Contributions to knowledge of water relations in hemiptera

Goodchild, A. J. P.

Award date:
1988

Awarding institution:
University of Bath

CONTRIBUTIONS TO KNOWLEDGE OF WATER RELATIONS IN HEMIPTERA

Submitted by A.J.P.Goodchild

for the degree of Ph.D. (Staff Candidature,Method B)

of the University of Bath

1988

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

TABLE OF CONTENTS

PREFACE

The work submitted herein for the Degree of Doctor of Philosophy (Staff Candidature - Method B) consists mainly of nine selected papers which have already been published in reputable journals. They are the sole work of the candidate, and have not previously been submitted to any university for the award of a higher degree. They are attached as Appendices 1 - 9, and the titles listed below. The thesis consists of an introduction to the subject in general, with the background to the published papers, and an account of experiments and ultrastructural studies carried out between 1978 and 1983, and not as yet published. The discussion assesses these results in the light of previous work, and reviews the literature of the subject up to the time of writing.

APPENDICES

1. A study of the digestive system of the West African cacao capsid bugs.(Hemiptera,Miridae) Proc.zool.Soc.Lond., 122, 543-572.(1952)
2. Some new observations on the intestinal structures concerned with water disposal in sap-sucking Hemiptera. Trans.R.ent.Soc.Lond., 115, 217-237.(1963)
3. Studies on the functional anatomy of the intestines of Heteroptera. Proc.zool.Soc.Lond., 141, 851-910.(1963)
4. Evolution of the alimentary canal in the Hemiptera. Biological Reviews, 41, 97-140.(1966)
5. Shield bug (Piezosternum calidum Fab.) infestation of Oyster Nut. E.Afr.Agric.& For.J., 33, 192-196.(1967)
6. Some unusual cell inclusions in the mid-gut of a Moth Bug, Gyarina nigritarsis Karsch.(Homoptera:Flatidae), and their possible significance in nutrition. Proc.R.ent.Soc.Lond.(A), 44, 11-16.(1969)
7. The rectal glands of Halosalda lateralis(Fallen)(Hemiptera:Saldidae) and Hydrometra stagnorum(L.)(Hemiptera:Hydrometridae). Proc.R.ent.Soc.Lond.(A), 44, 62-70.(1969)
8. Bionomics, aggregated feeding behaviour, and colour variations in the sap-sucking bug Mygdonia tuberculosa Sign.(Hemiptera:Coreidae). Rev.Zool.Afr., 91, 1032-1041.(1977)
9. The nature and origin of the mid-gut contents in a sap-sucking Heteropteran, Piezosternum calidum Fab.(Tessaratominae) and the role of symbiotic bacteria in its nutrition.Ent.exp.& appl.,23, 177-188.(1978)

SUMMARY

Measurements of sodium and potassium concentrations in the haemolymph and excreta, and an experiment to compare feeding, growth rate and excretion in 5th.instar larvae of the pentatomid bug Palomena prasina, are described, and an account given of investigations into the ultra-structure of the midgut of adults of a closely related species, Dolycoris baccarum.

It is shown that the haemolymph of P.prasina has a sodium:potassium ratio of approximately 0.4, substantially less than in other Exopterygote insects. It is suggested that this is an adaptation to phytophagy. Very little sodium, but a large amount of potassium, is discharged in the excreta, corresponding to the proportions in the food (runner bean pod) of 1:140.

The relationship between food intake, weight gain, and potassium excreted show significant correlations, the average per 5th.instar larva per day being approximately 35.5mg., 7.6mg., and 0.066mg. respectively. The points at which the regression lines cross the y-axis suggest that the "maintenance ration" per insect per day is 9mg.wet weight of bean pod (0.01 Kj.) and that approximately 15% of the excreta is not measured by the technique employed. A correction for the latter is applied to the results, and gives a result for volume of fluid excreta consistent with that calculated from independent data. Feeding efficiency is shown to be comparable with that of other phytophagous insects, but less than that of seed-feeding Hemiptera. Weight gain is 38.34% of food ingested and 40.6% of food assimilated. The former figure is high compared with biting-and-chewing feeders because no inassimilable material is ingested.

The structure of the midgut of adult D.baccarum shows that the anterior regions are adapted for secretion and solute absorption without mass fluid transport, but the posterior regions, which are isolated during larval life by an interruption of the gut lumen, resemble cells in other insects in which mass fluid transport is believed to occur. It is thought that movement from lumen to haemolymph occurs in the central tube of the caeca-bearing region, and from haemolymph to lumen in the hindmost part of the midgut, the excretory vesicle. The implications of this are discussed.

The material contained within the papers submitted as appendices is essentially summarised in the Introduction to this thesis.

INTRODUCTION

Insects belonging to the Order Hemiptera feed by piercing and sucking. Their feeding apparatus is among the most highly modified, from the primitive pattern found in the cockroach, of any group of insects. In place of the biting jaws and jointed maxillae and labium, with jointed sensory palps on the last two pairs of appendages, there remains only the proboscis-like labium, carrying in a channel on its morphologically anterior face a hollow piercing stylet. The stylet is composed of two pairs of delicate bristle-like structures, which interlock by means of longitudinal grooves and ridges. The inmost pair, which are interpreted as representing the maxillae, enclose channels which serve to convey saliva into the food, and the food back into the pharynx. These channels are formed by the apposition of grooves on the inner surfaces of the maxillae. The outer bristles, interpreted as the mandibles, support the maxillae, and have barbed tips which assist entry of the stylet bundle into the food. The mandibular stylets also move in and out alternately during penetration. Notwithstanding the specialised nature of this feeding equipment, the range of food source in different groups within the Order covers all kinds, excepting only the insoluble structural cellulose of plants. Although adaptable to many different purposes, the hemipteran feeding mechanism is supremely suited to feeding on plants, either cell contents or by tapping the vascular system. It probably first evolved in this situation, from forms like the present-day Psocoptera. Many textbooks treat Hemiptera as two Orders, Homoptera and Heteroptera, but since the feeding equipment is essentially the same, and is not likely to have evolved twice in unrelated lines, it does not seem unreasonable to regard these groups as branches of a single Order. Homoptera are all phytophagous, whereas Heteroptera include a great many carnivorous families. The distinguishing characteristics of the Heteroptera can be interpreted as adaptations to an omnivorous life amongst forest floor litter (flattened body form with tough fore-wings, proboscis capable of forward extension, long antennae, defence by glandular secretions rather than rapid flight), and so the sub-groups of Heteroptera in which phytophagy is found must be assumed to have returned to this habit secondarily. The distribution of food habit in Hemiptera is shown in Fig.1. The suggested relationships are based on midgut anatomy. Backus (1988), on the basis of stylet structure, mode of operation and innervation, suggests a slightly different arrangement,

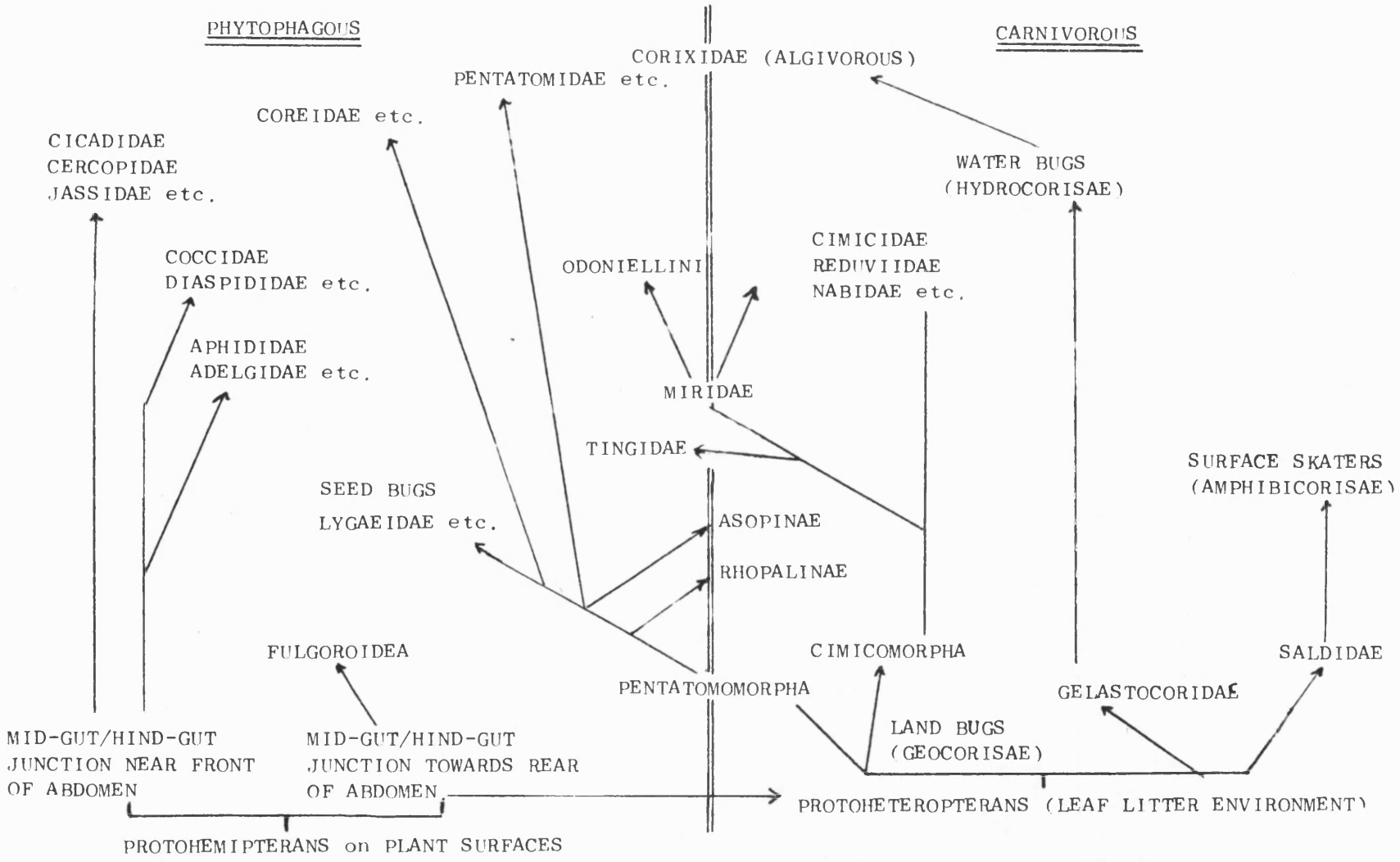


Figure 1. Trophic types and relationships in Hemiptera.

The complex anatomy of the alimentary canal of certain families of Homoptera has long excited the interest of entomologists (Snodgrass, 1935), and together with the production of liquid excreta, sometimes in copious amounts, has been interpreted as a means of excreting surplus water. The "filter chamber" formed by the apposition of the anterior mid-gut to the proximal parts of the Malpighian tubules and the posterior end of the mid-gut, enclosed within a sheath of the peritoneum, has been noted to increase in complexity with increased body size from one species to another. This suggests that the area/volume relationship is being maintained, and is of physiological importance. Cercopidae and Cicadidae feed by penetrating the xylem vessels of their host plants. Their ingesta is very poor in organic nutrients, and vast volumes must be passed in order to provide enough nourishment for growth and reproduction. Large tropical cercopids are gregarious and their excretory flow is like rain beneath the trees on which they live. In these insects, the ingesta are strongly hypotonic to the haemolymph. In the "filter-chamber" a large area of very thin epithelium separates the ingesta from the proximal Malpighian tubules and posterior mid-gut, and the higher osmotic pressure of the Malpighian tubule contents draws water through this. Passage along a narrow glandular hind-gut extracts solutes, resulting in excreta of similar ionic composition and osmotic pressure to the ingesta (Appendices 2 & 7; Cheung & Marshall, 1973a,b; Gouranton, 1968a,b; Marshall & Cheung, 1974,1975). At the other end of the size scale, very small insects such as the leafhoppers (Jassidae, etc.) may feed upon individual plant cells, have much reduced filter chambers, and may produce semi-solid excreta (Lindsay & Marshall, 1980; Appendix 4).

The other long-known "filter chamber" system is that of some Coccidae. In order to provide information for a review of the subject (App.4), I have examined material of a large species, Pulvinaria jacksoni, and illustrated it in the above-mentioned article. Though the structure has the feature in common with that of Cicadoidea, of the close association of anterior and posterior ends of the mid-gut, the Malpighian tubules are not involved, and the principle of operation appears to be different. The apposed ends of the mid-gut are twisted together, the anterior end inside the posterior, and the region of contact is fused and extremely thin. The whole structure is sunk into the bulbous rectum, in order, I believe, to supply mechanical support for the

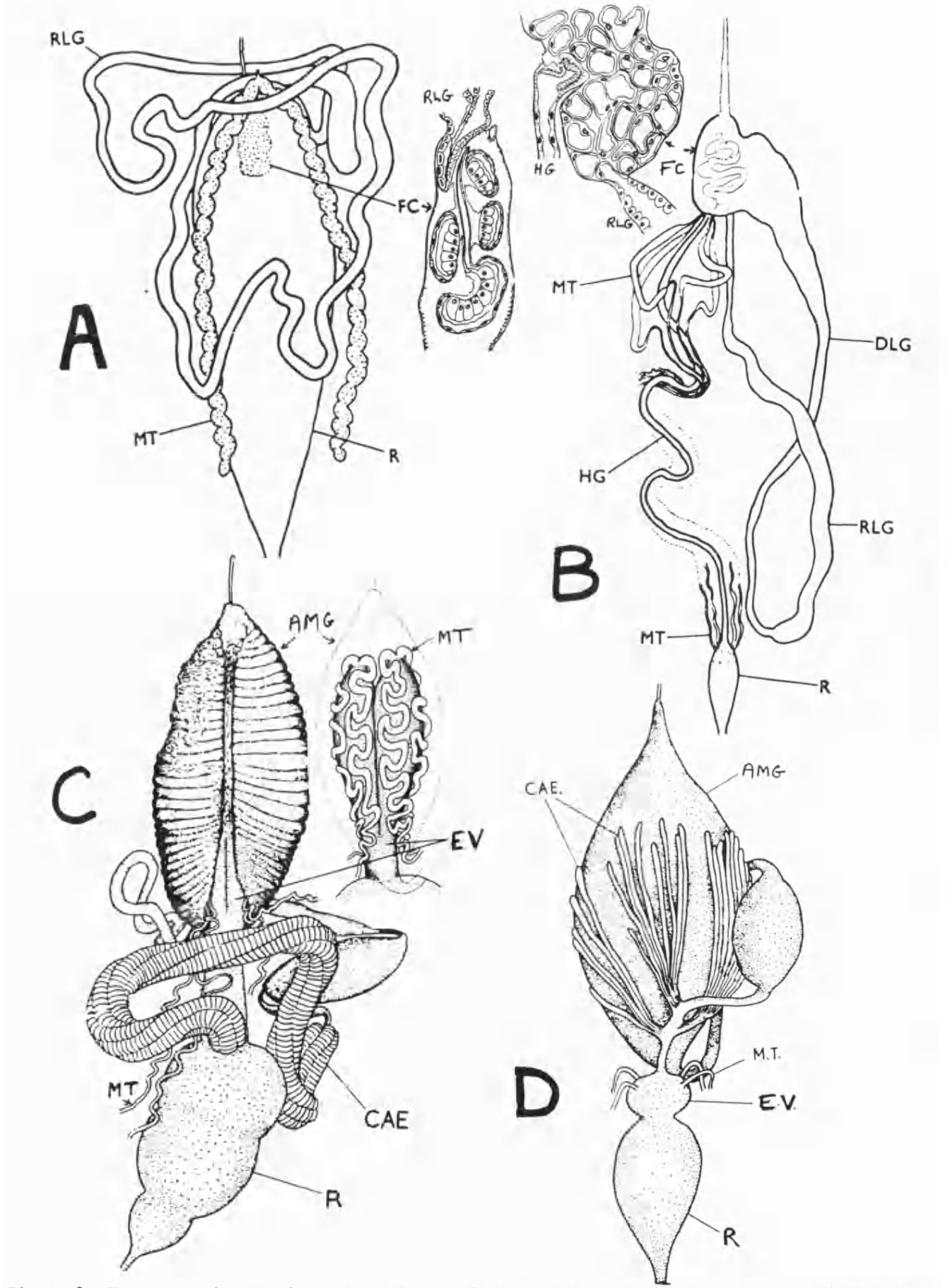


Figure 2. The supposed water-disposal pathways of plant-sucking Hemiptera. A. Coccoid (inset r., longitudinal section of "filter-chamber"), B. Cicadoid (inset l., longitudinal section of "filter chamber"), C. Pentatomidae Phyllocephalinae (inset r., excretory vesicle and proximal Malpighian tubules, ventral view), D. Lygaeidae. A & B from Appendix 4, C from App. 2, D from App.3. Abbreviations:- AMG anterior mid-gut, DLG descending loop of mid-gut, RLG returning loop of mid-g HG hind gut, CAE gastric caeca, EV excretory vesicle, FC filter chamber, MT malpighian tubules, R rectum.

development of an internal hydrostatic pressure, which would tend to "unwind" the coiled double tube. Water could pass through the thin intervening membrane from the anterior midgut to the posterior midgut and thence to the rectum. This mechanism, unlike the Cicadoid filter chamber, does not involve osmotic suction to induce the flow, and so there would be no urgent necessity to recover solutes. The fact that the Coccoid rectum is bulbous and not strongly glandular tends to support this interpretation. Cheung & Marshall (1973a) have provided evidence for the mechanism suggested for Cicadoidea, but no physiological experiments have yet been done on Coccoidea. It is only in these two groups of families (Cicadoidea including Cercopidae, Jassidae, etc., as well as Cicadidae) that the anterior and posterior ends of the midgut are close together and can form a "by-pass" system for dealing with excess water of a very dilute diet, and only in these groups is feeding from plant xylem vessels found. They are illustrated in Fig.2, A and B.
Other phytophagous Hemiptera, feeding on phloem sap, cell contents, or the endosperm of developing ovules, face a less extreme osmotic difference between their ingesta and their body fluids. Nevertheless, there exist among them certain distinctive features, correlated with the phytophagous habit, which must be presumed to have some physiological significance. Plant tissues generally have a higher water content than insects, and suctorial feeding will result in the ingesta containing mainly small molecules of sugars and amino acids. The absorption of these will lower the osmotic pressure in the gut, and lead to the need to excrete a more dilute material than that ingested.
These other Hemiptera occur in two major groups of Homoptera, the Aphidoidea and the Fulgoroidea, and in both the major divisions of the terrestrial Heteroptera (Geocorisae), the Cimicomorpha and the Pentatomomorpha. The special features of midgut anatomy are different in each case.
The Aphidoidea are unique among pterygote insects in lacking Malpighian tubules. Their gut is a simple tube, in some species the mid- to hindgut junction is anteriorly situated, but in others it is not (Ponsen, 1982a,b). Phloem sap is forced into their gut by plant turgor (Mittler, 1957, 1958). It has a relatively high osmotic pressure, chiefly from soluble carbohydrates, with little nitrogenous material. This mode of feeding results in a high carbohydrate content in the excreta ("honeydew"), but its osmotic pressure is regulated by varying the synthesis of polysaccharide sugars (Fisher, Wright & Mittler, 1984). Aphids compensate for

changes in dry matter content of phloem sap between day and night (Cull & van Emden, 1977). In some larger species of aphid, a simple form of filter chamber can be found (Ponsen, 1981), but in the absence of Malpighian tubules it is difficult to understand how it could operate. Many insects belonging to the Coccoidea have curiously modified Malpighian tubules, only two in number, with thick walls, and it is from such forms that the Aphidoidea may have evolved. They also form strange polysaccharides (Basden,1967).

The Fulgoroidea have a midgut quite unlike that of any other Hemipteran. It has been described (Appendix 2) from a species in which its distinctive features appear to be present in a primitively fully developed condition. The anterior and posterior ends of the midgut are widely separated, the hindgut a short bulbous rectum, and the Malpighian tubules normal in appearance and free in the haemocoel. The tubular midgut is coiled within a cellular sheath which isolates it from the haemocoel, except for a tubular diverticulum extending forward into the thoracic region. In the Tettigometrid Phalix titan, the sheath is two-layered, the outermost layer a thin membrane with scattered small nuclei, and the inner layer composed of flattened cuboid cells with large nuclei and strongly eosinophil cytoplasm. In a specimen of the large lantern-fly, Pyrops tenebrosus (App.2), the two layers of the sheath seemed to be separated, the inner layer adhering closely to the midgut tube. In other species (Appendix 6), the sheath is much less evident, and in the accounts given by earlier authors no explicit mention is made of such a structure. More recent anatomical studies (Mishra, 1980; Cheung, 1983) have confirmed the existence of an ensheathed intestine, but have not added any important new information. Whereas earlier authors put forward a variety of explanations for the anterior diverticulum of the midgut, with the establishment of the existence of a midgut sheath, it can be more elegantly explained as an adaptation for the inflation of the thorax at ecdysis, without damaging the sheath.

Among the phytophagous Heteroptera, the Cimicomorpha include the families Miridae and Tingidae, not a few of which are major crop pests. They are small insects, and their mode of attack on a plant is to destroy a group of cells with their toxic saliva, and suck out the contents. Physiologically, this may not be very different from sucking the juices from an insect prey, and indeed, some Mirid species will feed equally upon plants or upon insects.

The earliest work of the present writer (Appendix 1) was to study the feeding behaviour of the relatively large-bodied and distinctive group of Miridae, the tribe Odoniellini of the sub-family Bryocorinae, which include serious pests of cacao in West Africa and a pest of coffee in East Africa. One genus, Helopeltis, has a wide distribution in the tropics and attacks many different crops. Observing these insects in the act of feeding, it was found that an unusual kind of excretory process was taking place. After feeding on a soft cacao shoot, the mouth parts would be withdrawn from the plant, but the proboscis remained extended and resting on the plant surface, and a few large drops of clear fluid were deposited. The volume of fluid excreted in this manner was nearly as great as that passed via the anus. It appeared to come from the accessory salivary glands, which in Cimicomorpha have a thin-walled sac closely applied to the sides of the anterior midgut. In the cacao mirids, these sacs were greatly distended in insects dissected just after feeding. Sac-like extensions to the salivary glands are not present in the predominantly phytophagous Pentatomomorpha. In Cimicomorpha, they may have the function of recycling fluid from the midgut back into the insect prey, and it is perhaps significant that in blood-sucking forms they are small or absent.

