table 9 have been divided by 4 and are presented in table 10 as millimoles of sodium and potassium or millilitres of water per gram larva per day. In order to be able to use the data of table 10 in conjunction with data on amounts contributed by the tubules, measured on *Pieris*, the latter are also expressed on the basis of gram larva per day, recognizing that to assume proportionality of rates of flow to body mass over a

weight range of × 10 is hazardous. An average figure for tubule fluid from the rectal lead of *Pieris* (table 6) is 7.8 nl/min, and this is approximately 0.2 ml per g larva per day (6 tubules, body mass 0.37 g).

In view of the uncertainties indicated in the preceding paragraphs no attempt will be made to draw up complete balance sheets; instead, the data will be used to define the limits within which the rectal complex might operate. Certain propositions will presently be put forward regarding the other routes (other than through the lumen of the gut) by which material may enter or leave the rectal complex. The implications of these propositions will be examined in the light of the figures in table 10 (and other figures quoted elsewhere), and a proposition will be held to be rejected if it cannot be reconciled with the figures to within an order of magnitude.

TABLE 10. MOVEMENTS OF SODIUM AND POTASSIUM (mmol per g larva per day) AND OF WATER (ml per g larva per day) INTO AND OUT OF THE RECTAL COMPLEX: *MANDUCA*

	input from gut	output as faeces	balance by other routes input	balance by other routes output
high Na				
sodium: case A	0.1025	0.2100	0.1075	
case B	0.1325	0.2100	0.0775	—
potassium: case A	0.0325	0.0050	—	0.0275
case B	0.0100	0.0050	—	0.0050
water: case A	0.5875	0.3000	—	0.2875
case B	0.4000	0.3000	—	0.1000
high K				
sodium: case A	0.0100	0.0125	0.0025	—
case B	0.0125	0.0125	0	—
potassium: case A	0.0950	0.2625	0.1675	—
case B	0.1700	0.2625	0.0925	—
water: case A	0.4000	0.3750	—	0.0250
case B	0.3250	0.3750	0.0500	—

Before any propositions are put forward it is appropriate to consider what other possible routes there may be by which matter may enter or leave the rectal complex. There appear to be three such routes

(i) through the perinephric membranes;

(ii) through an opening in the perinephric membranes – this will be referred to as the direct route;

(iii) through the Malpighian tubules – this will be referred to as the tubule route.

The following preliminary comments may be made on these possibilities.

(i) The perinephric membranes are formed out of thin, flattened cells, virtually reduced to unit membranes and nothing else. Any interpretation of the mechanism of the rectal complex involving active transport by these membranes would hardly gain credence. An interpretation in terms of passive transport is not sufficiently different from diffusion or convection through an opening to warrant separate examination.

(ii) There is clear evidence that the inner perinephric space is in open, but restricted, communication with the haemocoel, and the possibility of exchanges by this route must clearly be taken into account.

(iii) It seems to have been tacitly assumed by all commentators upon cryptonephric systems that tubule fluid flows from the cryptonephric tubules to the free tubules, and that while the

cryptonephric tubules may remove matter from the rectal complex they do not bring any in. While there is no evidence of net inflow into the rectal complex by the tubule route the possibility appears to have been overlooked that a net outflow may co-exist with a periodic inflow and outflow, as in a tidal estuary. It is possible that fluid from the free tubules could be carried into the cryptonephric tubules and remain there long enough for solutes to be taken up into the perinephric space. This possibility will receive further consideration later, but not until two of the aforementioned propositions have been examined.

Proposition I: that on a high Na diet sodium enters the rectal complex by the direct route.

If there is no net flow between the perinephric space and the haemocoel, but only diffusion or a tidal flow, then the effect of uptake of sodium from the perinephric fluid and its transfer to the faeces must be to depress the concentration of sodium in the perinephric fluid so that it is below the concentration in the haemolymph. In fact, the reverse is true: perinephric fluid, 15 mmol/l (table 7); haemolymph, 14 mmol/l (table 3). If sodium is removed from the perinephric fluid then water must also be removed and there must be a net inflow. Suppose, then, that haemolymph enters the perinephric space, that sodium is removed by the rectal epithelium and that water is removed by the tubule route. In case B 0.0775 mmol per g larva per day would have to enter the rectal complex at a concentration of 14 mmol/l and $0.0775 \times 10^3/14$ ml per g larva per day, that is 5.55 ml of water, would have to be removed by the tubule route in addition to the 0.1 ml of water entering by the intestine in excess of that leaving in the faeces, a total of 5.65 ml per g larva per day. This is greater by a factor of $\times 28$ than the figure of 0.2 ml per g larva per day for the flow in the rectal leads. The proposition is therefore rejected for case B, and *a fortiori* for case A.