In the course of work on these insects, much literature was consulted and specimens of phytophagous Heteroptera dissected. The conclusion was reached that although the midgut of Odoniellini was quite different from that of other Cimicomorpha, it was also different from that of Pentatomomorpha. In becoming adapted to plant food of high water content, the Odonielline mirids had modified the organs characteristic of Cimicomorpha but had not reproduced the structures found in Pentatomomorpha. They had made use of the accessory salivary gland to excrete surplus water, and had lost all trace of rectal gland cells.

The writer's interest in alimentary structures associated with phytophagy having been thus aroused, attention was turned to the Pentatomomorpha. The distinctive features of this group of Heteroptera are the separate chamber of the midgut into which the Malpighian tubules open, referred to by earlier authors, mistakenly, as "ileum" but now recognised as belonging to the midgut and called "excretory vesicle", and the rows of pouch-like diverticula on the penultimate region of the midgut, the so-called "gastric caeca" (Plates 1 & 2). In shape the caeca vary from

bunches of long tubes in some Lygaeidae, through two rows of short finger-like caeca on opposite sides of the gut in other Lygaeidae and in Coreidae, Cydnidae, and Acanthosomatidae, to four rows of purse-shaped caeca in Pentatomidae and related families. In the latter the adjacent rows of caeca are in contact, so that the central tube is completely surrounded. In Pyrrhocoridae, Rhopalidae, some Lygaeidae and a few Pentatomidae, the caeca are rudimentary or absent, and this is associated with feeding on seeds, or carnivory. There is thus a correlation between presence of caeca and feeding upon hydrated plant tissue. In the early part of this century, the gastric caeca were the subject of many published works, most of which assumed without question that the function of the caeca was to harbour a pure culture of symbiotic bacteria. Although it is true that in almost all species which have been examined, the caeca contain vast numbers of bacteria, which can be cultured and always prove to be Gram-negative short flagellate rods of the genus Pseudomonas, the idea that the caeca evolved solely for that purpose is not convincing. The observation which set the writer thinking in terms of a water-regulating function was that of the presence in the living caeca of Coreidae and Lygaeidae of large, clear "bubbles" which discharged their contents as they passed from the caecum into the central tube. Similar bubbles could be seen in the excretory vesicle of some species, when living tissues were examined under the microscope, apparently nipped-off from the cell tips. Unfortunately these bubbles did not survive preparation for histological study. Although they have not been referred to in any published work on the caeca, some authors' illustrations of sections through bacteria-filled caeca show oval gaps which might indicate the presence of such bubbles.

Another pointer to a possible water-excreting function, again missed by early authors, was seen in the close anatomical relationship of the long caeca of some Lygaeid species to the anterior midgut (Fig.2D). Dissected alimentary canals illustrated by early authors were always displayed in an extended manner, which destroyed this relationship.

Though bacteria are almost invariably found in the gastric caeca, this need not mean that they were originally evolved to harbour them. The arguments against this, and a review of the relevant literature, are given in Appendix 3. Nevertheless, evolution has worked upon the possibly adventitious colonisation of this part of the midgut so as to make use

of the bacteria contained therein, in a manner which explains their intra-intestinal rather than extra-intestinal location, and which was not evident until extreme cases in certain tropical species had been discovered (Appendices 3, 9). That is, the bacteria are passed forward along the central tube to a bulb-like expansion of the anterior end of the caecal region, where they are digested. Exactly what benefit the insect derives from this is still obscure. In some species, in the Coreidae and Lygaeidae, this may be compatible with continued water excretion, but in Pentatomidae the main burden of water excretion is transferred to the excretory vesicle. In larval stages, and in some specialised forms in the adult as well, the midgut is interrupted by the obliteration of its lumen just anterior to the caecal region (and its associated bulb). In the most specialised forms, there is an interruption of continuity between caecal region and excretory vesicle, so that all possibility of caeca retaining an excretory function ceases. In these cases, it is of interest to note, the caeca tend to merge with the central tube and lose their separate identity. Some of these specialised forms are sap-suckers, and have the characteristic seen in many Homoptera of feeding gregariously and in one place for long periods. Two of these are illustrated in Plate 3. In both species, feeding continued long after the plant distal to the feeding site had shrivelled, showing that vascular tissues had been tapped.

The most extreme form of anatomical specialisation of the midgut is in the tropical family Phyllocephalinae (Fig.2C), most of which are associated with Graminae as host plants, but of which larval biology is almost unknown. In these insects, the excretory vesicle is greatly extended anteriorwards, together with the proximal parts of the Malpighian tubules, and lies dorsal to the anterior midgut which closely enfolds it. The adjacent epithelia are similar in histological structure, composed of rather flattened cells with sparse cytoplasmic contents (in this respect similar to the Cicadoid filter chamber). Although no physiological research has yet shown how this arrangement works, it is sufficiently specialised to merit the term "filter chamber". Unfortunately, there are no published observations on feeding behaviour, excretion or larval biology. It is quite likely that the larvae are subterranean root feeders, and this may also be the case for the Coreid Acanthocoris obscuricornis (Appendix 3) and Pyrops tenebrosus (Appendix 2). In the studies by Cheung (1979,1982,1983) on Pyrops candelaria in Hong Kong, only adults were used.

Thus it may be seen that the alimentary canal of phytophagous Hemiptera shows several intriguing features, the purpose and mode of operation of which need to be elucidated by physiological experiment. That so little has been done may be due to the mainly tropical distribution of the most interesting forms, and to their lack of importance as crop pests. At the most they may be occasional pests of crops of only local significance, and in poor countries there is neither the finance nor the time for purely academic studies. Furthermore, the most interesting species need to be reared on living plants, and the added complication and expense of this means that such techniques can only be justified for insects of great economic importance, such as aphids, mirids, etc. Most of the physiological research has been carried out on the more easily reared seed-sucking species (e.g. Berridge,1965; Miles, 1959,1960).

Even in their tropical homelands, the more specialised forms are not easily found, and it should be emphasised that much of the work presented in the appendices (e.g. nos.2,5 and 9) has only been possible because of serendipitous occurrence of infestations otherwise rarely encountered, or even single captures of the insects concerned. The large sap-sucking species (App.9) have a fluctuating distribution, and may only occupy a particular site for a year or two, during which time the incidence of hymenopterous egg parasites increases up to nearly 100%. In the British Isles, the Hemipteran fauna is depleted, even compared with continental Europe, and, as will be described in the next section, the only species readily available for study were two Pentatomidae.

While there are a number of studies on the physiology of salivary glands of Pentatomomorpha (Hori,1972; Miles,1964; Nuorteva & Laurema,1961; and Salkeld,1959), of their alimentary enzymes (Saxena,1958; Saxena & Bhatnagar,1961), and of their scent glands (Gilby & Waterhouse,1965; Waterhouse & Gilby,1964), there is virtually no information on the ionic composition and tonicity of excreta, relative to haemolymph. As it was intended to study the ultrastructure of the caeca and excretory vesicle, to see if they showed any of the features characteristic of water transport, it seemed desirable to find out the degree of osmotic work which had to be performed. Indeed, Marshall & Cheung(1975) showed that the excreta of a fulgoroid Homopteran was hypertonic to the haemolymph.

An experiment on efficiency of food conversion was also carried out, in order to see what effect, if any, the caecal bacteria had on this parameter, compared with other kinds of insect.

THE SOURCE OF RESEARCH MATERIAL

The most easily obtainable pentatomomorph Heteroptera in the neighbourhood of Bath turned out to be the species Dolycoris baccarum (Linn.) and Palomena prasina (Linn.), both Pentatomidae. The former (common name Sloe bug) is a dull mahogany-brown insect of 11-12 mm. adult body length, and the latter (common name Green shield bug) is bright leaf green and 12-13 mm. long. Despite many searches, the larger species of Coreidae (Mesocerus marginatus (Linn.) and Enoplops scapha (Fab.)). were not found. The Lygaeidae occurring in Britain are not large enough for the kind of work envisaged, nor is the Pied shield bug, Sehirus bicolor (Linn.)(Cydnidae), although it is quite common on White Dead Nettle (Lamium album).

D.baccarum and P.prasina are both sparsely and locally distributed, and the material of these species was obtained from one or two sites, each not more than 50 m^2. in extent, where adults could usually be found in spring (mid-May to mid-June). Though they were not found on the same site, both species seemed to have an association with the flowers of White Dead Nettle. P.prasina was also found in late spring on raspberry canes in a colleague's garden, and towards the end of the period of study larvae of both species were found, in late summer, on plants of annual mercury (Mercurialis annua)in my own garden, perhaps due to regular release of laboratory reared surplus adults, and to a summer when the garden was less resolutely weeded than usual.

P.prasina could be easily reared in the laboratory, on green bean pods. The adults collected in spring (rarely more than 3 or 4 pairs) were confined on pot grown kidney bean plants (Phaseolus vulgaris), and soon clusters of about 28 pale green, barrel-shaped eggs would be laid on the undersides of leaves, or even on the confining netting. In some cases, eggs had already been laid in the container in which the insects were collected in the field. Hatching occurred in about 8-10 days, and the hatchlings remained by the eggs for a further 5-6 days, before moulting to the second instar and commencing to feed. If the empty egg shells and the leaf were left for a week or two, and the leaf began to senesce, the cluster of shells was seen to be surrounded by a zone more transparent than the rest of the leaf, as if the larvae had taken their first feed from that region. At that time of year, it was difficult to obtain kidney bean pods, but the larvae were maintained successfully on ripe inflorescences of sainfoin (Onobrychis sativa)

until the new season's kidney beans became available. The method of culture was as follows. A two-pound biscuit tin was used, with a four-inch square hole in the lid closed with fine mesh nylon net. The bottom was lined with absorptive paper, and fresh bean pods stacked loosely thereon, about 250 gm. at a time, changed at 3 to 4 day intervals. The larvae made rapid growth, and deposited large amounts of watery excreta. New adults appeared in the culture in late July, which is two to three weeks before the new generation of adults could be found in the field. Soon after moulting to the adult, the excreta changed in character to a dense black paste, as the intestinal continuity was established and the mid-gut contents voided.

In contrast to P.prasina, a site was found on which D.baccarum adults could be collected in relatively large numbers both in spring and late summer (at the latter time they were found in a spot where Black Medick, Medicago lupulina, was dominant). However, no success was achieved with culture of larvae from the spring adults, although when caged on flowering shoots of Lamium album or Stachys sylvatica (Hedge woundwort, also of the family Labiatae), batches of pale pinkish eggs were laid, which failed to hatch. The characteristic brown "prickly" larvae of this species were occasionally found among herbage during the summer. Both species of bug enter reproductive diapause in the autumn, but remain active and feed. D.baccarum was kept in the laboratory on shoots of Lamium album, and the weights of marked individuals monitored at intervals. Weight was maintained while they had access to plant material, but dropped when it was withdrawn. In two out of four insects, weight was restored by access to a water-soaked pad. While exposed to a source of food, the insects were kept for two weeks under a long day lighting regime, but no mating took place. The insects were released, and the observations terminated, in mid-December.

Because of the relative availability of the different stages of the two species, larvae of P.prasina were used for experiments on excreta production and composition, while D.baccarum adults were used for studies on mid-gut ultrastructure. A few larvae of the latter species were also studied, and the autumn and spring adults were compared. Some light microscopy was carried out on P.prasina adults. All experiments were carried out at room temperature, approximately 20°C. Details of the methods used will be given with the account of each experiment.

THE PRODUCTION AND COMPOSITION OF EXCRETA

As already indicated, the liquid excreta of the larvae of Palomena prasina, is uncontaminated by midgut contents owing to the discontinuity of the gut lumen, but may include secretions of the caecal region and the excretory vesicle as well as the urine from the Malpighian tubules. It is for future, more refined research, to distinguish the contribution of these sources. For the purposes of analysis, the excreta were collected from the surfaces of bean pods in the culture box. Capillary tubes of accurately known 1 mm. bore size were used to pick up the globules by capillary attraction. Clean, discrete, average sized globules were selected, and confluent masses of excreta were avoided. Each capillary tube was used to pick up several globules, until capillarity ceased to be effective. The tubes were plugged with plasticine and stored in a frozen state pending analysis. As the results given in Table I suggest, periodical fluctuations in potassium content probably occurred, but this was not anticipated, and unfortunately no record was kept of the day on which particular samples were collected. Collections were made daily, and analysis carried out when a sufficient number of samples had accumulated.

The volume of samples was estimated from the length of column formed in the capillary tube. For most purposes no record was made of the number of excretory globules collected in a sample, but an estimate of individual globule size was made from some of the early samples by counting the globules as they were picked up. This showed that the average excretory globule has a volume of approximately 2.5 μl.

Some of the samples were used in an attempt to determine the osmotic pressure of the excreta, using a Wescor 5100B Vapour Pressure Osmometer. Difficulty was experienced in obtaining consistent results, even with material from the same sample, but those which were thought to be most reliable gave an osmotic pressure of 249 ± 8.5 mOsm./kg.(Mean$\pm$SE,N=4).For these measurements, samples were taken up in 5 mm. paper discs which were inserted into the machine. These discs were retained, and after drying were tested for uric acid by Folin & Wu's tungstate reagent, and for amino acids with ninhydrin. The former did not give a positive result, but the latter gave a faint reaction, indicating the presence of small amounts of amino acids.

The concentrations of sodium and potassium in the excreta were measured by using an EEL Flame Photometer. The excreta were transferred to small

clean glass vials, by sharply shaking the capillary tube over the mouth of the vial. The volume was estimated by measuring the column length, to the nearest 0.5 mm.,both before and after this operation. One millilitre of distilled water was added, to provide a suitable volume for injection into the photometer. The readings obtained were checked against freshly prepared standard solutions. A control was provided by simulated excretory globules formed by dropping distilled water on to pods, and then sampling those in the same way as the excreta. Control series A used fresh bean pods, and series B used pods which had been in the insect culture box (though not obviously contaminated with excreta). The difference between these two sets of controls was not significant. As far as possible, concentrations of sodium and potassium were measured on the same sample, and it is these readings which have been plotted in Fig.3 (p.19). The graph shows that there is no correlation between the concentrations of sodium and potassium. The results are listed in Table I, in which, for the sake of completeness, a number of readings where only the sodium or the potassium concentration was measured, are included.

A few determinations of cation concentrations in the haemolymph were also made, and are listed in Table I, but no measurement of osmotic pressure of haemolymph was made.

It will be seen from Table I that the potassium concentration in the excreta differs very significantly from either the control or the haemolymph, whereas the sodium concentration in the excreta differs from control and haemolymph only at a low level of significance. The net concentrations in the excreta, after subtraction of the control, are Potassium 2.947 µg/µl. and Sodium 0.0166 µg./µl. The high background level of sodium may be due to leaching from the glassware used in the experiment. The wide range of variation of potassium concentration in the excreta, with a much smaller variation in the haemolymph, and the opposite condition in the concentrations of sodium, may reflect a real situation. Being derived from only two or three egg masses laid at nearly the same time, the cultures were quite strongly synchronous, and these variations may indicate cyclical changes through the final instar, as observed by Berridge (1965) in Dysdercus fasciatus. The same author's figures for potassium mean (2.372 µg./µl) and maximum (3.43 µg./µl.) concentrations lie well within the range of the present work.

TABLE I

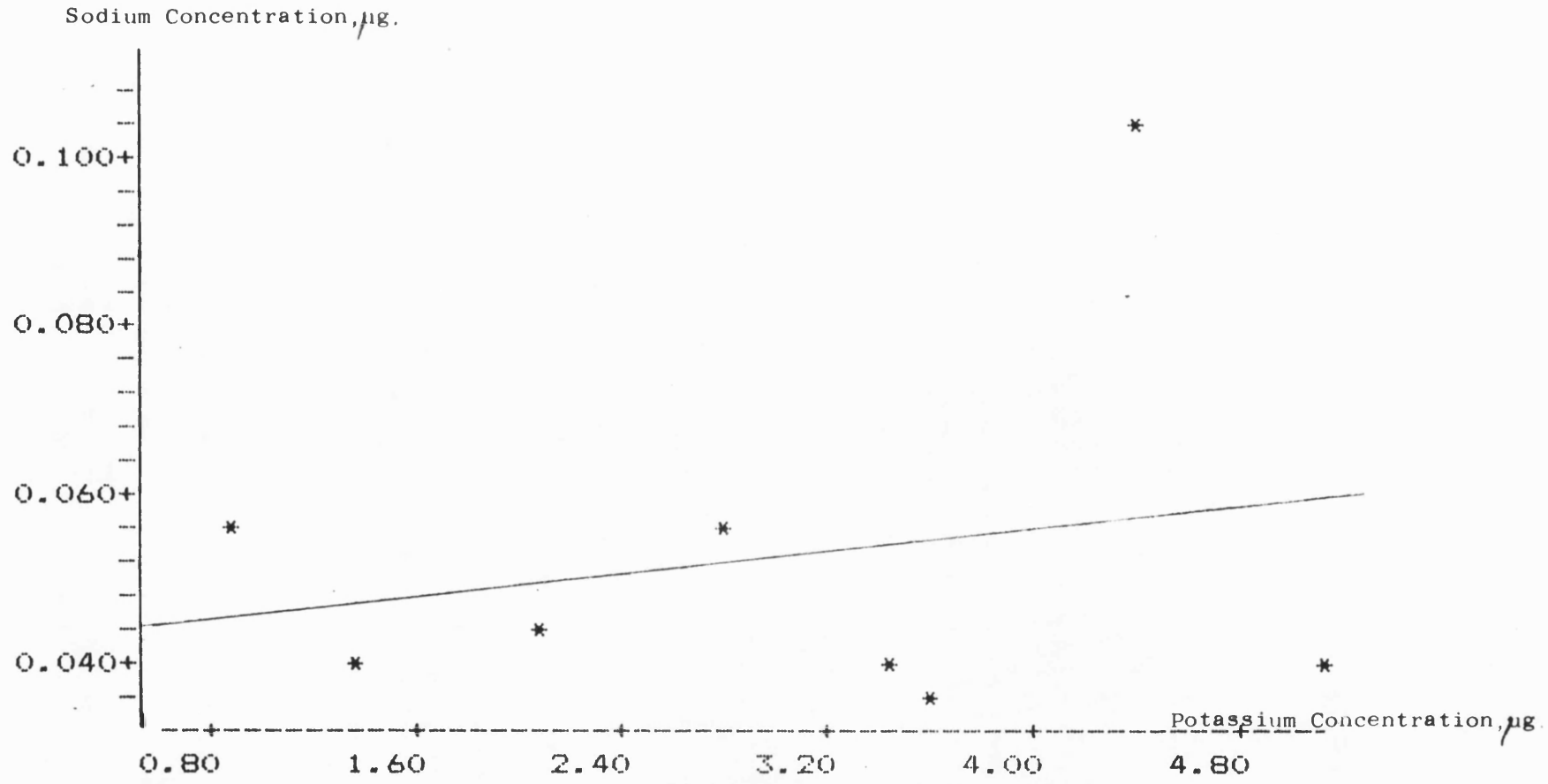
IONIC COMPOSITION OF EXCRETA AND HAEMOLYMPH OF PALOMENA PRASINA LARVAE

Sample	Volume(µl.)	Na (total)	Na (µg./ul.)	K (total)	K (µg./µl.)
1	26.70	1.797µg.	0.0673	-	-
2	18.85	1.977	0.1049	82.724µg	4.388
3	22.77	1.036	0.0455	46.753	2.053
4	18.06	1.034	0.0573	50.897	2.818
5	15.71	0.615	0.0391	21.850	1.391
6	13.74	0.512	0.0372	49.850	3.628
7	14.92	0.621	0.0416	51.800	3.472
8	19.63	0.760	0.0387	100.390	5.114
9	31.81	1.790	0.0560	27.620	0.868
10	8.64	-	-	31.540	3.651
11	20.80	-	-	58.080	2.790
12	16.50	-	-	40.360	2.446
13	33.77	-	-	61.840	1.831
14	37.31	-	-	142.490	3.996
Sample Mean Ion Concentration±SE			0.0542±0.0072		2.957±0.340
Control A (see text)					
1	29.02	1.045	0.0360	-	-
2	13.35	0.470	0.0352	-	-
3	32.76	1.640	0.0435	-	-
4	14.50	-	-	0.145	0.0100
Control B (see text)					
1	40.90	1.800	0.0440	-	-
2	25.54	0.950	0.0372	-	-
3	55.07	1.630	0.0296	-	-
4	27.94	-	-	0.285	0.0102
Control Mean Ion Concentration±SE			0.0376±0.0022		0.0101
Haemolymph					
1	5.50	1.828	0.332	5.446	0.990
2	6.28	1.016	0.162	5.311	0.846
3	8.25	0.997	0.121	7.223	0.876
4	5.10	-	-	5.119	1.003
5	5.50	-	-	3.458	0.629
H'lymph Mean Ion Concentration±SE			0.205±0.065		0.869±0.067

Significance of results.

Excreta vs. Control - Sodium t = 2.20 DF 13 .05>P>.02 Potassium t = 9.61 DF 13 P<.001

Excreta vs. Haemolymph - Sodium t = 2.76 DF 10 P = 0.02 Potassium t = 6.0 DF 16 P<.001



Regression Equation : Sodium Concentration = 0.043 + 0.00323 x Potassium Concentration µg.

r = 0.2098 Not Significant.

Figure 3. Graph of Sodium Concentration against Potassium Concentration (Excretory Droplets).

FEEDING, GROWTH, AND ION EXCRETION

A series of experiments was carried out to compare food consumption, insect growth, and excretory throughput. After some preliminary trials with different methods of confining the bugs on their food, the following system was arrived at. Glass crystallizing dishes were used, of internal dimensions 8.5 cm. wide by 4.5 cm. deep, closed by clear plastic Petri dish covers in which four one-inch holes had been made and covered with nylon mesh. Two lengths of runner bean pod were cut for each dish, of such a length as to fit firmly across the width of the dish, not touching the base or lid. They were set at right angles to each other. The pods were weighed before insertion, and groups of P.prasina nymphs placed in the dish, having been weighed as a group. The insects were chosen from the culture box as being apparently healthy, active, and unfed or only partly fed, as shown by the state of distension of the body. The insects were left in with the bean pods for 72 hours, and then the group of nymphs was weighed to the nearest 0.1 mg. in a pre-weighed vial, and the pod lengths were weighed to the nearest milligram. The insect's feeding left no visible lesion, and the pods were still fresh looking. Each replicate of three or four dishes had one dish without insects, as a check on evaporative losses from the pods. Unfortunately, there was no check on evaporation from the insects. After the weighings, the dishes were set aside, upside down, to dry out, and at the end of the series of replicates, the amount of sodium and potassium deposited by the insects was measured by flame photometry in the same way as the excretory droplets. The dried deposit, a scarcely visible smear, was eluted with 20 ml. of hot distilled water (10 ml. for the control dishes), and further diluted if necessary to bring the photometer reading into the range of the standard solutions. As the surface of the pods was not rinsed into the dish, any excreta adhering to them was lost. (In notes made at the time, only one dish (replicate 6c, Table II) was recorded as having a significant amount of excreta visible on the pods.) As will be seen from Table II, eight sets of replicates, yielding 22 individual records of insect weight gain in relation to food consumed, were obtained. As the insects were returned to the culture box after the experiment, some of them may have been used more than once. This is known to have occurred in the replicates 6a/7b and 6b/7a, and because of their immaturity, the insects used in 1a and 1b were probably used again later.

The results from this experiment are tabulated (with details of the insects used in each replicate) in Table II, and expressed graphically in Figures 4, 5, and 6 (pp.24-26). In additional to statistical treatment of these results, they may be compared with those of the previous experiment, and with the analysis of composition of runner bean pod given in Paul & Southgate (1978). Comparison may also be made with the figures published by Berridge (1965) on excretion in the cotton stainer bug, Dysdercus fasciatus.

It is clear that in both the experiments, as in Berridge's work, large amounts of potassium are excreted, but very small amounts of sodium. In fact, from Table II, the sodium excreted is not significantly different from the background. Figure 4 shows, as did Figure 3, that there is no connection between the concentrations of potassium and those of sodium.

The concentration of sodium in the excreta (corrected mean 0.0166 µg./µl.) may be compared with that in the food (0.020 µg./mg.) and that in the haemolymph (0.205±0.065 µg/µl.). The insect is therefore excreting less sodium than it ingests and retaining in the body a level ten times above the ingesta. There are a few points on Figure 4, and one (sample 2) on Figure 3, where sodium concentration is unusually high. Apart from experimental error, two interpretations of sample 2 are possible. One is that the excretory globules which formed the sample had all lost water by evaporation (a 50% loss of volume would bring the point well into line with the others). Even if this sample were the first of the series to be collected (as stated above, no record was kept of the order in which samples were collected), and the globules had been exposed for longer than the others, such a diminution in volume would have been noticeable, and the globules would not have appeared normal. The other possibility is that there may be a point in the instar when a natural rise in sodium excretion occurs. Berridge records that the sodium content of the excreta rose from 0.013 µg./µl. in the middle of the fifth instar, to 0.100 µg./µl. at the end. He also found that potassium excretion was highest in the middle of the instar, and the lack of any correlation between the concentrations of sodium and potassium in the excreta of P.prasina suggests that it is similar in this respect.

With regard to potassium excretion, the opposite condition prevails, in that this cation is more concentrated in the excreta (mean = 2.947 µg/µl.)

TABLE II

RELATIONSHIP of WEIGHT GAIN of PALOMENA PRASINA, WEIGHT LOSS of FOOD, and EXCRETA COMPOSITION

Experiment	Bean Wt.loss,gm.	Net Wt.loss,gm.	Bug Wt.gain,gm.	Na excreted,µg.	K excreted, µg.
1.(20-23/07/82)					
Control (no bugs)	1.756	-	-	9.10	7.05
a)(Seven 3 & 4 instar)	2.005	0.249	0.0690	20 73	188.23
b)(" " ")	2.246	0.490	0.0787	27.70	542.76
2.(23-26/07/82)					
Control (no bugs)	1.816	-	-	28.41	27.50
a)(Four 4th.instar)	1.836	0.020	0.0355	12.79	243.04
b)(" " ")	1.910	0.094	0.0333	18.60	227.85
3.(27-30/07/82)					
Control (no bugs)	1.675	-	-	13.64	8.82
a)(2,4th.& 2,5th.inst.)	2.136	0.461	0.1169	9.09	611.76
b)(Three 5th.instar)	2.051	0.376	0.0593	9.09	235.29
c)(" " ")	1.921	0.246	0.0423	9.09	513.43
4.(30/07-02/08/82)					
Control (no bugs)	1.731	-	-	12.66	11.47
a)(Three 5th.instar)	1.986	0.255	0.0856	21.52	639.34
b)(" " ")	1.970	0.239	0.0972	32.91	747.54
c)(" " ")	2.111	0.380	0.0931	6.33	1318.03

TABLE II (continued)

Experiment	Bean Wt.loss,gm.	Net Wt.loss,gm.	Bug Wt.gain,gm.	Na excreted,µg.	K excreted,µg.
5.(03-06/08/82)					
Control (no bugs)	1.534	-	-	2.27	4.05
a)(Three 5th.instar)	1.685	0.151	0.0664	18.52	518.18
b)(" " ")	2.093	0.559	0.0926	44.70	1076.47
c)(" " ")	1.828	0.294	0.0695	8.99	267.57
6.(13-16/08/82)					
Control (no bugs)	1.371	-	-	14.29	14.10
a)(Five 4th.instar)	1.658	0.287	0.0842	8.33	554.66
b)(Three 5th.instar)	1.706	0.335	0.0593	7.14	576.00
c)(2,4th.& 2,5th.ins)	1.625	0.254	0.0641	5.95	112.00
7.(16-19/08/82)					
Control (no bugs)	1.540	-	-	4.76	10.13
a)(Three 5th.instar)	2.067	0.527	0.0563	7.06	486.49
b)(Five 4th.instar)	1.897	0.357	0.0231	7.06	213.33
c)(Four 4th.instar)	1.656	0.116	0.0197	4.71	16.44
8.(24-27/08/82)					
Control (no bugs)	1.532	-	-	14.29	11.25
a)(Four 5th.instar)	2.162	0.630	0.1125	7.06	966.67
b)(" " ")	2.009	0.477	0.0770	11.76	760.84
c)(" " ")	1.767	0.235	0.0764	7.06	529.58
Means ± SE		0.320±0.032gm.	0.0687±0.0077gm.	13.92±2.34µg.	515.70±66.9µg.
Control Means ± SE				12.43±2.79µg.	11.80±2.49µg.

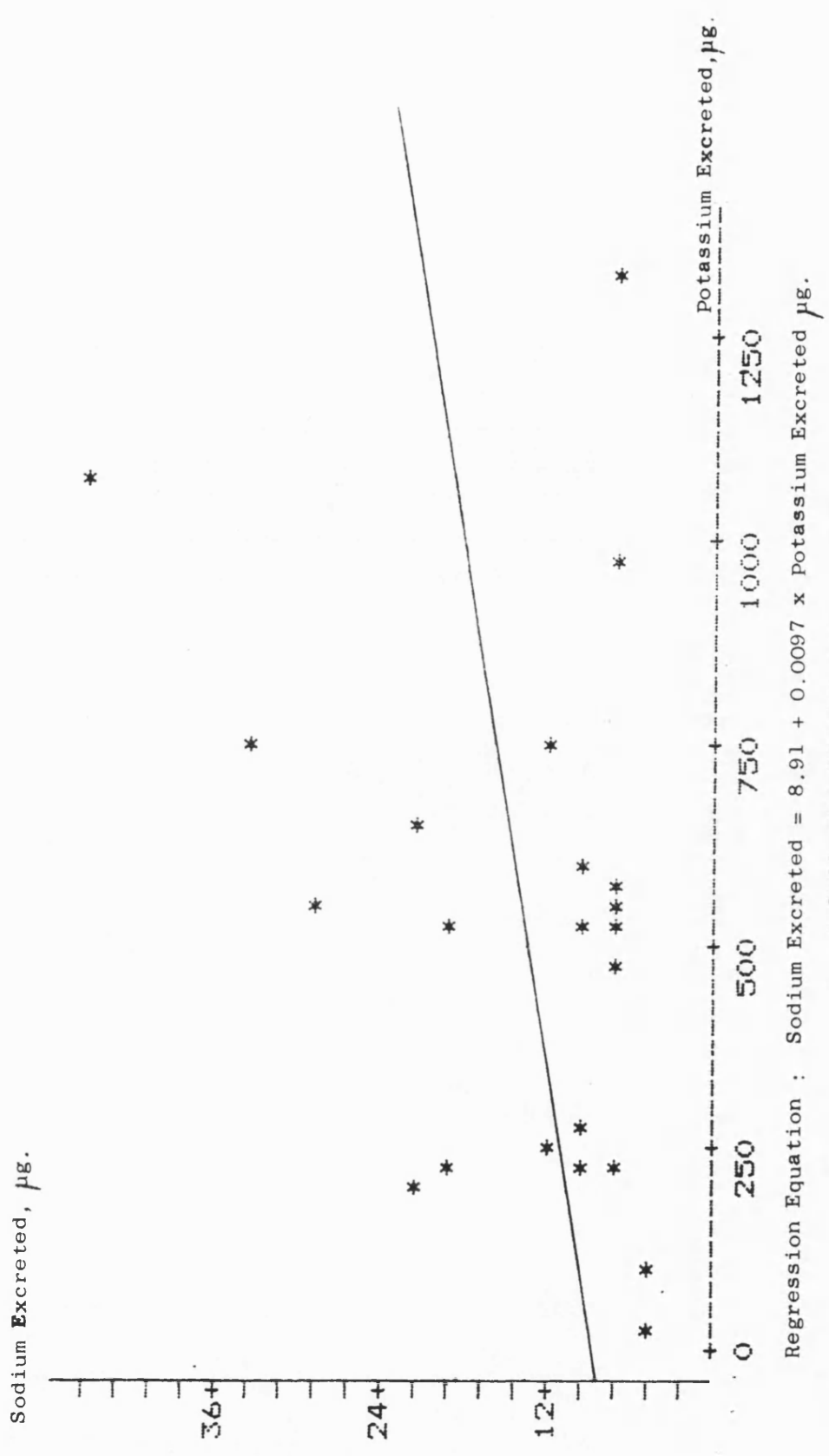
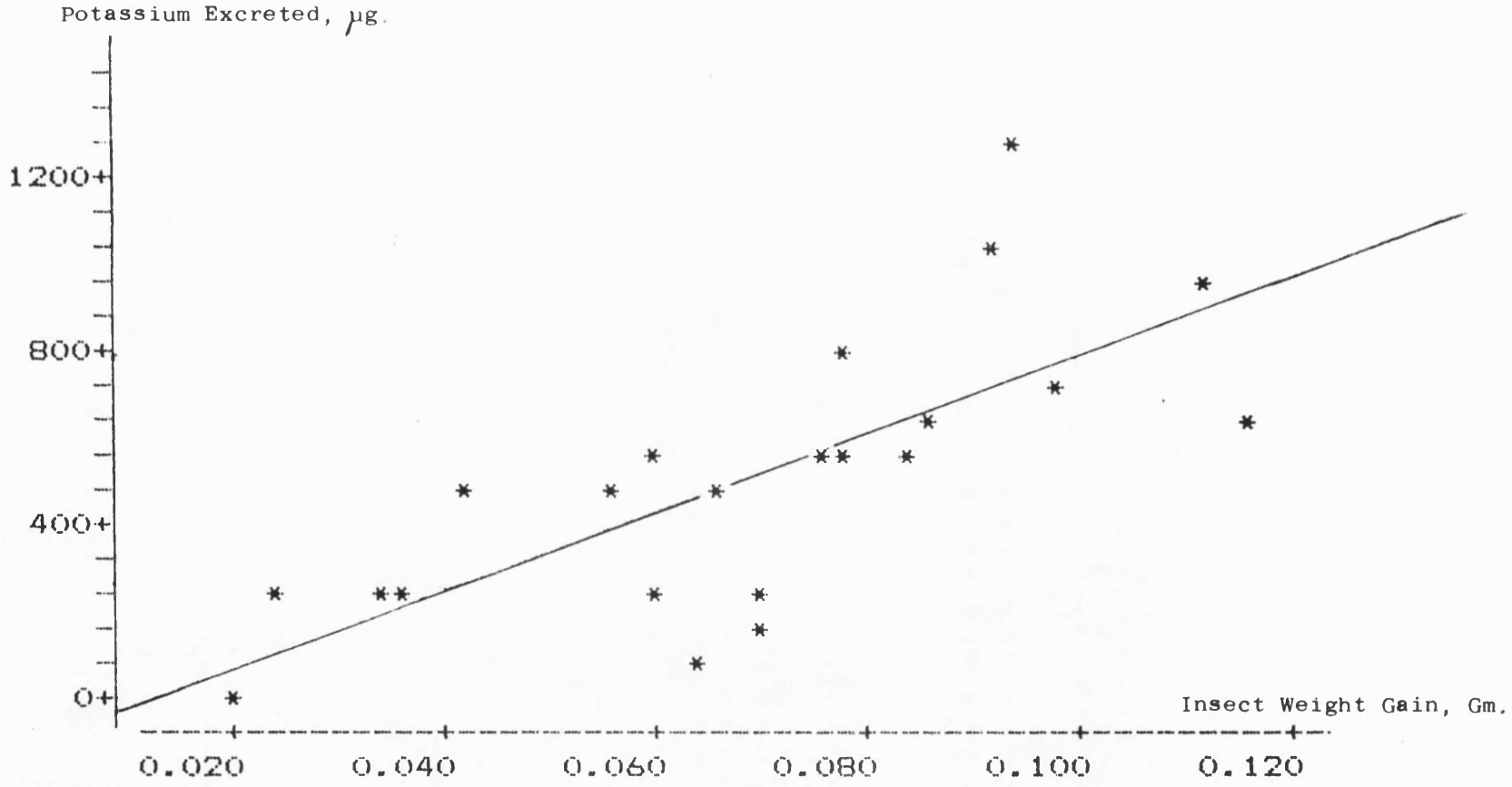


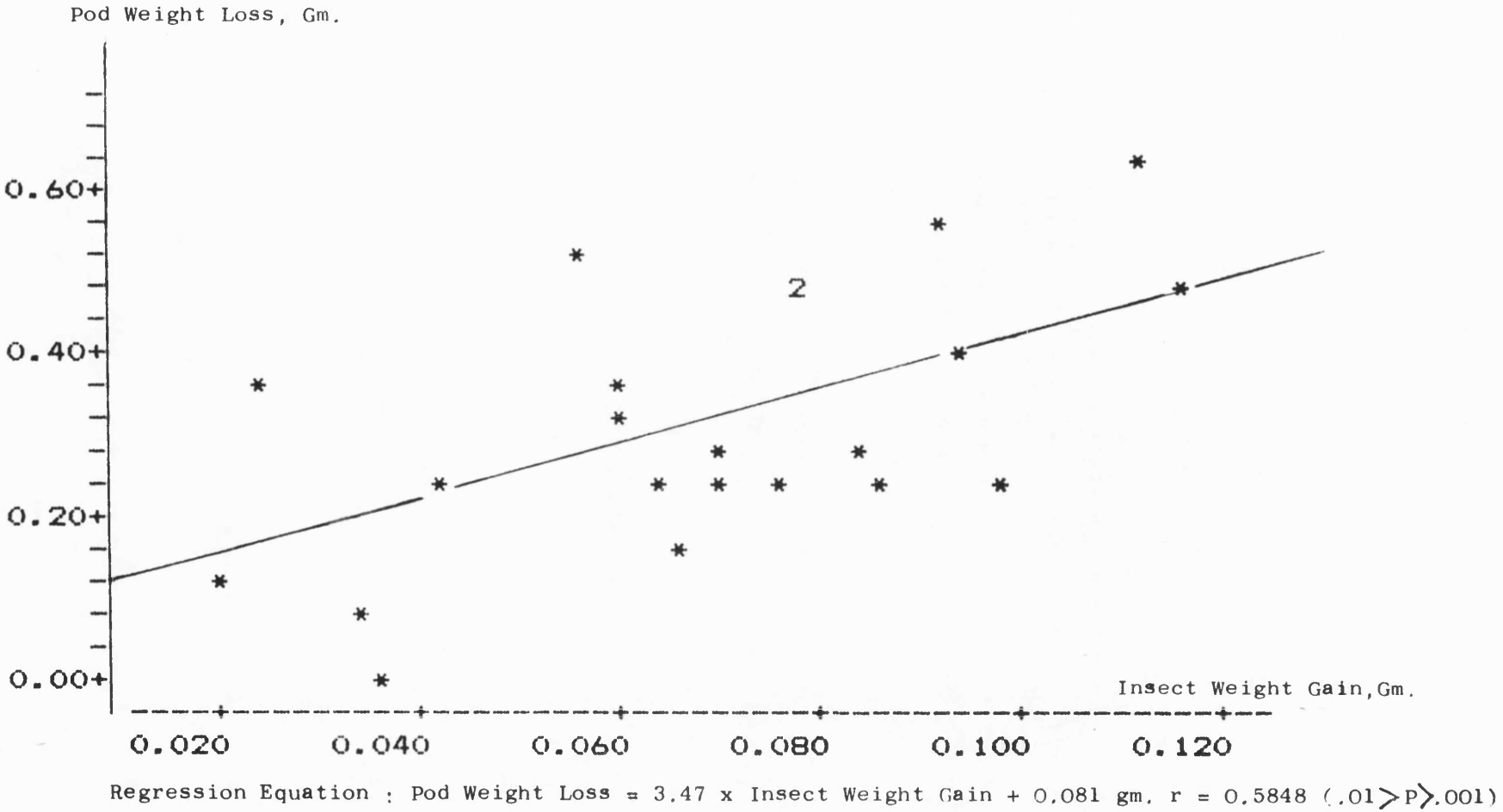
Figure 4. Graph of Sodium Excreted against Potassium Excreted (Pod Feeding Experiment).



Regression Equation : Potassium Excreted (µg.) = 8787 x Insect Weight Gain (gm.) - 88 µg.

r = 0.7197 P<.001

Figure 5. Graph of Potassium Excreted against Insect Weight Gain.



Regression Equation : Pod Weight Loss = 3.47 x Insect Weight Gain + 0.081 gm. r = 0.5848 (.01>P>.001)

Figure 6. Graph of Pod Weight Loss against Insect Weight Gain.

than in the haemolymph (mean = 0.869 µg./µl.). In the food, there are 280 mg./100gm.(= 2.80 µg./mg.). As was the case in the excreta analysis, the potassium deposited in the pod-feeding experiment is very much greater than the control. In the former, the control showed only a trace of potassium, and the higher level in the control of the latter may be due to the smearing of cut ends of pods against the wall of the dish. It will be noted that the figures for potassium in the excreta and in the food are very close. After allowing for experimental error, for the fact that concentration in the ingesta may be higher than the published figure for the whole bean (which includes insoluble fibre), and for loss of water from the insect by evaporation, it may be concluded that most of the potassium ingested is excreted, in contrast to the retention of sodium.

In the graphic analysis of the relation between potassium deposited and insect weight gained (Figure 5), the regression is highly significant. It seems to indicate, however, that when weight gain is zero, potassium deposition is negative, to the extent of 88 µg. This could be explained as the result of a systematic underestimation of the magnitude of the potassium deposited, namely that adhering to the pods. Although in most replicates there were no visible deposits on the pods at the conclusion of each experiment, thin dried films would not be detected. It will be noticed that in the one case where visible deposits of excreta were seen (Replicate 6c), the measured amount of potassium deposited in the dish was correspondingly low (112 µg.). The amount left on the pods would vary from one replicate to another, but the 88 µg. could be taken as the average over all, and if this were to be added to each of the figures for potassium deposited, the graph would pass through the origin. Although such underestimation of total potassium must inevitably occur, other interpretations of Figure 5 are possible. One is that the insects do not deposit excreta until an average weight gain of 10 mg. has been achieved. On the face of it, this is plausible. However, the usual habit of these insects is to retain a full rectum during starvation, and to excrete before commencing to feed. The insects were not starved before the experiment, and although in principle an insect must feed before it can excrete, the effect would be cancelled out by others which excrete, having fed elsewhere.