Proposition II: that on a high K diet potassium enters the rectal complex by the direct route.

Following the same arguments as used for proposition I and again taking case B, the concentration of potassium in the haemolymph is 33 mmol/l (table 3) so the volume of water to be removed by the tubule route would be $0.0925 \times 10^3/33$, that is 2.80 ml, from which would have to be subtracted the apparent input of 0.05 ml giving a net volume of 2.75 ml per g larva per day to be removed by the tubule route, too much by a factor of more than $\times 13$. This proposition is likewise rejected.

With the rejection of these propositions it is difficult to avoid the conclusion that both sodium and potassium must enter the rectal complex by the tubule route.

The suggestion that sodium and potassium can enter the rectal complex by the tubule route is not so implausible as might first appear. In the opened larva the intestine and rectal complex are often seen to be in lively motion and undergoing strong contractions which, in the case of the rectal complex, could have the effect of compressing the thin-walled outer tubules and expelling their contents through the rectal leads, fluid from the free tubules flowing in during relaxation. Observation of small oil droplets injected into the rectal lead of the opened larva shows that there is just such movement of tubule fluid into and out of the cryptonephric tubule. Indirect evidence that sodium and potassium may be taken up from the cryptonephric tubules was sought by means of an experiment in which the rectal lead was cut and tubule fluid was collected from both cut ends; the fluid issuing from the proximal (iliac plexus) end may be regarded as representative of the fluid which enters the cryptonephric tubule on the flow and the fluid from the distal (rectal complex) end as representative of the fluid which leaves on the ebb. This experiment was carried out on *Pieris* (*Manduca* not being available) on high Na and high K diets, with results as set out in table 11. On high Na diet the sodium concentration is

54 mmol/l lower in the fluid which leaves than in the fluid which enters. On high K diet the potassium concentration is 10 mmol/l lower in the fluid which leaves, but this difference is not statistically significant.

In order to assess the physiological significance of these differences in concentration it is necessary to have some idea of the volume and frequency of the tidal flow. No direct measurements of volume have been possible but from its geometry it is estimated that the capacity of the dilatation of the outer tubule is about 50 nl. From observations on the opened larva the frequency of contraction is about 10/min. The tidal flow is therefore 500 nl per tubule per minute. For all six tubules it is 4.3 ml per day, and if the larva weighs 0.37 g the total tidal flow is 11.7 ml per g larva per day.

On the basis of this figure two further propositions will now be examined.

TABLE 11. CONCENTRATIONS OF SODIUM AND POTASSIUM (mmol/l) AND FREEZING-POINT DEPRESSION (Δ, °C) OF FLUID COLLECTED FROM THE PROXIMAL (ILIAC PLEXUS) AND DISTAL (RECTAL COMPLEX) CUT ENDS OF THE RECTAL LEAD OF THE MALPIGHIAN TUBULE, IN RELATION TO DIETARY REGIME: PIERIS

		proximal			distal		
		Na	K	Δ	Na	K	Δ
high Na		45	150	0.805	5	150	0.665
		118	110	0.825	19	200	0.885
		91	214	1.130	10	255	1.115
		110	166	1.005	84	144	0.935
		79	141	0.825	13	127	0.655
		67	128	1.245	28	200	0.845
		97	170	1.055	31	172	1.055
		43	152	0.905	28	188	0.935
	av.	81	154	0.974	27	179	0.886
high K		8	215	0.835	3.5	205	0.805
		3	265	0.905	4	200	0.835
		3.5	225	0.915	3.5	185	0.865
		3	185	0.950	2	255	0.930
		2	190	0.905	3	240	0.885
		2.5	210	0.855	1.5	225	0.815
		2.5	265	0.950	2	235	0.850
		7	225	0.895	7.5	150	0.750
		2	205	0.875	1	205	0.805
	av.	3.7	221	0.898	3.1	211	0.838

Proposition III: that on a high Na diet the tidal flow mechanism is adequate to account for the input of sodium into the rectal complex.