A third possibility is that the relationship between excreted potassium and weight gain is not linear. This model could only be verified by means of an experiment where all deposited potassium was measured.
The comparison of insect weight gain and pod weight loss which is plotted in Figure 6, also shows a highly significant regression. In this case, the regression line crosses the vertical axis in such a way as to indicate a positive amount of feeding at zero weight increase. This amount, 0.081 gm. wet weight of bean pod, probably represents basal, or maintenance, metabolism of the insects (in most replicates, three 5th.instar) for three days. Paul & Southgate (1978) give the calorific value of raw runner bean pod as 114 Kj/100gm. The maintenance requirements of each larva for one day will therefore be =(0.081 x 1.14)/9 Kj = 0.01026 Kj. In fact it is probably less than this, because the water content of the ingested material is likely to have been greater than the overall value for the whole pod, since a substantial part of the pod's dry matter consists of water-poor fibre.
The parameters by which the efficiency of food conversion is measured are Efficiency of Conversion of Ingested Food (ECI), which is Weight Increase x 100 divided by Food Intake, and Efficiency of Conversion of Digested Food (ECD), which is Weight Increase x 100 divided by Food Intake less Faeces. In most insects, feeding by biting and chewing, the faeces amount to a significant quantity, and ECD is much higher than ECI. Calculations can be based on dry weight determinations or on calorific value. In order to extract these parameters from the data in Table II, a number of corrections and approximations need to be made. All weights are wet weights (except cation determinations). From Paul & Southgate (1978) we know the water content of bean pod as 91.6% (if fibre is excluded), and we assume a water content of 85% for insect tissues (in line with figures given by Reynolds et al.,1985). The matter of faeces, small though the amounts are, can be estimated by assuming that certain ions, phosphate and magnesium in particular, are present in the same proportion to potassium as their occurrence in the food, and that nitrogenous excretion is similar to that of D.fasciatus as given by Berridge (1965). A further correction is applied for the assumed 88 µg. of potassium not measured, and then a back correction subtracting a proportion of the weight of faeces from the final pod weight (or rather, adding it to the figure for weight loss).
The faecal mass works out at potassium 591.9 µg., phosphate 304.7 µg.

magnesium 56.8 µg., and of nitrogenous material allantoin 111 µg., amino acids 48 µg., and urea 21 µg. In D.fasciatus, Berridge (1965) found that 13.4% of nitrogenous excreta was unidentified substances or mucopolysaccharide. This would add a further 27.8 µg.
The grand total of faecal output of 1161.2 µg. To allow for the small amounts of other ions (sodium, chloride, bicarbonate) and organic materials, this could be rounded off to 1200 µg. or 1.2 mg.
The relevant figures can now be listed, as follows -
Food Intake, dry weight, corrected for faeces left on pods = 8.4/100 x (0.320 + 88/592 x 0.0012) gm. = 0.0269 gm.
Weight Increase = 15/100 x 0.0687 = 0.0103 gm.
Therefore, ECI = 0.0103/0.0269 = 38.31% and ECD = 0.0103/(0.0269 - 0.0012) = 40.1%.
These results will be discussed in a later section, but it can be stated that they fall within the range of other kinds of phytophagous insect for which this information is available.
As a check on the consistency of the results, it is interesting to calculate the volume of liquid excreta produced during the feeding experiment, using two independent methods. One is to divide the potassium deposited (corrected for that left on pods) by the mean potassium concentration in the excreta, i.e. 591.9/2.947 = 200.8 µl. The other is to deduct the water content of the insect weight gain from the water content of the pod weight loss, i.e (0.320 x 0.916) - (0.0687 x 0.85) = 234.7 mg.(approx. 234.7 µl.). Allowing for loss of water by evaporation, which would reduce the first figure, there is a satisfactory level of agreement. In terms of individual fifth-instar larva, it means that they discharge about 22 µl. per day (about 9 globules). Data for evaporative loss by P.prasina are not available, but adult Dolycoris baccarum were found to lose 12 µl./day when starved in summer (but only 2 µl./day in autumn). Berridge (1965) reports water loss of between 4 and 10 mg. per day in fifth-instar D.fasciatus.

THE ULTRASTRUCTURE OF THE MID-GUT OF D. BACCARUM

The mid-gut of pentatomomorph Heteroptera forms the major section of the alimentary canal, and is divided into four distinct regions (Plate 1) Anteriorly, there is a large sac-like expansion, followed by a tubular region which leads to a second expanded bulb. Posterior to that is the region which in most species bears the gastric caeca, and finally the compact excretory vesicle. In larval stages of many (perhaps most) Pentatomidae and in larvae and adults of a few other species, the gut is discontinuous between the second bulb and the gastric caecal region, and there is a distinct swelling of the latter at its anterior end, just posterior to the discontinuity. An abbreviated designation of gut regions is commonly adopted by authors on the subject, anterior sac being M1, tubular region M2, second bulb M3 and caeca-bearing tube M4. The excretory vesicle is not reckoned in this scheme, but may sometimes be referred to as the ileum. The bulb connected to the caecal region has not been referred to by other authors, who have either not noticed it, or it has not been relevant to their work. I refer to this as the M4 bulb, or M4B. The insects available for electron microscopy were two females and two males collected in June 1978, four females and three males collected in September 1978 and held in the laboratory, in the Palomena prasina culture box, until 3rd.November, and three adults and four 5th.instar nymphs collected in August 1979.

The insects were killed by decapitation and the alimentary canal rapidly dissected out in the insect's own haemolymph (two of the nymphs were dissected in saline, for comparison). The parts were fixed in ice-cold 2.5 % glutaraldehyde in cacodylate buffer (pH 7.3) for 2 hours, then post-fixed in 1 % osmium tetroxide in cacodylate buffer for one hour, dehydrated with changes of acetone and embedded in Spurr's resin. Sections made on a Reichert ultramicrotome were stained with uranyl acetate and lead citrate and examined in a JEM 100C electron microscope operated at 60 Kv. For light microscopy, 1 µm. resin sections stained with toluidine blue were used. The sections illustrated in Plate 2 were of a nymph of P.prasina, fixed in Heidenhain's Susa solution and embedded in paraffin wax, sectioned at 8 µm. and stained with Weigert's iron haematoxylin and eosin.

The mid-gut of Pentatomoidea as observed through the light microscope, like that of most insects, is composed of columnar epithelium, the cells approximately 25-50 µm. tall and 10-20 µm. wide. The tips are often slightly bulbous, with a brush border, and paired nuclei 10 µm. in diameter are situated towards the base. In M1, transverse ridges of epithelium extend into the gut lumen at the anterior end. Posteriorly, the cells become cuboid or even flattened, and this continues into M2 and M3. M4 is a narrow tube, in P.prasina the cells are only 6 µm.high and 4 µm. wide, and the lumen 7 µm. wide. The gastric caeca have walls only about 1 µm. thick except where the nuclei are situated. The cells of the excretory vesicle, under light microscopy, contain one or two very large vacuoles, and those of the rectum are mostly of "rectal gland" type, which in these insects means flattened with large single nuclei(Plate 2).

The ultrastructure of the insect mid-gut, as outlined by Martoja & Ballan-Dufrancais (1984), consists of cells with a microvillous apical border, a basal zone of infoldings which may be labyrinthine, a fine-grained uniform cytoplasm with small rounded mitochondria mainly situated near the apical and basal borders, and a few large lipoid inclusions. The different levels of the Pentatomid mid-gut exhibit these features in varying degrees.

In M1 (Plate 4), there is no apparent basal infolding, but a well developed apical border of microvilli. Lipoid globules are present, and also a number of clear vacuoles, smaller than the lipoid bodies, and some with membranous inclusions. The cytoplasm is of varied texture and electron density. Mitochondria are abundant near to the apical border and in the dense regions of the cytoplasm, but sparse towards the base. The less dense cytoplasm is more extensive towards the cell base, with many coarse granules, which might be identified as glycogen by comparison with other published work. There are clear vacuoles here also. The M1 region, and the two succeeding ones, M2 and M3, were examined in only a few of the specimens, so the differences may not be valid. However, in M2 there is a more distinct basement membrane (Plate 5)with small clear vacuoles near to it. Several lipoid globules are present in the middle of the cell, and lines of small electron-dense bodies (SG) run out towards the apical border. The latter is not evenly covered with microvilli, and there is evidence of secretion by the discharge of vacuoles. Mitochondria are less evident, seeming to have

been thrust aside by the secretory vacuoles. Micro-organisms (MO) can be seen in the cytoplasm, but the significance of these is unknown.(In comparing Plate 5 with Plate 4 or succeeding Plates, it must be borne in mind that it is at a lower level of magnification).
In the M3 region (Plate 6), the cell structure is similar to that of M2 in many respects. There is a thick sinuous basement membrane (BM) with vacuoles most abundant in the basal part of the cell. Membranous whorls can be seen in some of the vacuoles. Elongated or dumb-bell shaped mitochondria are mainly found near the apical border, which bears many well-developed microvilli. Lipoid spheres are not apparent, but some electron dense, possibly pre-secretory, granules can be seen. In the lumen of the intestine, there is a layer of fibrous secretion, and many of the caecal bacteria (this is an adult insect, in which the midgut has become continuous). In Plates 5 and 6, the tortuous nature of the intercellular junction (IJ) is well shown, and the occasional small vacuole can be seen actually interrupting the cell junction.

Plate 7 depicts the structure of the M4 bulb, an organ which is only recognisable in the immature insect (e.g. Plate 1). There is a strong impression of great metabolic activity, mitochondria are abundant in both the apical and basal regions of the cells, and vacuoles and lipoid spheres are present. The cytoplasm is densely granular, and there are stacked sheets of rough endoplasmic reticulum. There is a distinct basement membrane and the apical border bears many microvilli. Fibrous secretion is present in the lumen.
The small columnar cells of the M4 central tube (Plate 8) are the first, as one proceeds posteriorward along the gut, to show signs of basal infolding, but most characteristic of this region are the long intercellular canals between the basal parts of the cells. The apical parts contain some vacuoles, and the intercellular membrane follows a path of great complexity. The short microvilli of the apical border seem to extrude threads of the fibrous secretion from the spaces between them. The secretion seems to entangle caecal bacteria. In the preparations studied, not many mitochondria can be detected. In the gastric caeca (Plate 9A) cells, there is a thin basement membrane like that of M1, a few small vacuoles and mitochondria, and the apical membrane seems to lack microvilli but is indented by the mass of bacteria. In places it appears to break down, so that bacteria invade the cytoplasm. There is no sign of anything which could suggest a major water transport

role for these cells. The most posterior part of the mid-gut, the excretory vesicle, is subject to considerable changes in volume, being greatly swollen in some of the insects dissected, and much less so in others. At present, the exact relationship of these changes to the moulting or feeding cycle is not known. Material for light microscopy was fixed by removing some part of the insect's cuticle (to facilitate penetration), and immersing in fixative without dissection. This shows (Plate 2) the organ in a state of distension, whereas for electron microscopy the parts have to be dissected into small enough pieces before fixation, and in doing so their fluid content is released and the organ shrinks. This capacity for stretching shows up under electron microscopy as short intercellular junctions with lobes extending both into the lumen and basally into the haemolymph. Other parts of the alimentary canal, notably the M1 region and rectum, may be observed on dissection to be filled or empty, but while they may appear collapsed, contraction of the wall is much less evident.

Plates 9B to 13 show the characteristic features of the cells lining the excretory vesicle. The tortuous intercellular junction is present here as before, the microvillous apical border descends into deep clefts which do not correspond to cell boundaries, the basal infolds are deep, labyrinthine with many sinuses and cisternae, the basement membrane much folded (and appears to be thinner in nymphs than in adults). In some adults the apical border seemed to lose its villi and the corresponding layer of mitochondria. Mitochondria generally are small, rounded or irregular rod-like, peripherally distributed but not to any great extent into the basal infolds. In the 5th.instar nymphs, very large vacuoles are present in the cells. Probably because of the extensibility of the cell layer, the serosal membranes are folded in a complex manner in the electron micrographs. Well developed muscles and abundant tracheoles are found on the haemolymph side. A further regular feature is the variable electron-density between cells (e.g. Plate 11). In the adults captured in autumn and maintained in the laboratory culture, the basal infolds were not distended with fluid and the protuberant cell bases closed up, so that a double layer of basement membrane seemed to run up into the cell layer (Plates 12B,13).

The rectum (gland) cells are flattened except at the position of the nucleus. They have a chitinous intima on the lumen side, beneath which are many infoldings of the cell membrane, Betwen these are elongated

mitochondria, which are also found closely surrounding the nucleus. The apical infolds form strings and clusters of subspherical cisternae (Plate 14). These features are shown at a higher magnification in Plate 14A. Some difficulty was experienced in preparation of the rectum material, due to the resin sections splitting along the line of the chitinous intima. Malpighian tubules (not illustrated) seemed to be as found in other insects, somewhat flattened, domed cells, with microvilli on the apical border, about one-quarter of the maximum cell depth, and closed parallel basal infolds of the same depth as the microvilli. The cytoplasm was uniformly granular, with a number of small vacuoles.

PLATES 1 - 14

(Between pages 34 and 35)

KEY TO LABELLING OF PLATE 1

M1 - Anterior sac of midgut.

M2 - Tubular middle section of midgut.

M3 - Posterior bulb of midgut.

M4 - Caeca-bearing region of midgut.

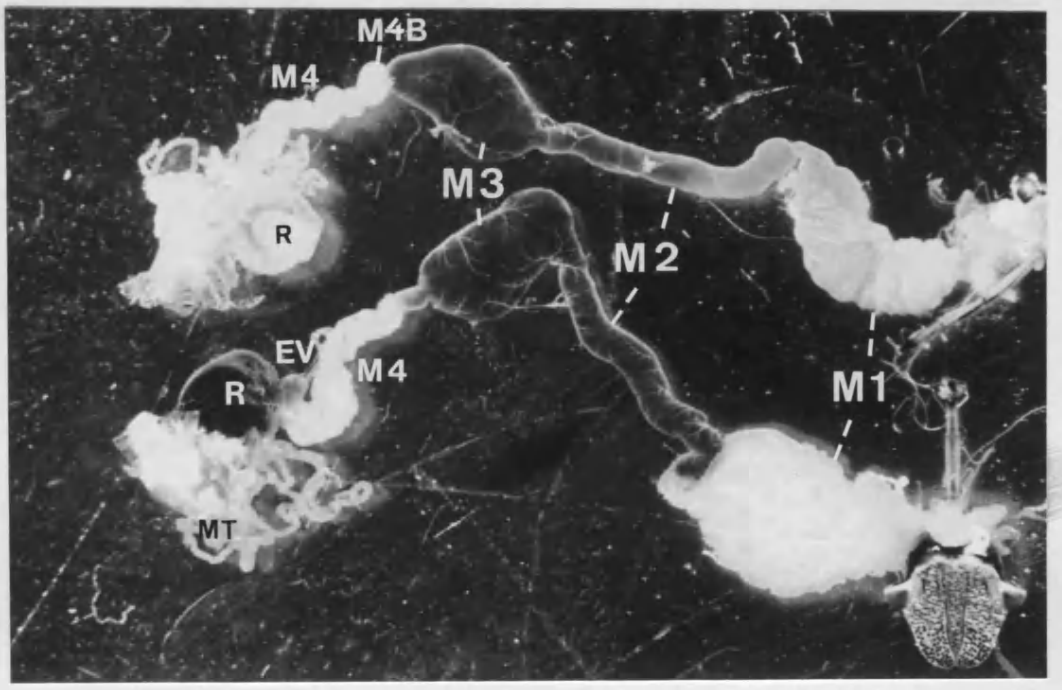
M4B - Bulb at anterior end of M4 (larva only).

CAE - Gastric caeca.

EV - Excretory vesicle.

R - Rectum (hindgut)

MT - Malpighian tubules.



4·0 mm
2·5 mm

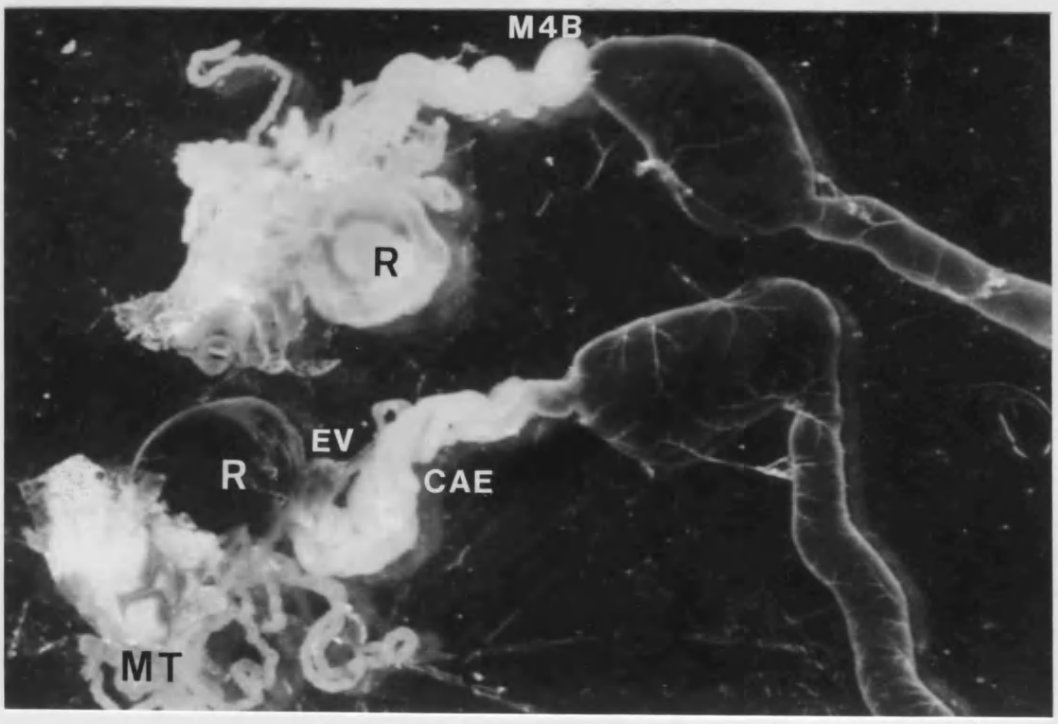
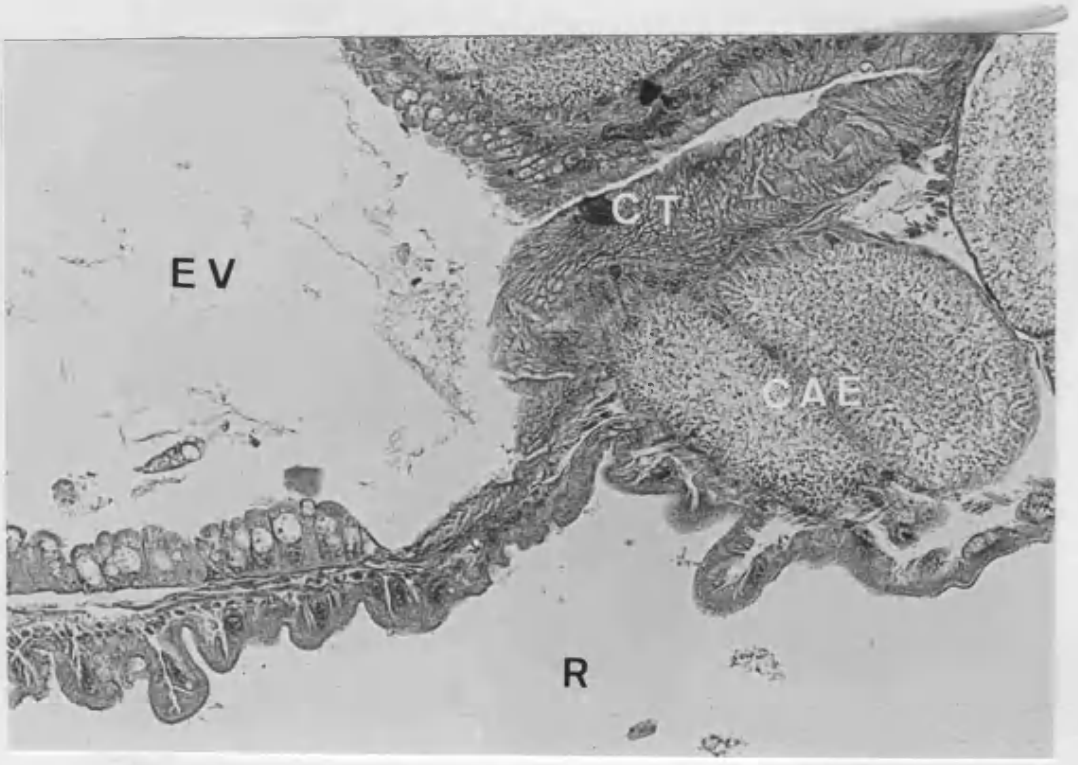


PLATE 1. Dissected midgut-rectum of Palomena prasina
Below - M2 to Rectum at higher magnification.
Upper specimen in each illustration is of a larva, lower is of a young adult. Note obliteration of M4B and dark material in rectum of latter.

For key to labelling, see opposite page



0·2mm

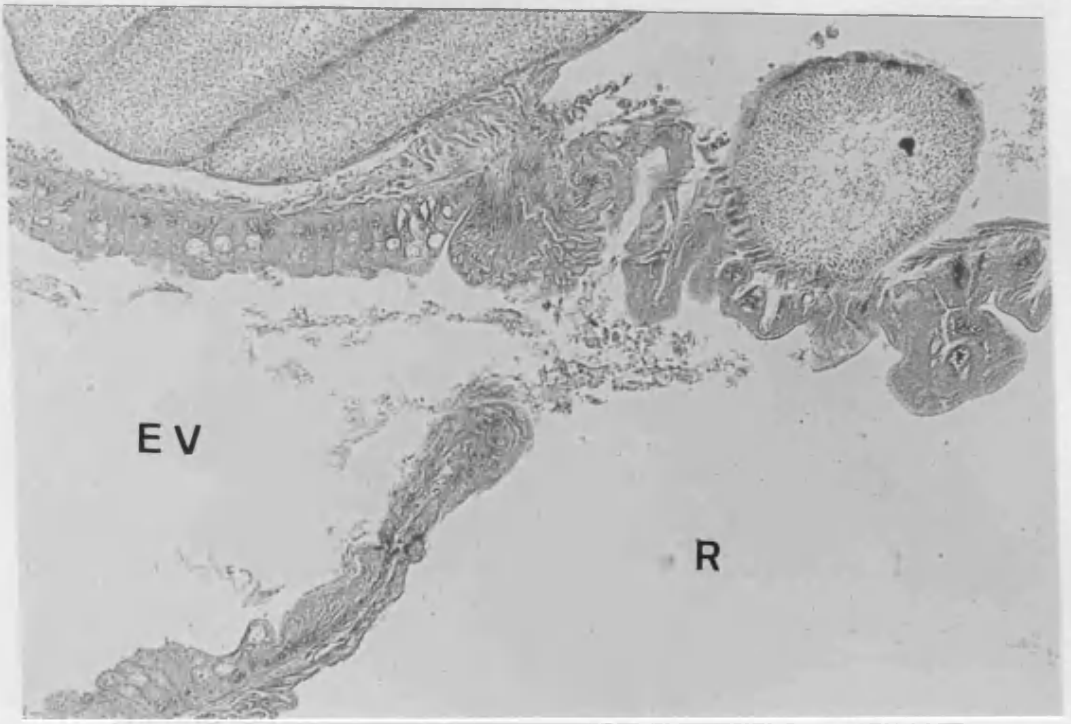


PLATE 2. Posterior midgut of Palomena prasina.
Above - section through junction of central tube of gastric caecal region with excretory vesicle.
Below - section through opening of excretory vesicle into rectum. CT = central tube CAE = caeca
EV = excretory vesicle R = rectum. Anterior of insect to left.

Approx. half natural size

PLATE 3. Sap-sucking tropical Pentatomomorpha.
Above - Mygdonia tuberculosa (Coreidae). Cluster of 3rd.instar larvae awaiting ecdysis, with group of feeding 4th.and 5th.instar larvae in background.
Below - Piezosternum calidum (Tessaratomidae), part of a large colony of immature adults, feeding prior to dispersal.

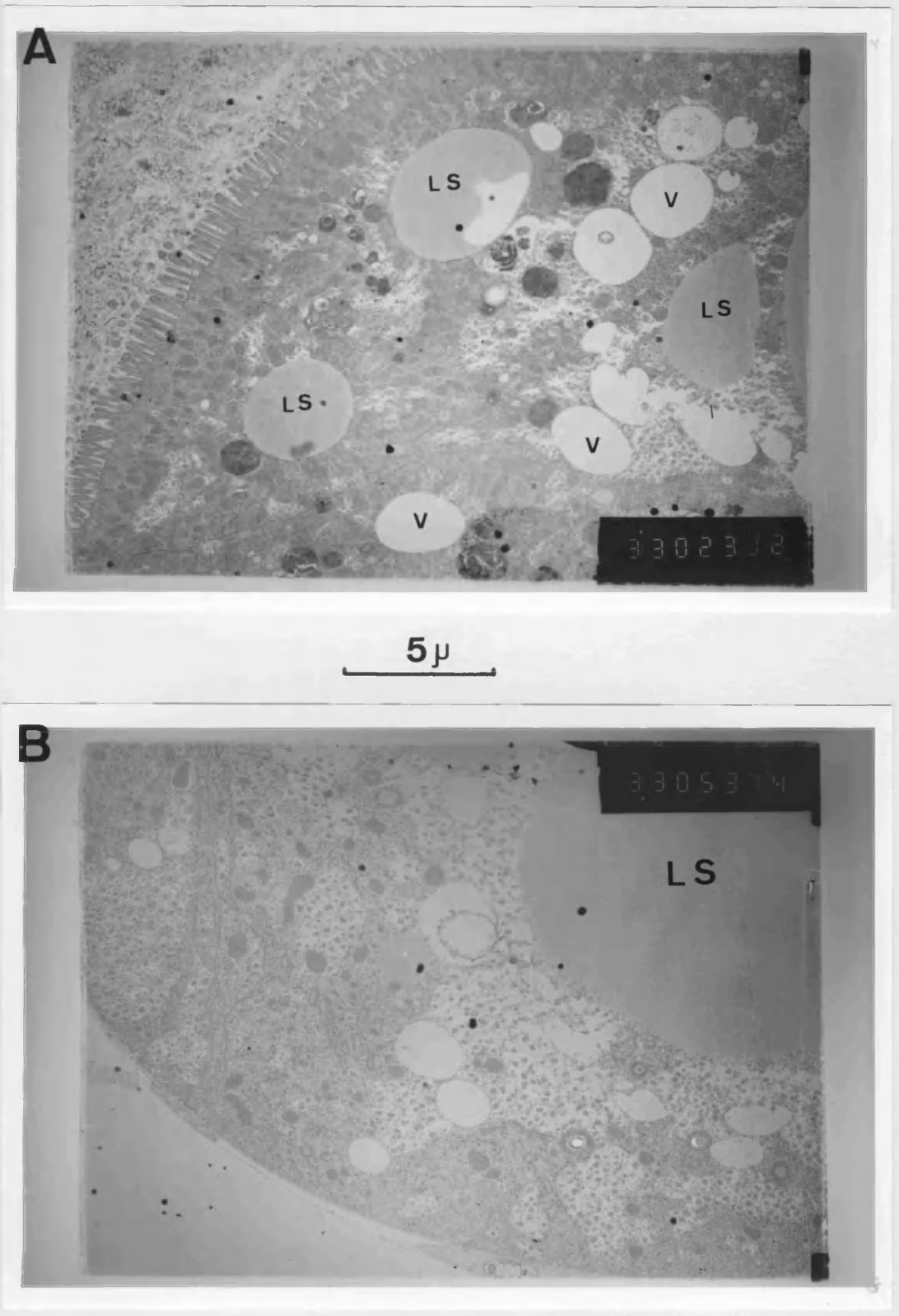
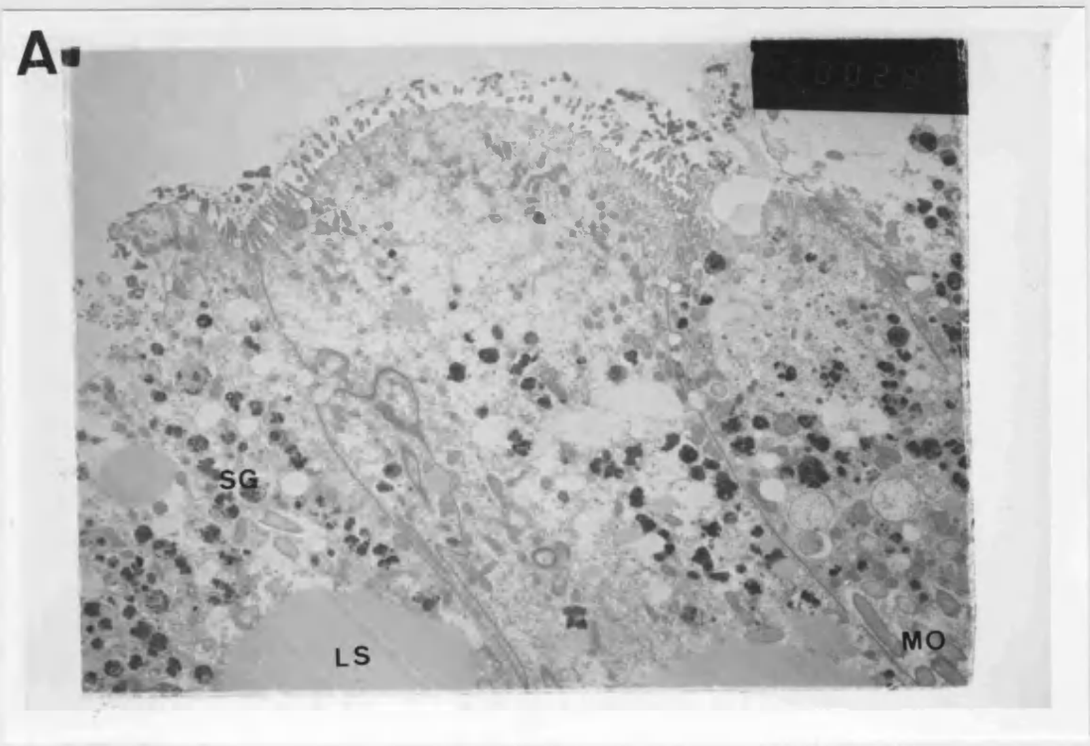


PLATE 4. Apical(A) and basal(B) regions of the cells of the anterior midgut(M1) of a fifth-instar larva of Dolycoris baccarum. For description see text. LS = lipoid spheres, V = vacuole.



10 μ

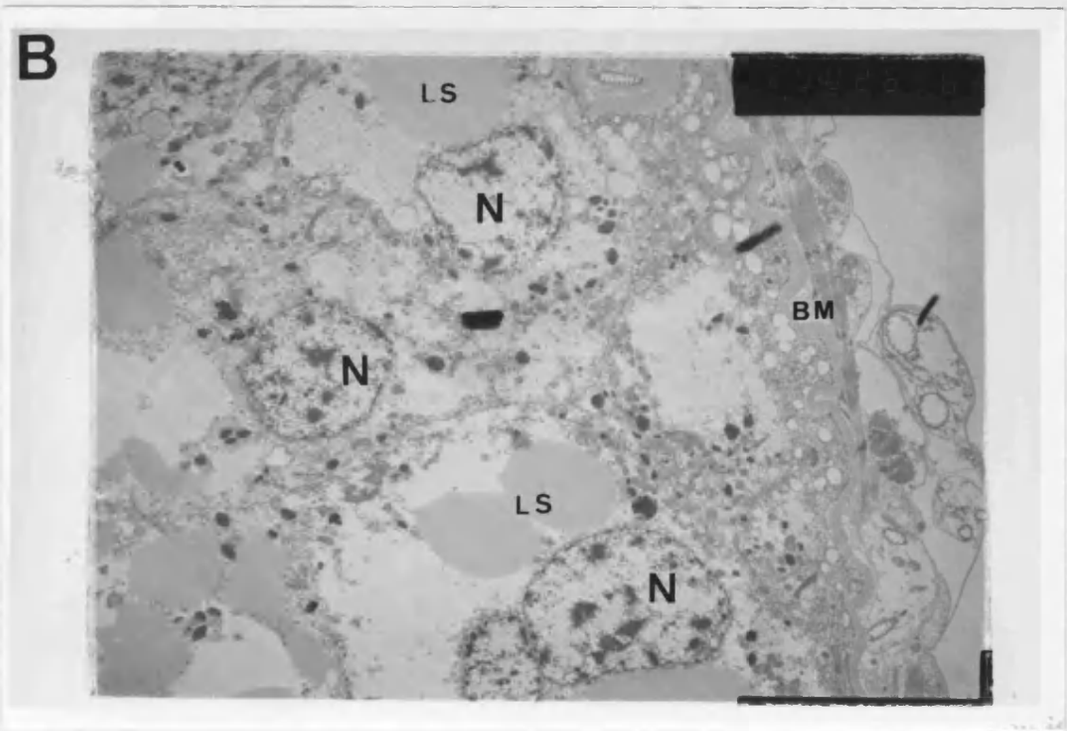


PLATE 5. Apical(A) and basal(B) regions of the cells of the tubular midgut(M2) of a spring-caught male of Dolycoris baccarum. For description see text.
LS = lipoid spheres, N = nuclei, BM=basement membrane
SG = secretory granules, MO = micro-organisms

PLATE 6

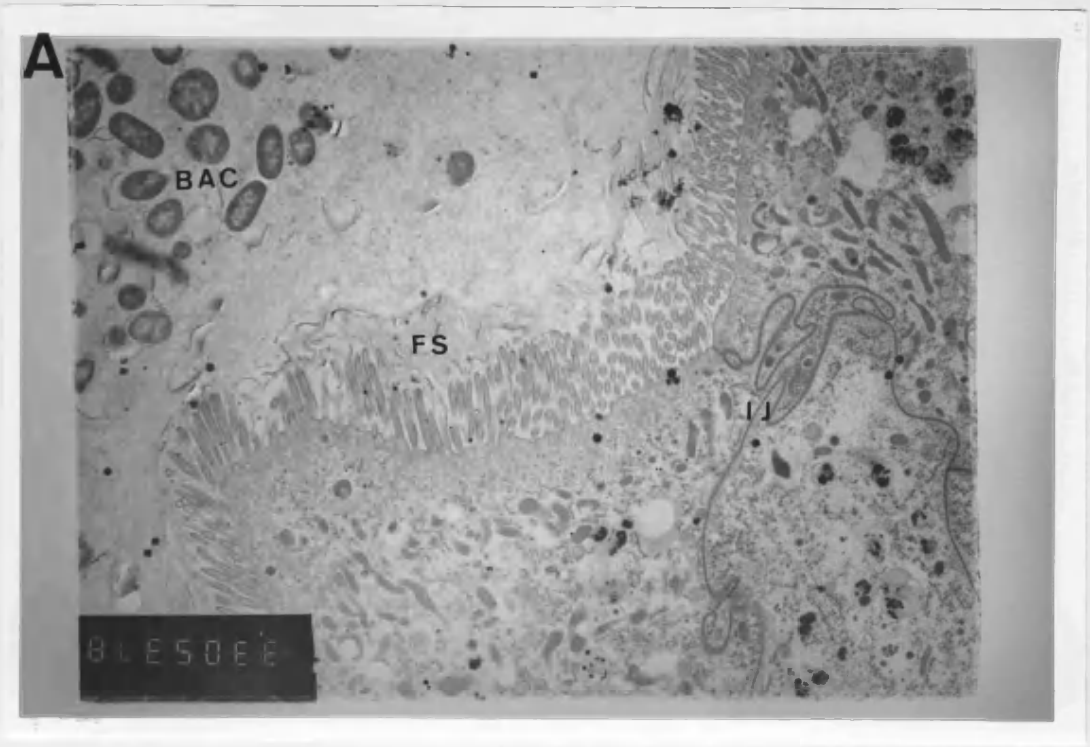


PLATE 6. Apical(A) and basal(B) regions of cells of the third midgut(M3) of a summer-caught female of Dolycoris baccarum. For description see text. BAC = bacteria, BM = basement membrane, MUS= muscles surrounding gut, N = nucleus.

FS = fibrous secretion, IJ = intercellular junction

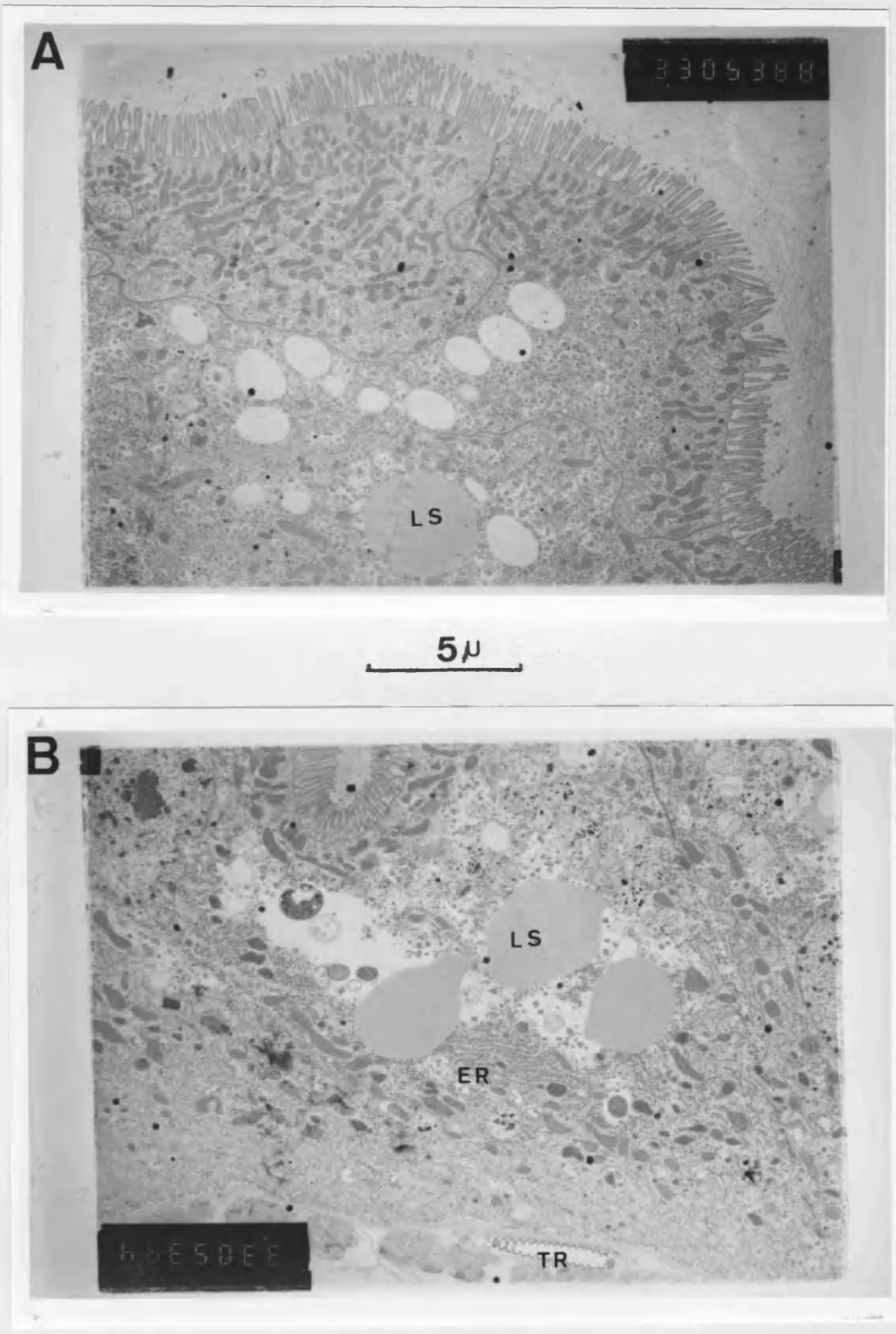
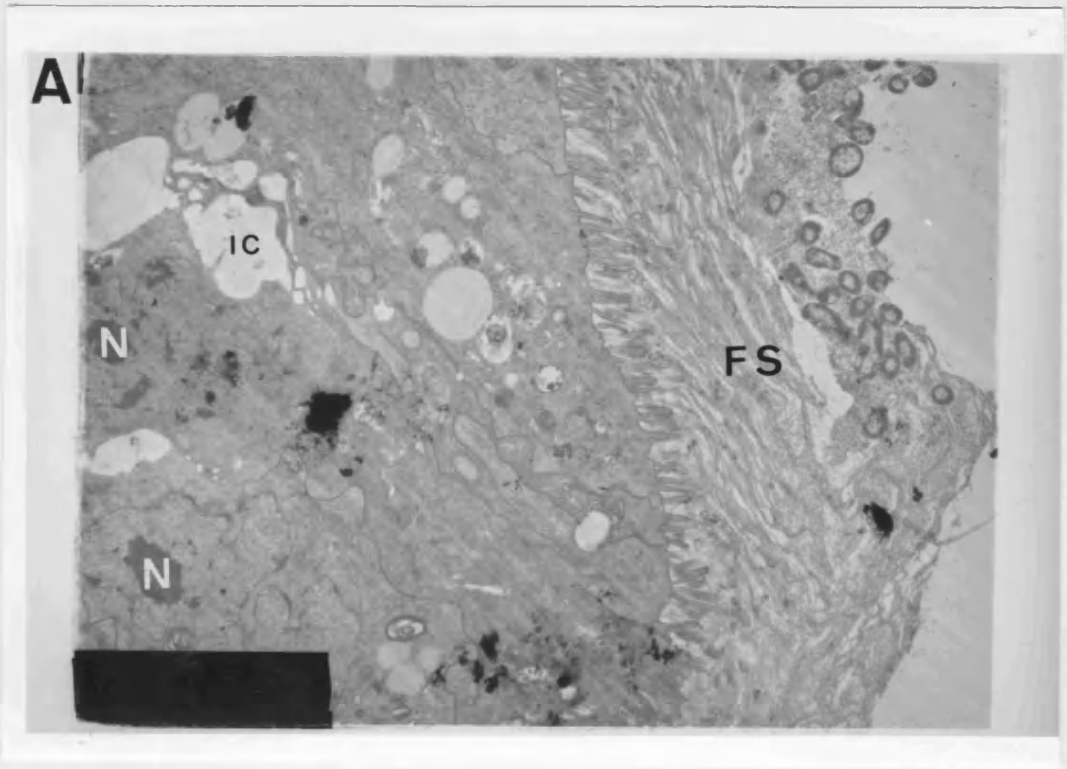


PLATE 7. Apical(A) and basal(B) regions of cells of the bulb anterior to the caecal tube(M4B) in a fifth-instar larva of Dolycoris baccarum. For description see text. ER = endoplasmic reticulum, LS = lipoid spheres, TR = trachea.



5 µ

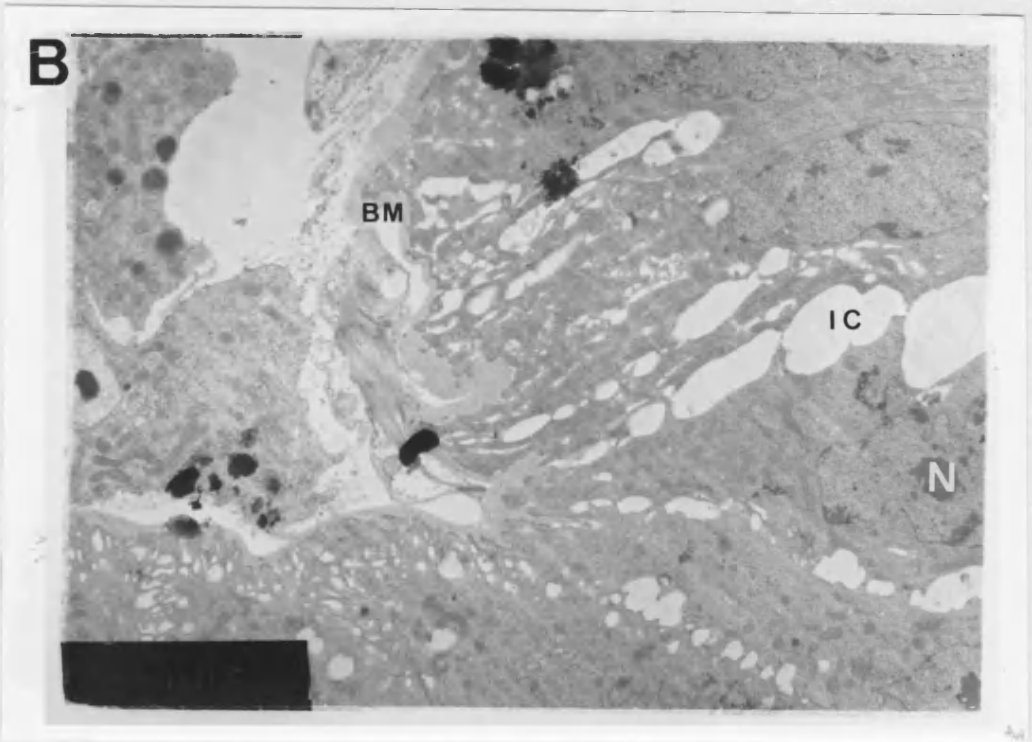
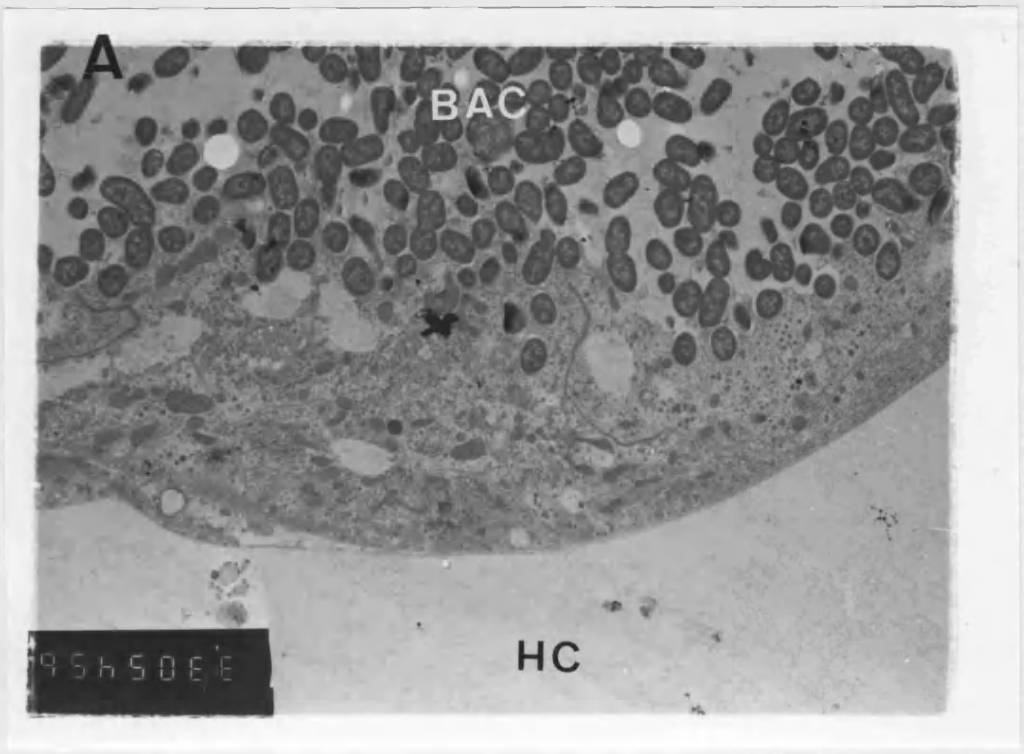


PLATE 8. Apical(A) and basal(B) regions of cells of the central tube of the caecal region of the midgut (M4CT) in a spring-caught male of Dolycoris baccarum. BM = basement membrane, FS = fibrous secretion, IC = intercellular channel, N = nucleus.