The tidal flow has been estimated as 11.7 ml per g larva per day, and the reduction in sodium concentration is 54 mmol/l (table 11); the amount of sodium removed from the tubule fluid and available for transfer to the rectal lumen is therefore 0.63 mmol per g larva per day. This is well in excess of the requirement, 0.1075 mmol (table 10, case A). The proposition is accepted.

Proposition IV: that on a high K diet the tidal flow mechanism is adequate to account for the input of potassium into the rectal complex.

For potassium the reduction in concentration is 10 mmol/l but, as already mentioned, this figure is not statistically significant. Nevertheless it is of interest to know what figure for concentration difference, taken in conjunction with a tidal flow of 11.7 ml per g larva per day,

would account for the input of potassium, which is 0.1675 mmol per g larva per day (table 10, case A). The concentration difference would have to be 14.3 mmol/l. This is within an order of magnitude of 10 mmol/l, but in view of the statistical non-significance of this figure it would not be proper to judge the proposition either as accepted or as rejected.

7. DISCUSSION

In as much as the principal aim of this investigation was to elucidate the mechanism of the rectal complex, its most significant outcome is the concept that ions can enter the rectal complex by way of the Malpighian tubules. This concept is based not upon any theory as to mechanism but on the principle of the conservation of matter and the extreme physiological inplausibility of alternative routes. Its validity could best be tested by tying off the rectal leads and seeing whether or not the salt-loaded larva was able to transport ions into the faeces. But in view of the difficulties of surgery this experiment seemed too ambitious to be worth attempting.

Even so, a concept so novel would not readily gain acceptance unless some feasible mechanism for its realisation were put forward. The mechanism here proposed is tidal flow of tubule fluid between the free tubules and the cryptonephric tubules, coupled with ion uptake by the crypto-nephric tubules. That there is such tidal flow is a matter of direct observation, but for the rate of flow there is only a crude estimate. There is indirect evidence of sodium uptake but not of potassium uptake. The proposed mechanism lacks the support of direct evidence; but at the same time there is nothing in the findings to suggest that it is unfeasible.

After that brief assessment of the results presented in this paper it may be permitted to introduce other considerations of possible relevance.

First, it may be recalled that for the uptake of sodium the tidal flow mechanism has considerable margin of capacity over requirement (§ 6, proposition III). Early in the course of this work, before the concept of tidal flow emerged, it was observed that in larvae on high K diet the rectal leads and the dilatations of the outer tubules were turgid, as compared with their relatively flaccid condition on high Na diet. One of the factors upon which the effectiveness of the proposed mechanism could depend is a hydrostatic pressure in the free tubules adequate to force fluid into the cryptonephric tubules; the observation may be interpreted as indicating that the smaller concentration difference on a high K diet as compared with a high Na diet requires to be associated with a tidal flow of greater volume, and that this is achieved by greater distension of the dilatation under higher hydrostatic pressure.

Second, the tidal flow mechanism makes possible a build-up of high osmolarity within the rectal complex. Such build-up requires that the fluid leaving the cryptonephric tubules must be of lower osmolarity than that which enters. The figures in table 11 show small but significant differences in osmolarity on both high Na and high K diets.

Third, the dilatation of the outer tubule is a structural feature whose functional significance is not immediately obvious. In the context of the tidal flow mechanism the dilatation clearly serves to increase the volume of the tidal flow; out of this context its functional significance remains obscure.

At this juncture it is convenient to extend the discussion to include the rectal complex of the mealworm. Although in this insect it is not possible to speak of outer and inner tubules, the disposition and differentiation of the cryptonephric tubules have this in common with lepidopteran larvae that the distal portion of the tubule has thick walls and a narrow lumen whereas the

proximal portion has thin walls and a wide lumen. The rectal complex of the mealworm is not provided with any external musculature whose contraction could cause the collapse of the proximal, thin-walled tubules; but, interestingly, at the junction between the free tubules and the cryptonephric tubules there is a muscular region which undergoes periodic contractions. Lison (1937) supposed that this activity served to draw fluid out of the cryptonephric tubules; but the present author (Ramsay 1964) observed that the cryptonephric tubules were normally in a state of turgor and that the principal effect of this muscular activity was to produce a to-and-fro movement of the fluid in the tubules. No interpretation of this activity in terms of the mechanism of the rectal complex was entertained at that time. It now seems possible that tidal flow coupled with uptake of potassium is the basic mechanism of the rectal complex in the meal-worm, with inward secretion of potassium at the leptophragmata in the role of a supercharger.

Wigglesworth (1932) drew attention to the importance of the rectal glands of insects for the withdrawal of water from the faeces, and later he wrote of the cryptonephric condition 'The precise significance of the arrangement is not known: perhaps this serves to add the absorptive powers of the Malpighian tubules to those of the rectal epithelium' (Wigglesworth 1934). Later still (Wigglesworth 1972 and personal communication) he endorsed the view that conservation of water provided the selection pressure under which the cryptonephric condition was originally evolved, pointing out that the cryptonephric condition is vestigial or absent in the aquatic members of those families which characteristically possess it.

These facts are not in dispute. But on the other hand a large number, possibly a majority, of the possessors do not seem, *prima facie*, to have any serious problem of water conservation. This would be true of the whole vast range of leaf-eating caterpillars, of which *Pieris* and *Manduca* are examples; the need for water conservation would seem to affect only the relatively few genera, such as the flour moth *Ephestia* and the clothes moth *Tineola* which live on dry food.

All the insects in which the cryptonephric condition is found appear to have one feature in common, namely that they have a high throughput of food. Living on the leaves of plants or on stored products they swallow large quantities of the readily available food which passes rapidly through the alimentary canal and is voided in an incompletely digested state. If they are to maintain salt and water balance the salt and water content of the faeces must be virtually the same as the salt and water content of the food. The experiments described in this paper have shown that the larvae of *Pieris* and *Manduca* can carry heavy salt loads while effectively main-taining the normal concentrations of sodium and potassium in the haemolymph; and that *Manduca* can rapidly restore the normal sodium concentration after an injection of sodium chloride into the haemocoel, whereas in *Schistocerca* and *Carausius*, which have a high through-put of food but no cryptonephric system, the ability to restore the normal concentration of sodium is relatively poor.

These considerations suggest that to regard the cryptonephric condition as being exclusively related to the removal of water from the faeces is perhaps to take too narrow a view; the crypto-nephric condition may be related to the requirements of salt balance as well as of water balance.

The difficulties of salt and water balance are exacerbated by high throughput of food, but they arise because of movements of salts and water into and out of the gut. The problem of water balance is easily appreciated in the case of those insects such as the mealworm which live on dry food; the dry food must necessarily be moistened in the midgut to make digestion possible and before the faeces leave the body the water added in the midgut must be expeditiously recovered.

The problem of salt balance facing lepidopteran larvae is less easy to understand. For biochemical reasons which one can hardly guess at it must presumably be of selective advantage to these insects to set up a potassium-rich environment in the midgut, and their problem would then seem to be the rapid recovery of potassium from the faeces. But there is more to it than that; these larvae are able not only to recover potassium but also to eliminate sodium and potassium in large amounts. If they were indiscriminate feeders, their choice ranging from higher plants to seaweed, one could say that their excretory systems were admirably adapted to meet the variety of stresses imposed by their feeding habits. But the exact opposite is the case; lepidopteran larvae are notable for their exclusive attachment to species-specific host plants. The remarkable flexibility of their regulatory powers would seem to be superfluous; yet it is difficult to believe that this flexibility, whatever the circumstances in which it was evolved in the past, would have survived prolonged disuse.

A possible explanation may be sought in the magnitude of the internal ion fluxes, relative to the total amounts of the ions in the body, and coupled with discontinuities in the course of normal digestion. The midgut constitutes a large proportion of the total body volume and at any moment it must contain a large proportion of the total body potassium. Intake of food is interrupted by necessary pauses (e.g. for ecdysis) during which the fluxes must cease. It may be that the abrupt stopping and starting of massive internal fluxes make it necessary for the insect to have on call greater powers of regulation than would be required to keep a continuous process in adjustment.