5μ

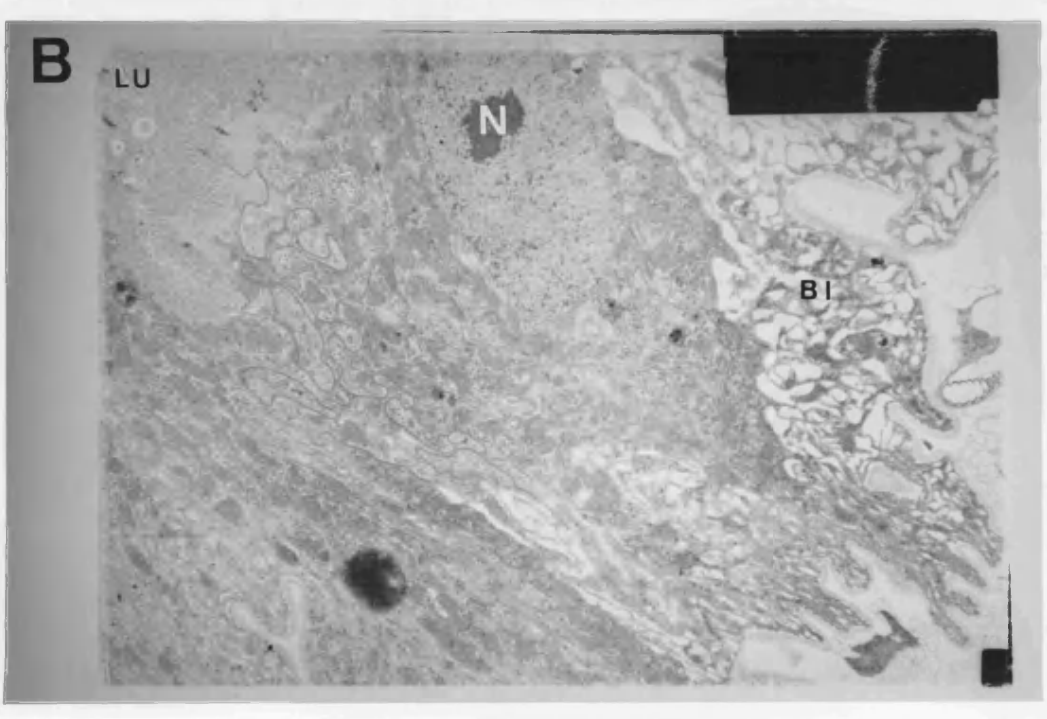
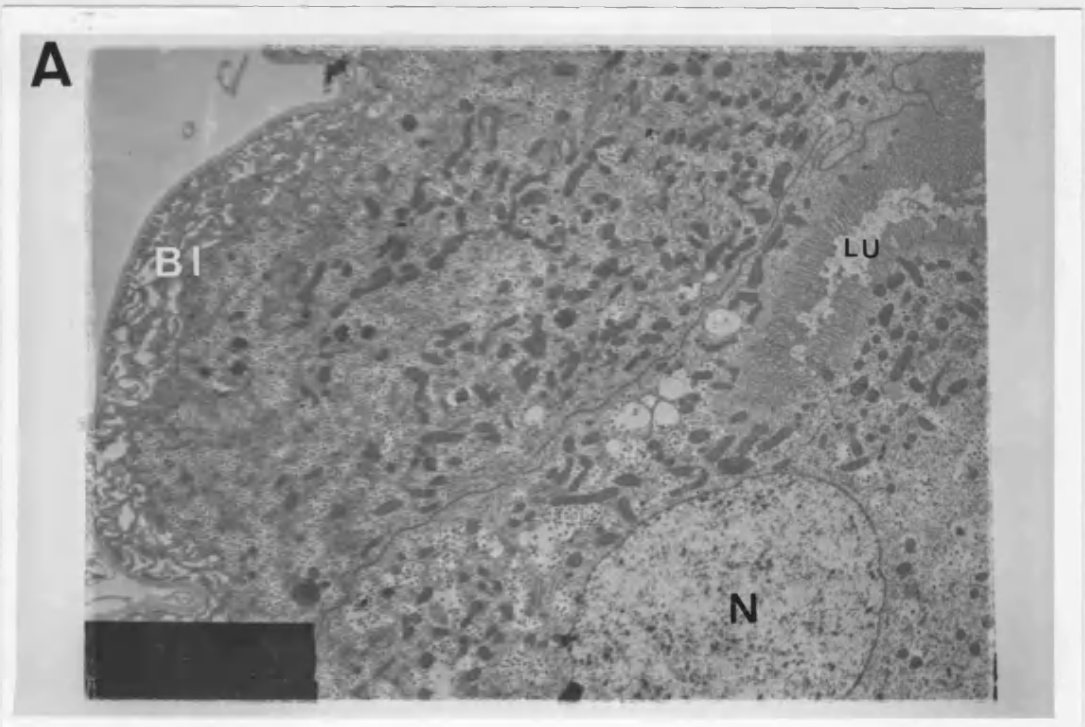


PLATE 9A. Wall of gastric caecum of a fifth-instar larva of Dolycoris baccarum. BAC = symbiotic bacteria HC = haemocoel.

PLATE 9B. Wall of excretory vesicle of a spring-caught female of Dolycoris baccarum. N = nucleus, BI = basal infoldings, LU = lumen of vesicle



5μ

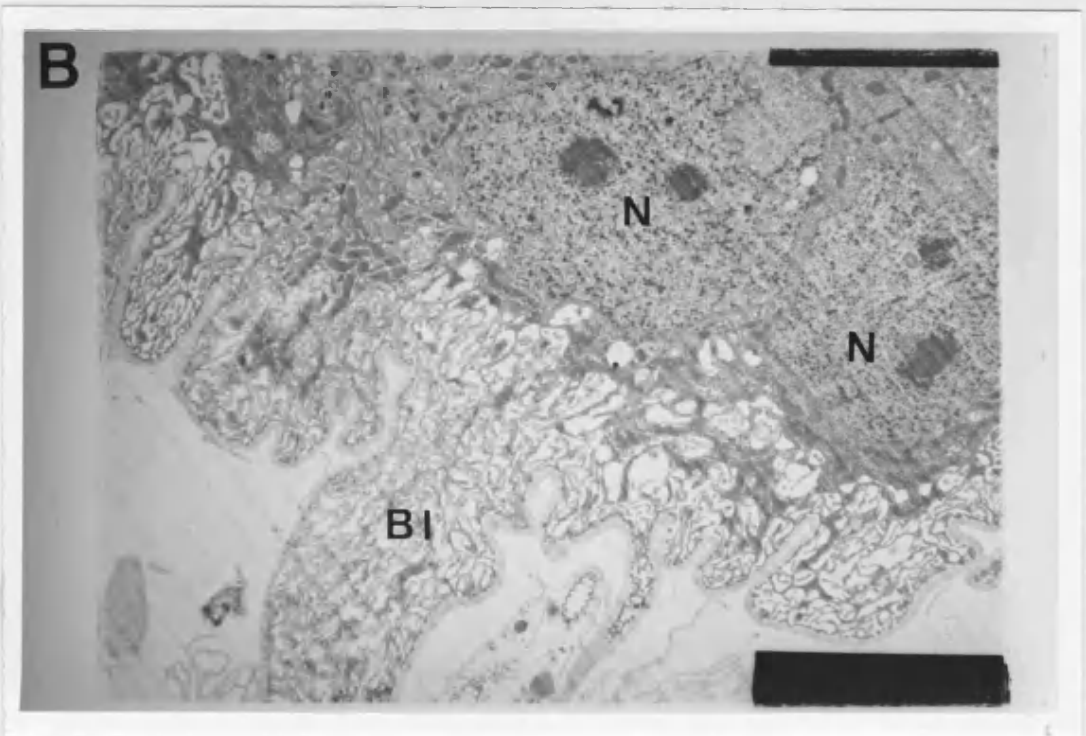
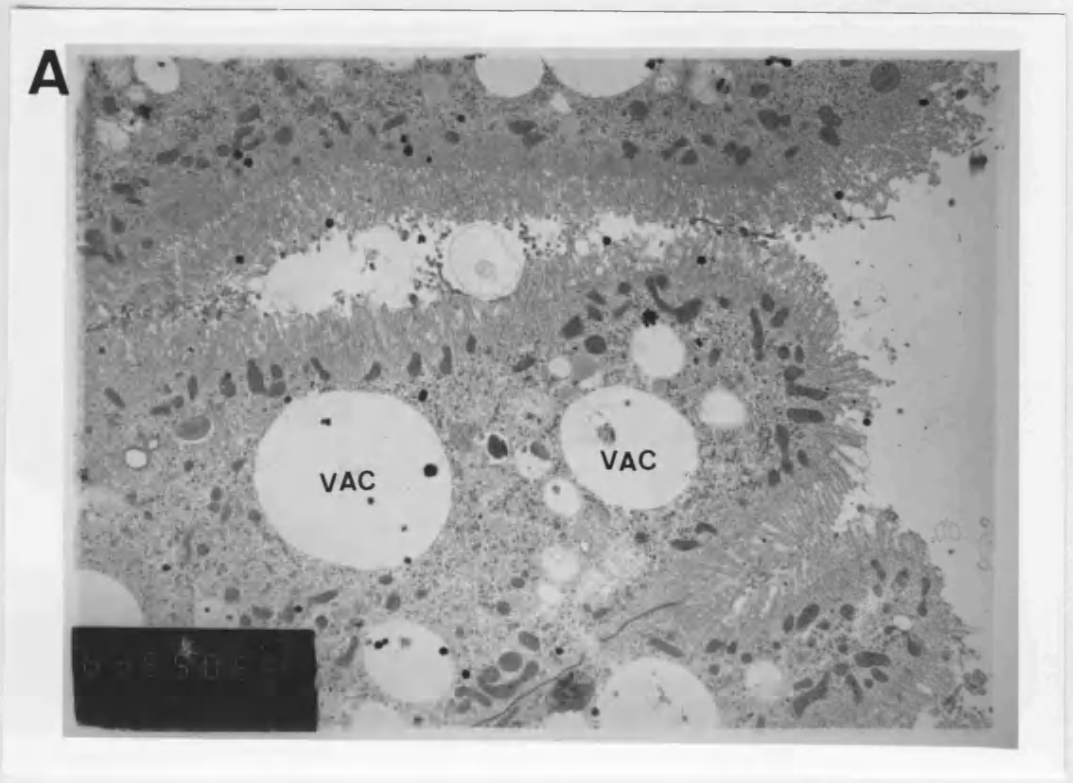


PLATE 10. Wall of excretory vesicle of spring-caught males of Dolycoris baccarum(in B, basal region only) BI = basal infoldings, LU = lumen of vesicle,N=nuclei.



5μ

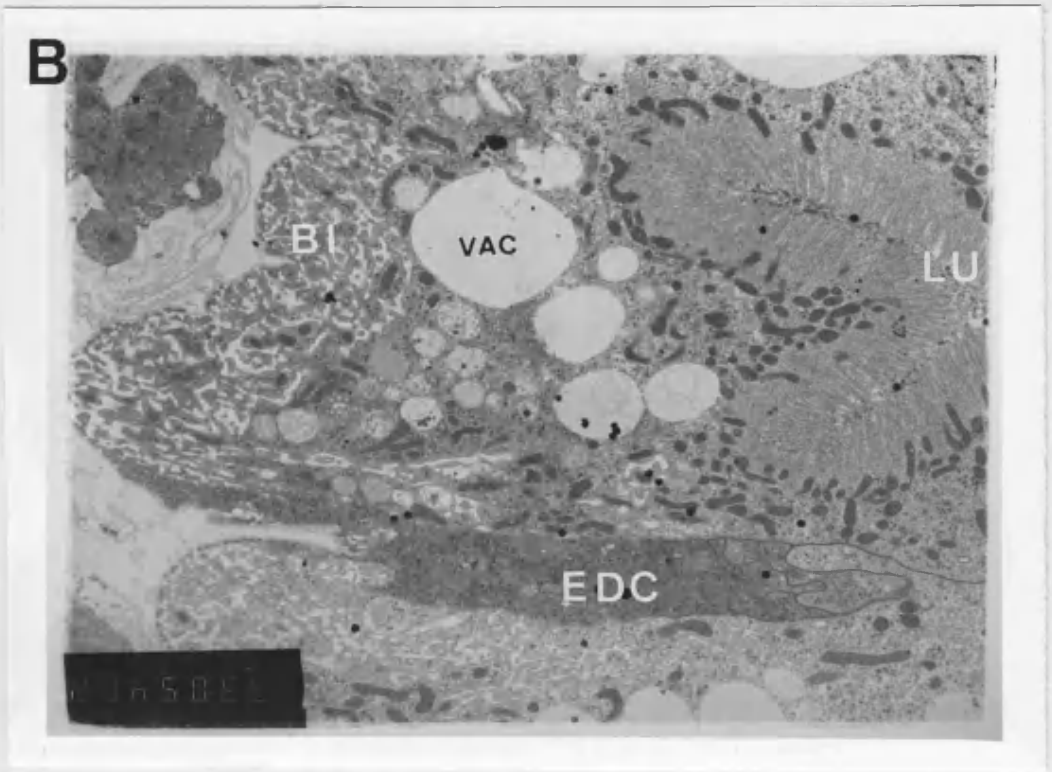
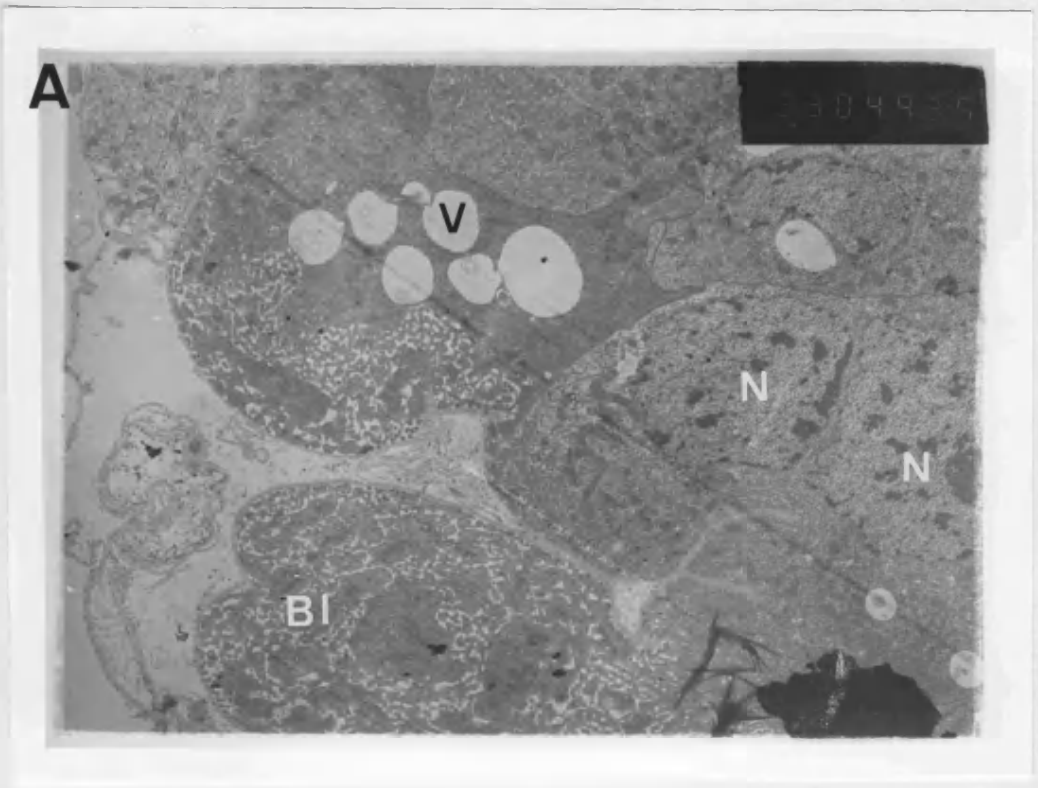


PLATE 11. Apical(A) and basal(B) regions of the cells of the excretory vesicle in a fifth-instar larva of Dolycoris baccarum. BI = basal infoldings, LU = lumen of vesicle, EDC = electron-dense cell, VAC = vacuoles



5μ

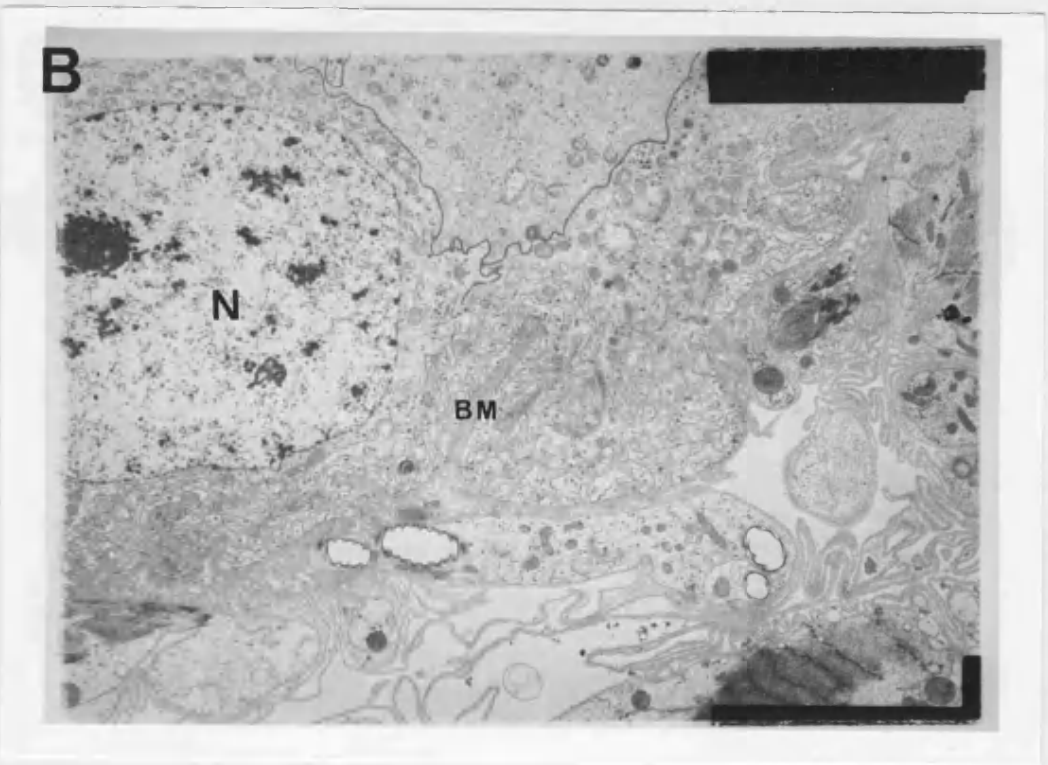
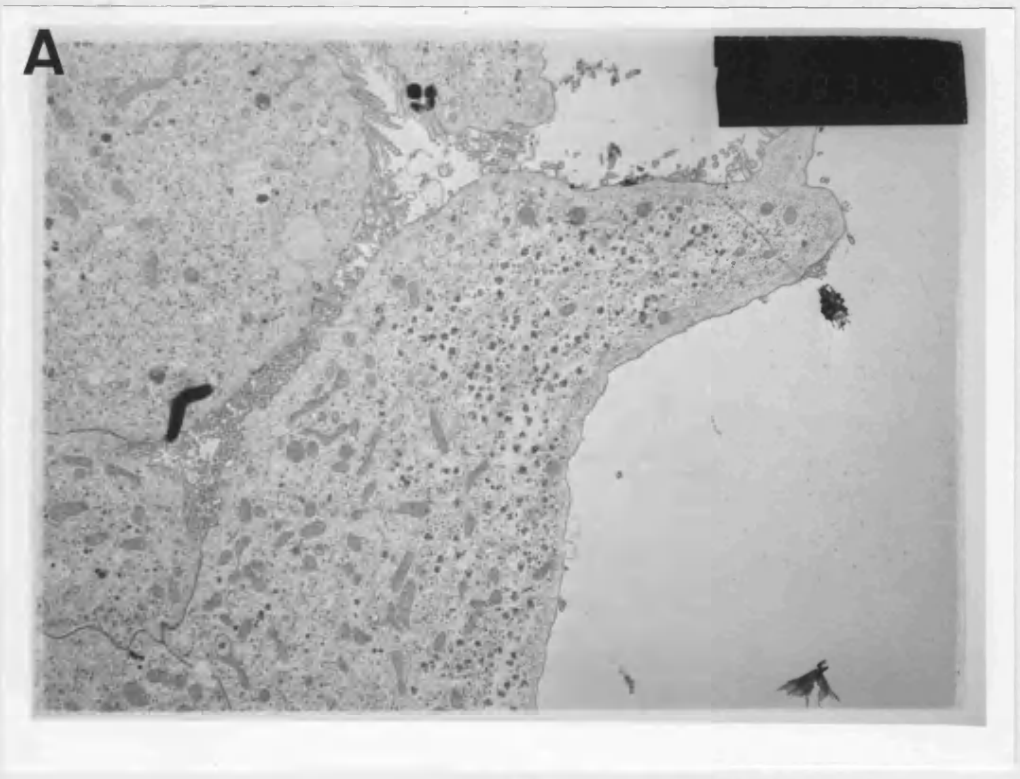


PLATE 12. Basal regions of cells of excretory vesicle of (A) unfed fifth-instar larva, and (B) autumn--caught male of Dolycoris baccarum. BI=basal infolds, BM = basement membrane, N.= nuclei, V = vacuoles.