Tentatively, then, the following view is put forward regarding the biological significance of the cryptonephric condition in lepidopteran larvae. Selection pressure on lepidopteran larvae living on leaves is for rapid growth; this is achieved by a high throughput of food and by a digestive system which involves massive internal fluxes of ions; the Malpighian tubules have been pressed into the direct service of the rectal epithelium to provide a powerful and quickly acting mechanism to compensate for any maladjustment of these fluxes; the high osmolarity of the fluids in the rectal complex of some larvae is a later evolutionary development related to the recovery of water from the faeces and associated with the habit of living on dry food.

References

Cholodkowsky, N. 1887 Sur la morphologie de l'appareil urinaire des lépidoptères. *Arch. Biol.* **6**, 497–514.

David, W. A. L. & Gardner, B. O. C. 1966 Rearing *Pieris brassicae* (L.) on semi-synthetic diets with and without cabbage. *Bull. Ent. Res.* **56**, 581–593.

Grimstone, A. V., Mullinger, A. M. & Ramsay, J. A. 1968 Further studies on the rectal complex of the mealworm *Tenebrio molitor*, L. (Coleoptera, Tenebrionidae). *Phil. Trans. R. Soc. Lond.* B **253**, 343–382.

Harvey, W. R. & Nedergaard, S. 1964 Sodium-independent active transport of potassium in the isolated midgut of the Cecropia silkworm. *Proc. natn. Acad. Sci. U.S.A.* **51**, 757–765.

Henson, H. 1937 The structure and post-embryonic development of *Vanessa urticae* (Lepidoptera). II. The larval Malpighian tubules. *Proc. zool. Soc. Lond.* B **107**, 161–174.

Hoyle, G. 1954 Changes in the blood potassium concentration of the African migratory locust (*Locusta migratoria migratorioides* R. & F.) during food deprivation, and the effect on neuromuscular activity. *J. exp. Biol.* **31**, 260–270.

Irvine, H. B. 1969 Sodium and potassium excretion by isolated insect Malpighian tubules. *Am. J. Physiol.* **217**, 1520–1527.

Ishimori, N. 1924 Distribution of the Malpighian vessels in the wall of the rectum of lepidopterous larvae. *Ann. ent. Soc. Am.* **17**, 75–86.

Lison, L. 1937 Sur la structure de la région cryptosoléniée chez les coléoptères *Tenebrio molitor*, L. et *Dermestes lardarius*, L. *Bull. Acad. R. Belg.* **23**, 317–327.

Ramsay, J. A. 1953 Active transport of potassium by the Malpighian tubules of insects. *J. exp. Biol.* **30**, 358–369.

Ramsay, J. A. 1955a The excretory system of the stick insect, *Dixippus morosus* (Orthoptera, Plasmidae). *J. exp. Biol.* **32**, 183–199.

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

Ramsay, J. A. 1964 The rectal complex of the mealworm *Tenebrio molitor*, L. (Coleoptera, Tenebrionidae) *Phil. Trans. R. Soc. Lond.* B **248**, 279–314.

Ramsay, J. A. & Brown, R. H. J. 1955 Simplified apparatus and procedure for freezing-point determinations upon small volumes of fluid. *J. sci. Instrum.* **32**, 372–375.

Saini, R. S. 1962 Histology and physiology of the cryptonephridial system in insects. Ph.D. Thesis, Cambridge University.

Saini, R. S. 1964 Histology and physiology of the cryptonephridial system of insects. *Trans. R. ent. Soc. Lond.* **116**, 347–392.

Treherne, J. E. 1957 Glucose absorption in the cockroach. *J. exp. Biol.* **34**, 478–485.

Tobias, J. M. 1948 Potassium, sodium and water exchange in irritable tissues and haemolymph of an omnivorous insect, *Periplaneta americana*. *J. Cell comp. Physiol.* **31**, 125–142.

Wigglesworth, V. B. 1932 On the function of the rectal glands in insects. *Qu. J. micr. Sci.* **75**, 131–150.

Wigglesworth, V. B. 1934 *Insect physiology*. London: Methuen.

Wigglesworth, V. B. 1972 *Principles of insect physiology*, 7th ed. London: Methuen. (See pp. 574, 575.)

Winston, P. W. & Bates, D. H. 1960 Saturated solutions for the control of humidity in biological research. *Ecology* **41**, 232–237.