5μ

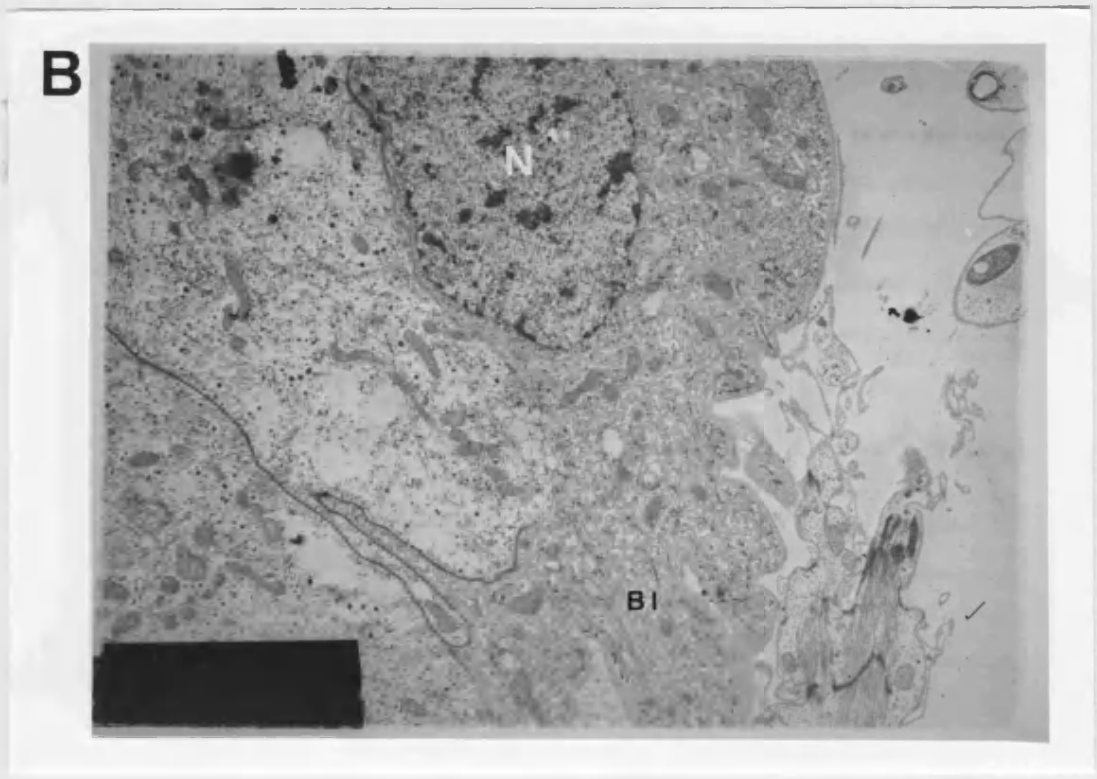
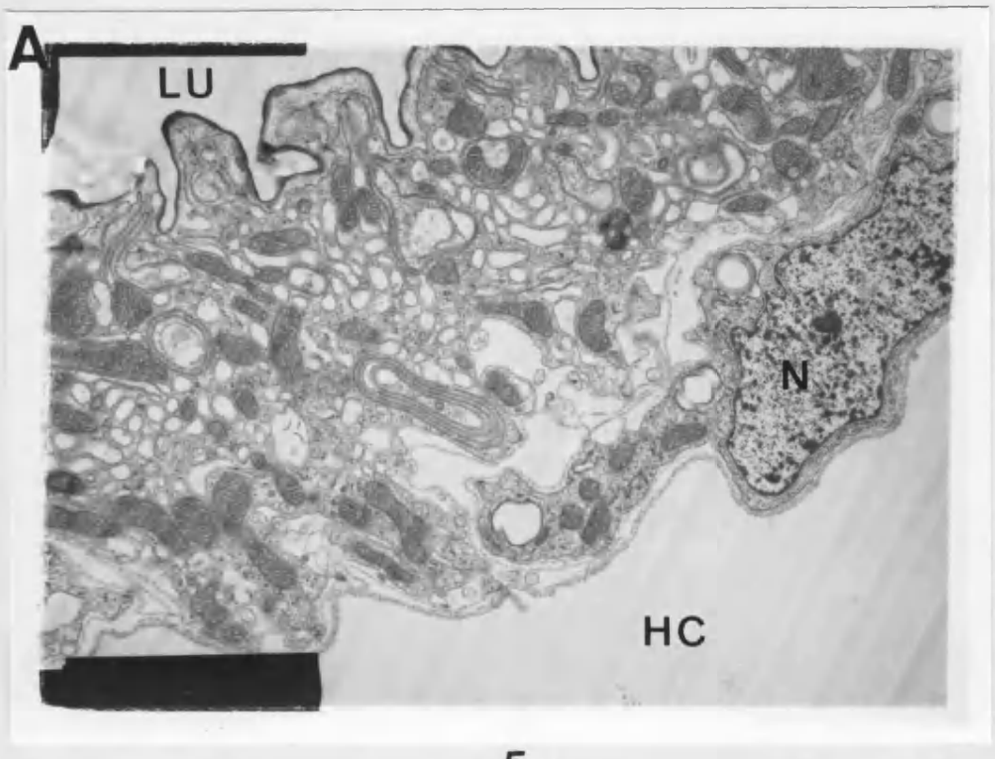


PLATE 13. Apical(A) and basal(B) regions of cells of the excretory vesicle of an autumn-caught female of Dolycoris baccarum, injected with water 30mins.before fixation. Note slight opening of basal infolds(BI).



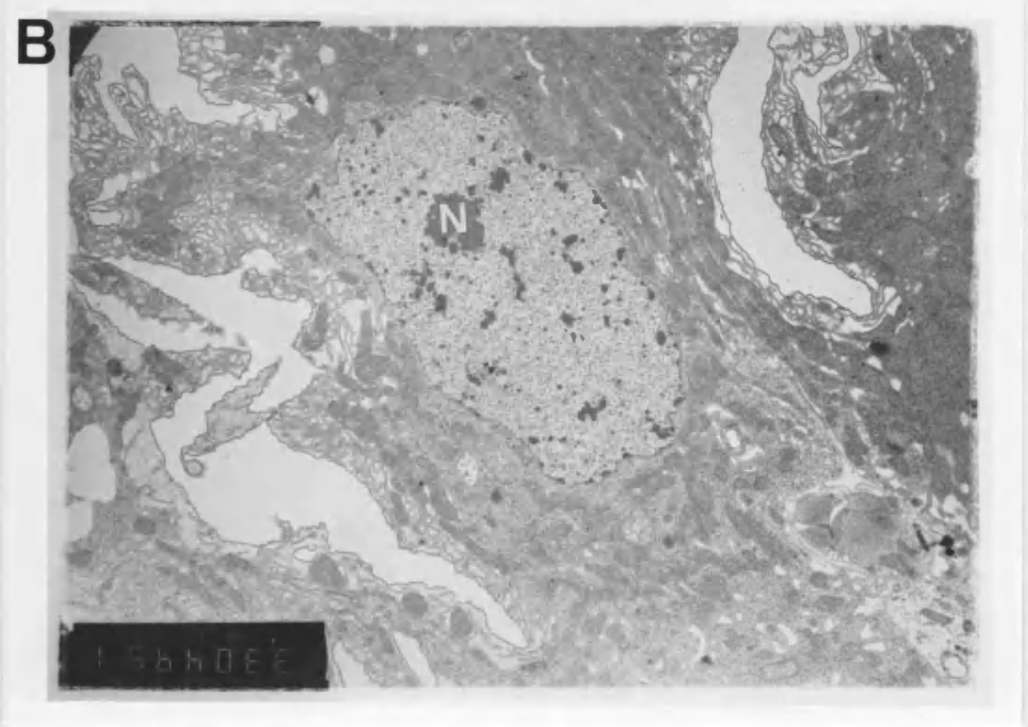


PLATE 14. (A) Wall of rectum of a fifth-instar larva, and (B) apical region of a rectal gland cell of a spring-caught male of Dolycoris baccarum.
LU = lumen of rectum, HC = haemocoel, N = nuclei.

DISCUSSION

The experiment on feeding and growth of Palomena prasina, and the measurements of composition of excreta and haemolymph, have yielded information which can be considered under two distinct headings, namely those of haemolymph composition, its relationship to diet, and its regulation by the excretory organs; and on the other hand, efficiency of food conversion. The literature in these subject areas does not usually overlap, though parallel studies might be of great interest, since efficiency of utilisation of a food source must relate to the metabolic work needed to compensate for ion imbalance, water content, and detoxification of plant defence compounds.

In both these areas, as it happens, phytophagous Hemiptera feature only to a small degree. A survey of haemolymph composition by Florkin & Jeuniaux (1974) lists many kinds of insect, but of phytophagous Hemiptera there is mention only of Jassidae and (useful coincidence) Palomena prasina adults. The latter reference (again by happy chance !) gives only figures for sodium and potassium concentrations. However, further data are available from Berridge (1965), Cheung & Marshall (1973a), Downing (1980), Lindsay & Marshall (1981), and Marshall & Cheung (1975). These are summarised in Table III, and for comparison, figures for insects of other orders are given in Table IV.

The pattern, in insects as a whole, is found to be that the lower forms, Apterygota and Exopterygota, have haemolymph sodium concentration greatly exceeding that of potassium, in both larvae and adults. This relationship holds good in the endopterygote orders Megaloptera, Neuroptera, Mecoptera and Diptera, and also for most Coleoptera and adult Hymenoptera. The Lepidoptera have what is regarded as a specialised haemolymph composition, with low levels of sodium and high levels of potassium, and this may also be found in larvae of Coleoptera Phytophaga and of Hymenoptera. In these forms there is a reduction in the importance of chloride as a contributor to haemolymph osmotic pressure, and its replacement by amino acids or other organic components. The osmotic contribution of potassium remains about the same as in unspecialised haemolymph types. Adults of these groups are less extreme, and may have equal concentrations of the two cations, or sodium may be slightly in excess. It is thought that the specialised type of haemolymph evolved along with phytophagy on Angiosperm plants. Lepidopteran larvae are among the most voracious and effective phytophages in existence, and the most primitive Hymenoptera are not far behind.

TABLE III

SODIUM AND POTASSIUM CONCENTRATION IN HAEMOLYMPH AND EXCRETA OF HEMIPTERA

INSECT	HAEMOLYMPH		EXCRETA		AUTHOR
Heteroptera	Na(mEq/l)	K(mEq/l)	Na(mEq/l)	K(mEq/l)	
Palomena prasina larva (plant-sucker)	8.9±2.8	22.3±1.7	0.05-2.93	22-131	This work.
Palomena prasina adult (" ")	22	42	-	-	quoted by Florkin & Jeuniaux(1974)
Dysdercus fasciatus larva (seed-sucker)	22.1-31.3	22	0.57-4.33	10-88	Berridge (1965)
Corixa punctata adult (detritivorous)	112	31	-	-	quoted by Florkin & Jeuniaux (1974)
Notonecta obliqua adult (carnivorous)	155	21	-	-	
Triatoma megista adult (haematophagous)	133	50	-	-	" " "
Homoptera					
"Jassidae" adults (plant phloem ?)	59	21	-	-	" " "
Eurymela distincta adults (plant phloem)	60.7±5.5	9.8±1.1	40.6±3.9	27.0±3.9	Lindsay & Marshall(1981)
Pyrops candelaria adults (plant cambium)	18.5±1.7	19.2±1.0	6.0±1.1	116.4±8.0	Marshall & Cheung (1975)
Cosmocarta abdominalis larva (xylem)	12.8±1.9	20.9±1.8	0.44±0.1	4.82±1.1 *	" " "
Cyclochila australasiae adults (xylem)	21.2±1.8	28.4±1.3	1.2±0.2	7.1±1.7 *	Cheung & Marshall (1973a)
			14.4±0.6	42±5 **	
Myzus persicae adults (Aphididae, phloem)	0.23	13±0.8	0.57	103±14.4	Downing (1980)

* The excreta composition of these two xylem feeders approximates closely to that of their ingesta.

** Figures for Malpighian tubule fluid before entry into the filter chamber complex.

Addendum - ~~Dysdercus~~ Oncopeltus fasciatus adult	21·6	6·2	-	-	Staddon & Everton (1980)

TABLE IV

SODIUM AND POTASSIUM CONCENTRATION IN HAEMOLYMPH OF NON-HEMIPTERAN INSECTS

Figures from Florkin & Jeuniaux (1974) except *Pieris brassicae* (Nicolson, 1976)

INSECT SPECIES	SODIUM (mEq/l)	POTASSIUM (mEq/l)
Orthoptera:		
Locusta migratoria larva	60	12
adult	67.4	9
Coleoptera:		
Dytiscus sp. larva (carnivore)	115	20
Dytiscus marginalis adult (carnivore)	165.2	6.4
Tenebrio molitor larva (graminivore)	71 - 75	38.7
Tenebrio molitor adult (" ")	87.2	30.1
Rhynchophorus palmarum larva (phytophage)	3.9	22 - 44
Rhynchophorus palmarum adult (" ")	27.3	26.9
Hymenoptera:		
Apis mellifera larva (nectar, pollen)	10.9	30.5
Apis mellifera adult (" ")	47.1	27.1
Vespula germanica larva (carnivore)	26.0	56.4
Vespula germanica adult (" ")	93.0	18.2
Lepidoptera:		
Bombyx mori larva (phytophage)	6.0	39.4
Bombyx mori adult (" ")	14.3	36.1
Barathra brassicae larva (phytophage)	4.3	53.6
Barathra brassicae adult (" ")	15.6	43.9
Pieris brassicae larva (" ")	8.0	48.8
Pieris brassicae adult (" ")	30.5	29.2

It can be seen from Table III that Jassidae and Eurymelidae (closely related forms of small leaf-hopper) have the primitive sodium:potassium ratio in their haemolymph, but the fulgorid P.candelaria, the cercopid C.abdominalis, and the cicadid C.australasiae have a ratio of less than unity. In the phytophagous Heteroptera (unfortunately data are available only for Pentatomomorpha, not for e.g. Miridae), the ratio is slightly above unity in D.fasciatus but is in the region of 0.5 in P.prasina. Although the concentrations quoted by Florkin & Jeuniaux (1974) for adult P.prasina are approximately double those found in larvae in the present work, the ratio is nearly the same.

Where figures for excreta composition are available, the excreta are generally found to contain less sodium and more potassium than the haemolymph. This applies to E.distincta, even though the haemolymph concentrations of these cations have the primitive sodium excess, and though it might seem not to apply to C.abdominalis and C.australasiae in respect of potassium excreted, it is so in relation to the fluid secreted by the Malpighian tubules into the filter complex. Final excreta figures relate to the osmotic withdrawal of water from the ingesta diluting the Malpighian tubule secretion, and the re-absorption of ions in the hind-gut. Aphids (Downing, 1980) are clearly a special case, lacking Malpighian tubules, and manipulating the osmolarity of their excreta by synthesising unusual oligosaccharides (Fisher, Wright & Mittler, 1984).

It is interesting to note that the fulgorid P.candelaria, which does not have a filter chamber, and as stated earlier has some features of gut structure in common with Heteroptera, is the Homopteran which comes nearest to the haemolymph and excreta composition of pentatomomorph Heteroptera.

The much lower figure for sodium excretion in P.prasina compared with D.fasciatus may be the consequence of the lower concentration of that cation in the food (2mg./100gm. in runner bean pod against 99mg./100gm. in cotton seed). The sodium:potassium ratio is also markedly different (1:140 against 1:10). The lower proportion of potassium in cotton seed may be typical of seeds, other examples being wheat (1:14), rice (1:18) and haricot beans (1:27), whereas fruits range from blackberries (1:52) to marrow (1:210) (figures from Paul & Southgate, 1978).

Some aspects of the excretion process in D.fasciatus have important implications for this discussion, and will be considered in detail.

In Berridge's studies of this insect (1965), they were observed to feed on moist cotton seed for the first four days of the final larval instar, then to cease feeding and seek water during the remaining four days of the instar. Liquid excreta only were produced, during days 1 - 5, though the rectum was full of liquid during days 6 - 8. Its contents formed a water store (a possibility suggested in Appendix 3, p.880), and from initial hypotonicity to the haemolymph, lost volume and increased tonicity through the non-feeding period. The rectal contents never became hypertonic to the haemolymph. The potassium content of the excreta increased rapidly from the second to the fourth day of the instar, then declined. Sodium content also varied, but in the opposite sense to potassium, the highest concentration in the rectum being on days 5 and 6. The lowest sodium levels in the excreta corresponded to the period of maximum potassium excretion and the severe depletion of haemolymph volume (44%) and total body water which accompanied it. At this time also, haemolymph sodium was at its maximum concentration and chloride was the principal component of haemolymph osmotic pressure. At other times during the instar, amino acids and other non-electrolytes were the main source of osmotic pressure. On day 6, when haemolymph sodium was declining, and rectal contents ceased to be voided, the sodium concentration in the excreta reached a maximum of 14mM. Absorption of sodium accompanied the re-absorption of water in the non-feeding period. Although haemolymph sodium concentration varied during the instar (and similar changes in P.prasina would partly account for the wide range of the few determinations of that cation), potassium concentration was closely regulated. It would seem that Dysdercus fasciatus is not very well adapted to a diet of dry seeds, being unable to absorb water from the rectal contents to form hypertonic excreta, as is the case with Orthoptera, Coleoptera and Lepidopteran larvae on the same diet. The intestinal discontinuity and rudimentary caeca indicate its descent from feeders on more hydrated plant material. Unlike biting and chewing feeders, seed-feeding bugs attach themselves to a seed and feed continuously for long periods. It is common at certain times of the year in West African forests, for larvae of Dysdercus spp. to be found wandering around with a seed of the silk-cotton tree, Ceiba pentandra, attached to their mouth parts. Such a manner of feeding may prevent the insect from interrupting

its meal in order to drink. It therefore adopts the remarkable habit of "borrowing" water from its body reserves so that it can rapidly excrete surplus potassium. Because of the preliminary softening and fluidisation of the cotton seed endosperm which is necessary before ingestion can begin (Saxena,1963), I believe that the rate of potassium excretion in D.fasciatus is simply following the rate of food ingestion. One cannot be sure whether the Malpighian tubules are secreting at their maximum rate, or whether rate of ingestion is the limiting factor. Nevertheless, it seems that potassium (and also magnesium and phosphate) must be cleared from the haemolymph without delay, and cannot wait until drinking is possible. This may be compared with the massive excretion of potassium in the newly adult Pieris brassicae (Nicolson, 1976), when the haemolymph has to change to suit the adult tissues (Table IV). In this case, it is accompanied by a 74% drop in haemolymph volume and a discharge of 89% of the body potassium, and occurs over a four-hour period after ecdysis. Although a net loss of potassium occurs, it must be noted than potassium is also being re-absorbed by the hind-gut, to maintain a flow of fluid from the Malpighian tubules (tubule secretion 151 mM. potassium, 48 mM. sodium; urine excreted 90 mM. down to 40 mM. potassium, 1.5 mM up to 7 mM. sodium during post-ecdysis transition). Ramsay (1964), referring to high osmotic pressure in the perinephric space of the rectal complex of Tenebrio molitor being largely due to non-electrolytes, points to the need for ion transporting epithelia to have a "natural" ionic ratio and strength on one side.

Thus it may be concluded that the excess potassium intake which is a consequence of phytophagy leads to physiological adaptations which minimise its effect on the tissue environment. One adaptation is to tolerate a change in cation ratio in the haemolymph which minimises the gradients against which active transport has to take place, either inwards (sodium) or outwards (potassium). It will probably be found that as more species of pentatomomorph Heteroptera are studied, the trend shown in P.prasina will be repeated. Studies on cimicomorph species would also be valuable. In lepidopteran larvae and other higher insects, the replacement of electrolytes by non-electrolytes in haemolymph osmotic pressure may be a response to possible toxic effects of a high potassium concentration. The tendency in this direction found by Berridge (1965) in D.fasciatus may also prove to be widespread. Whereas in most insects potassium is recycled via the rectal glands, it is possible that the

rather weakly developed rectal gland of phytophagous Pentatomomorpha does not do so, but is only concerned with sodium recovery. The manner in which a supply of potassium to the Malpighian tubules may be maintained will be discussed later.

It is convenient at this point to consider the implications of the ultrastructural studies, returning to the matter of food conversion efficiency last of all. As stated in the Introduction, the main objective of these studies was to find out if any of the cells of the caecal region or excretory vesicle possessed the structural characteristics typical of water transporting epithelia. It is now generally recognised (Berridge & Oschman, 1972) that the cells of which such epithelia are composed have, in addition to apical microvilli (which may be abundant or sparse), numerous deep basal infoldings and/or intercellular canals of which the lateral membranes may be extensively folded. Mitochondria may be associated with any or all of these cell boundaries, their distribution giving an indication of the sites of greatest metabolic activity. Parallel studies on ultrastructure and physiology have been carried out on mammal gall-bladder (Kaye et al., 1966) and insect rectum (Berridge & Gupta,1967). Various theories have been put forward to explain the mechanism of fluid transport (Curran & MacIntosh,1962; Diamond & Bossert,1967,1968). In principle they all depend on osmotic flow in response to active transport of ions.

The application of this to insect physiology has been largely concerned with elucidating the excretory cycle, whereby a potassium-rich fluid is secreted by the Malpighian tubules, more or less isosmotic with the haemolymph, and is converted into a hypertonic excreta by absorption of salts and water by the rectal glands. The rectal gland of many insects is a complex multi-layered organ, and the inter- and intracellular pathways agree well with the requirements of the theoretical models, though the ability of some insects' rectal glands to absorb water without apparent ion movement is still not fully explained (see review by Phillips et al.,1986). In all Hemiptera so far studied, the rectal gland is a simple single layer of cells. In certain Homoptera (Marshall & Cheung, 1973) and in aquatic larvae of Diptera (Meredith & Phillips, 1973), the narrow tubular rectal gland absorbs or (saline mosquitoes) secretes ions without concomitant water flow, and in these species the rectal glands have been found to lack basal infolds and lateral canals. More recently, it has become evident that (e.g. Dow, 1981) that there is

also a circulation of water within the midgut of some insects. Some contributions have been made (Ferreira, Ribiero & Terra, 1981; Richards, 1975) to a comparative study of ultrastructure and physiological function. The structure may offer only meagre clues as to the direction in which water is being transported, since that will depend on the location of the ion pumps, and their orientation. By analogy with the observations of Kaye et al.(1966) on rabbit gall-bladder, where the intercellular channels were enlarged when transport was occurring, the insect midgut cells in which the basal or intercellular channels are distended might be assumed to be those in which absorption from the lumen is taking place, while those in which the basal channels remain narrow and linear could be the site of secretion into the lumen. The direction of water flow within the midgut is shown by experiment to be from behind forward, secreted by the posterior midgut and absorbed by the enteric caeca or anterior midgut. Dow (1981) regarded the Malpighian tubules as the posterior source of water in the starved locust, but there is no ultrastructural information for this insect. However, the model is supported by Dow's (1986) interpretation of the observations of Ferreira et al.(1981), and by the structure (Richards,1975) and physiology (Ramsay, 1950) of the larval midgut of the mosquito Aedes aegypti. In the cetoniid beetle Protaetia acuminata adult (Cheung & Low, 1975), the anterior midgut has cells of "absorptive" type, the posterior midgut has cells of "secretory" type, and the cells of the middle midgut have no basal infolds, so must be considered "neutral". There is no physiological information for this insect.

To return to the interpretation of the ultrastructure of Dolycoris baccarum, we are dealing with a long, regionalised, and for part of its life discontinuous midgut, to which generalisations derived from the insects referred to above, which have uniform, relatively short midguts, can only be applied with caution. The anterior part of the midgut, in larval life isolated from the caecal region, shows no evidence of water transport. In it, enzymes are secreted, and nutrients absorbed. In the posterior part of the midgut, the caeca, contrary to expectation, show no trace of water transport structure, but the cells of the central tube of M4 are evidently of the absorptive type, and the fibrous secretion so strongly developed here, may function as an ion trap or semi-permeable barrier analogous to the chitinous intima of the hindgut. It may be composed of mucopolysaccharide (detected by Berridge (1965) in

the excreta of Dysdercus fasciatus) and may be comparable to the "fuzzy coat" found by Marshall & Cheung (1970) within the midgut tube of the fulgorid Homopteran Pyrops candelaria. A protective function must also be a possiblity in D.baccarum, since it is the adult stage which has been studied, and at this stage the midgut has become continuous, and residues from the anterior regions are passing along the M4 central tube. The peritrophic membrane of insects has been regarded as a protection against abrasion of the midgut cells by harsh insoluble particles in the food. In Pentatomomorpha such as D.baccarum, the protection afforded by the fibrous secretion is perhaps of a chemical nature. In the larvae of these insects, the M4 central tube is less well defined, and the openings of the caeca are wide. Though it seems probable that bacteria are carried along the central tube forwards to the M4 bulb for digestion, the evidence of the present work is not sufficient to confirm or to explain this.

That the posterior region of the midgut should be capable of absorbing fluid was unexpected, but is nevertheless explicable as a means of reserving the potassium needed by the Malpighian tubules. In the cacao capsid bugs (Appendix 1) absorption of fluid in the posterior midgut was observed (shown by increased concentration of certain insoluble crystals present in the gut contents). Since those insects have no rectal gland cells at all, it was supposed that this was to provide water for the excretory process, but in the light of present-day knowledge it is clear that potassium is also involved. In view of the large amounts of potassium which are excreted by P.prasina (and almost certainly also by D.baccarum), and the possibility referred to earlier that the rectal gland cells absorb little, it is reasonable to conclude that the M4 tube cells transport a potassium-rich, probably isosmotic, fluid into the haemocoel. In the larvae, M3 would probably have this function. With regard to the excretory vesicle, the exaggerated basal infolds strongly suggest a fluid transport function. It would be reasonable to suppose that this is directed inwards, as a secretion into the lumen, from the anatomical relationships seen in many specialised Pentatomomorpha, reaching the extreme in the Phyllocephalinae (Appendices 2 & 3). The mechanism of secretion, observed in living tissues of some species as a pinching off of swollen cell tips, or under light microscopy as a highly vacuolated cytoplasm (Plate 2 and figures in Appendix 3), may be by the discharge of the vacuoles seen in the electron micrographs.

On the other hand, the deep clefts lined with microvilli, which are another characteristic feature of this organ, may provide the site for a standing osmotic gradient, as required by the theoretical models referred to earlier. As a result of the physiological experiments, the writer does not seek to interpret the alimentary modifications found in phytophagous Hemiptera solely in terms of water disposal, but also of potassium excretion. It is not expected, therefore, that the secretion of the excretory vesicle would necessarily be hypotonic to the haemolymph, and indeed, it may be hypertonic. The interpretation proposed is that the excretory vesicle in phytophagous Pentatomomorpha acts as an accessory to the Malpighian tubules in the secretion of a potassium-rich fluid, perhaps coming into action at the times in the instar when peak outflow of potassium is taking place.

Comparison with such information as is available about other Hemiptera does not contradict the above conclusion. Only one other description of the ultrastructure of the midgut of a phytophagous Heteropteran has been published, that by Cheung (1977) on the Citrus Stinkbug, Rhynchocoris serratus. This author did not include the excretory vesicle in his investigations, nor does he make any reference to a discontinuity between M3 and M4, or to a separate M4 bulb, although his account was based on both adult and larval material. He does, however, mention a discontinuity between M4 and the excretory vesicle ("lumen blocked by a thick plug of cells"). He found "membranous whorls" in the M3 region, which he interprets as the remains of degenerate cells from more anterior parts of the midgut, trapped at that level "because of the dense mass of bacteria filling the lumen". His account differs from the present work in his finding of basal infoldings in the cells of the anterior regions but not, apparently, in those of the central tube of M4. This description suggests that R.serratus digestive system functions in a manner more like that of the Tessaratomid, Piezosternum calidum, than that of D.baccarum or P.prasina. In Piezosternum calidum (Appendix 9) it seems as if the whole anterior midgut has the function attributed to the M4 bulb, in the breakdown and digestion of bacteria from the caeca.

In the midguts of Homoptera, cells have been described which have many deep basal infolds, in addition to strongly developed apical microvilli (Cheung & Marshall,1973b, 1982; Lindsay & Marshall, 1980; Marshall & Cheung, 1974). Such cells are situated at both anterior and posterior ends of the midgut, and their structure does not unequivocally indicate

whether they are secretory or absorptive. The closest resemblance to cells in D.baccarum are those in the fulgorid Pyrops candelaria (Cheung & Marshall, 1982). It is possible in this species that the posterior midgut is secretory, and the anterior midgut absorptive, from the appearance of the cells. The midgut is not interrupted, and fluid secreted posteriorly could travel forwards. There may be a return flow within the midgut sheath, and the nutrients swept forward may accumulate in the anterior midgut diverticulum. In another fulgoroid, the flatid bug Gyarina nigritarsis, the diverticulum has evidently some involvement in nutrition (Appendix 6). It is a matter of interest, that the rectal gland cells of P.candelaria (Cheung, 1979) also closely resemble those of D.baccarum.

In the other forms of Homoptera, belonging to the cicadid, cercopid, and eurymelid families, which possess filter chambers, the midgut is usually filled with swollen mineral storage-excretion cells. It is not unreasonable to suppose that absorption at the anterior end, and secretion at the posterior end of the midgut, of a potassium-rich fluid, will assist the function of the filter chamber by increasing the osmotic gradient within it. It is much less likely that a forward movement of fluid within the midgut will occur. The figures given by Cheung & Marshall (1973a) for the urine within the filter chamber cannot discriminate between that originating in the Malpighian tubules and that contributed by the posterior midgut (as, indeed, is the case also for the figures for Palomena prasina). It is therefore possible that in these insects also the midgut is an accessory in potassium excretion.

One may conclude that the function proposed for the structures seen in the caecal region and excretory vesicle of Dolycoris baccarum, can be supported by a rational interpretation of the structures reported to be present in other phytophagous Hemiptera.

To turn to the food conversion efficiency aspect of the present work, the subject has been reviewed by Waldbauer (1968) and more recently by Slansky (1985). The earlier review had no entries under phytophagous Hemiptera, but this gap has been filled by McNeill (1971) working on a grass-feeding mirid, Leptopterna dolabrata in this country, and by Chaplin & Chaplin (1981) on the seed-feeding lygaeids Oncopeltus fasciatus and Lygaeus kalmii in the U.S.A. These studies obtained data for respiration but not for faecal output, calculating ECD on the basis that Food eaten - Faeces produced = Weight gain + Respiration.

Chaplin & Chaplin used milkweed (Asclepias syriaca) seeds as the food source, and weighed both food and insects daily. McNeill studied a population in the field, obtained average dry weights and energy content for each instar, and sampled daily on fixed 0.25m^2 quadrats to assess population density. Respiratory rate and a relationship between feeding rate and body size were obtained by laboratory studies. L dolabrata leaves lesions on the grass blades on which it feeds, and the area of these was measured and their number counted, to give the amount of food eaten. Calorific values were obtained for plant tissue from which the cell walls had been separated. McNeill states that ECD reaches a peak when the insects change from leaf-feeding to seed heads in June.

Table V gives a summary of ECI and ECD percentages, on energy and dry weight bases, for phytophagous Hemiptera and, for comparison, other phytophagous insects. The figures given for L.dolabrata are averages over five successive seasons study. They, and the figures for P.prasina, seem to lie in the same range of magnitude as other insects, but the figures for the seed-sucking lygaeids O.fasciatus and L.kalmii seem to be remarkably high. In comparing them with other Hemiptera, Chaplin & Chaplin (1981) publish figures from McNeill (1971) which they have presumably extracted from graphs of seasonal variation of ECD given by that author for L.dolabrata. Chaplin & Chaplin show that while feeding on grass blades, L.dolabrata has ECI of 12%, and ECD of 60%, and when seed heads become available and are preferentially fed upon, ECI rises to 42% and ECD to 70%. This brings the figures up to the level of Aphididae, but still below the Lygaeidae. The implication is that the food obtained from seeds requires less metabolic work for the same amount of nourishment than that obtained from more hydrated plant tissues or from plant sap. The task of drawing liquid food up the minutely narrow stylet channels (the powerful cibarial muscles being the energy-demanding part of the system) must not be very different. The smaller volume drawn in from seeds would probably have a higher viscosity. So energy must be consumed in excreting excess water and salts.

Although ECD for P.prasina is similar to most other phytophagous insects, ECI is higher, and this reflects the fact that suctorial feeding excludes insoluble material which would be discarded in the faeces.

Apart from the aphids, for which successful artificial and controlled feeding techniques have been developed (Auclair, 1965), and throughput can readily be measured both as to composition (Mittler, 1958) and amount

TABLE V

COMPARISON OF EFFICIENCIES OF CONVERSION OF INGESTED (ECI) AND DIGESTED (ECD) FOOD IN DIFFERENT INSECTS

INSECT	DRY WEIGHT BASIS		ENERGY BASIS		AUTHOR
Heteroptera	ECI (%)	ECD (%)	ECI (%)	ECD (%)	
Palomena prasina, 5th.instar, c.20°C	38.3	40.1	-	-	This work.
Lygaeus kalmii, 5th.instar, 20°C	-	-	72.5	93.5	Chaplin & Chaplin (1981)
Oncopeltus fasciatus, 5th.instar,20°C	-	-	84.6	94.9	" " "
Leptopterna dolabrata, all stages.	-	-	17.8	55.0	McNeill (1971)
Sigara alternata (Corixidae) ?adults	-	-	23	65	quoted by Chaplin & Chaplin (1981)
Homoptera					
Philaenus spumarius(Cercopidae)	-	-	24	54	" " "
Acyrtosiphon pisum (Aphididae)	-	-	48	58	" " "
Eucallipterus tilliae (")	-	-	44	72	" " "
Macrosiphum liriodendri (")	19	47	24	77	quoted by Slansky (1985)
Orthoptera					
Achaeta domestica	28	40	38	52	" " "
Coleoptera					
Leptinotarsa decemlineata larva, 20°C	32	-	32	-	" " "
Lepidoptera					
Spodoptera sp. 5th.instar on kidney bean.	28	40	33	44	" " "
Pieris rapae, 5th.instar.	17	45	20	46	" " "
Malacosoma disstria, 4th. & 5th.instars	16.7	47	-	-	Futuyma & Wasserman (1981)
Manduca sexta 5th.instar, artificial diet	38	65	44.5	72	Reynolds, Nottingham & Stephens (1985)

(Wright, Fisher & Mittler, 1985), the study of feeding efficiency of plant feeding Hemiptera is not easy. The ability of P.prasina to grow apparently normally on an isolated plant organ, and the ability of such an organ to remain acceptable as food for a reasonable time, is fortunate. As my failure to culture D.baccarum shows, there are many species for which such a study would be extremely difficult if not impossible. For many species which feed on vegetative tissues such as shoots or leaves, which must remain attached to the plant in order to remain acceptable as food, and on which no distinct lesions are caused, the task of measuring food intake would present considerable problems.

Very many species of pentatomomorph Heteroptera are reputed to feed on a range of plant species, and their performance on different foods, in relation to ionic composition and plant defences, would be of great interest. In connection with the present work, it is far from clear what are the natural food sources in this country of P.prasina. It might be that they are opportunist and take whatever is available, even including rotting debris, but larvae reared on Phaseolus pods have shown no signs of feeding when offered a variety of alternatives - tomato, courgette, pea pod, broad bean, or unripe hazelnut (one of its reputed wild hosts). Probably they become adapted to what is most readily available in their early life. This, again, poses a problem in making comparative studies of feeding efficiency. In parts of the world where they are plant pests, several studies have been made on the performance of pentatomomorph Heteroptera on different plant species, or different organs or stages of development of the host plant, principally to assess the importance of weeds as alternative hosts (Hori & Kuramochi,1986; Hori, Kuramochi & Nakabayashi, 1985; Hori, Okamoto & Kuramochi, 1984; Kotaki et al.,1983; Panizzi & Hertzog, 1984; Panizzi & Slansky, 1985; Naresh & Smith,1983; and Shiga & Moriya,1983). Results are recorded in terms of weight gain, feeding rate and mortality, but data on feeding efficiency and food composition are not given.

Finally, it is necessary to consider, in the light of the present work and the literature of recent years, whether any light can be shed on the function of the caeca and the caecal bacteria. Although this work shows no ultrastructural evidence of a fluid transport role, I remain convinced that this would be found in the less specialised Coreidae and Lygaeidae. Insofar as the caeca in Pentatomidae are specialised to culture bacteria which are subsequently broken down and absorbed, it is the role of these

bacteria which is in question. Since the caeca are, in the growing phase of most Pentatomomorpha, cut off from the anterior part of the midgut, there is no possibility of their intervening to modify the ingesta, so as, for instance, to detoxify plant defence chemicals. If they are able to recycle nitrogenous excretory products, as some evidence from bacteria cultured from the Tessaratomid Piezosternum calidum (Appendix 9) seemed to suggest, this might be revealed by a very rigorous analysis of rate of nitrogenous excretion per unit of growth, compared with an insect lacking symbionts (ideally a colony of the same species reared aseptically).

It may be that the caecal bacteria form a store of nutrient which is drawn upon during sexual maturation. Many observations on this and other species (personal, unpublished) have found the caecal row to be shrunken in mature adults. It is not easy to see why the insect cannot simply use its fat body as a nutrient store during larval development, but it may be compared with the massive invasion of the haemocoel of certain fulgoroid Homoptera (Appendix 6) by yeast-like symbionts.

A nutritive function of some kind is the only explanation which is consistent with the vast numbers of bacteria present, and the evidence for their breakdown in the M4 bulb (the bacterial cell-wall splitting enzyme, N-acetylglucosaminidase, has been detected in this part of the gut). Other suggestions, such as that the caecal bacteria prevent the invasion of the gut by other bacteria, or that they synthesise essential nutritional factors, could be dealt with by much smaller numbers of bacteria, residing amongst the cells of a simple tubular midgut. Srivastava & Rouatt (1963) found a variety of bacteria in the gut of an aphid (though, interestingly, not Pseudomonas), though the insects did not seem to be harmed by their presence. One may question the need for such an elaborate arrangement as the gastric caeca, solely to allow one bacterial species to exert dominance over others.

To conclude, future work might concentrate on the larvae of Palomena prasina, to establish by close observation the feeding rhythm, and to analyse haemolymph and excreta, and observe the ultrastructure of the caecal region and excretory vesicle, at known times in the instar. I do not believe that it would be technically difficult to cannulate the anus and to introduce fluids of known composition, as Phillips (1964) did with the desert locust, nor even to isolate Malpighian tubules so as to analyse their secretion separately from any contribution from the excretory vesicle.

ACKNOWLEDGEMENTS

I wish to express my appreciation of the help, encouragement, and advice generously given by my colleagues Stuart Reynolds and Keith Charnley, also the enthusiastic help with statistics given by Nigel Franks, and my gratitude to the manufacturers of Tippex correction fluid, without which this work would have been much more difficult.

REFERENCES

(* Titles marked thus were consulted in Biological Abstracts)

AUCLAIR,J.L.(1965) Feeding and nutrition of the Pea Aphid, Acyrthosiphon pisum (Homoptera: Aphidae) on chemically defined diets of various pH and nutrient levels. Ann.ent.Soc.Amer. 58, 855-875.

BACKUS,E.A.(1988) Sensory systems and behaviours which mediate Hemipteran plant-feeding: a taxonomic review.J.Insect Physiol. 34,151-165.

BASDEN,R.(1967) The occurrence and composition of the sugars in the honeydew of Eriococcus coriaceus(Mask.). Proc.Linn.Soc.New South Wales, 92, 222-226.

BERRIDGE,M.J.(1965) The physiology of excretion in the Cotton Stainer, Dysdercus fasciatus Sign. I-III. J.exp.Biol., 43, 511-552.

BERRIDGE,M.J.& GUPTA,B.L.(1967) Fine-structural changes in relation to ion and water transport in the rectal papillae of the blowfly, Calliphora. J.Cell Sci.,2, 89-112.

CHAPLIN,S.B.& CHAPLIN,S.J.(1981) Comparative growth analysis of a migratory and a non-migratory insect: the milkweed bugs. J.anim.Ecol.,50, 407-420.

CHEUNG,W.W.K.(1977) Ultrastructural and functional differentiation of the midgut of the Citrus Stinkbug, Rhynchocoris serratus Don. (Heteroptera: Pentatomidae) J.Chinese Univ.Hong Kong, 4, 339-354.

CHEUNG,W.W.K.(1979) An electron microscopic observation of the rectum of the Lantern Bug, Pyrops candelaria Linn.(Homoptera: Fulgoridae). J.Chinese Univ.Hong Kong, 5, 521-533.

CHEUNG,W.W.K.(1983) Light and electron microscopic observations of the midgut sheath and anterior diverticulum of the Lantern Bug, Pyrops candelaria Linn.(Homoptera:Fulgoridae) Cytologia, 48, 333-342.

CHEUNG,W.W.K.& LOW,K.W.(1975) Ultrastructural and functional differentiation of the midgut of the sugar cane beetle, Protaetia acuminata(F)(Coleoptera:Cetoniidae). Int.J.Insect Morphol.& Embryol.,4, 349-361.

CHEUNG,W.W.K. & MARSHALL,A.T.(1973a) Water and ion regulation in cicadas in relation to xylem feeding. J.Insect Physiol.,19,1801-1816.

CHEUNG,W.W.K. & MARSHALL,A.T.(1973b) Studies on water and ion transport in Homopteran insects: ultrastructure and cytochemistry of the cicadoid and cercopoid midgut. Tissue & Cell,5,651-669.

CHEUNG,W.W.K. & MARSHALL,A.T.(1982) Ultrastructural and functional differentiation of the midgut of the Lantern Bug, Pyrops candelaria Linn.(Homoptera:Fulgoridae). Cytologia, 47, 325-339.

CULL,D.C. & VAN EMDEN,H.F.(1977) The effect on Aphis fabae of diel changes in their food quality. Physiol.Entomol.,2, 109-115.

CURRAN,P.F. & MACINTOSH,J.R.(1962) A model system for biological water transport. Nature (London),193, 347-348.

DIAMOND,J.M. & BOSSERT,W.H.(1967) Standing-gradient osmotic flow. A mechanism for coupling of water and solute transport in epithelia. J.Gen.Physiol.,50, 2061-2083.

DIAMOND,J.M. & BOSSERT,W.H.(1968) Functional consequences of ultrastructural geometry in "backwards" fluid-transporting epithelia. J.Cell Biol., 37, 694-702.

DOW,J.A.T.(1981) Countercurrent flows, water movements, and nutrient absorption in the locust midgut. J.Insect Physiol.,27,579-585.

DOW,J.A.T.(1986) Insect midgut function. Adv.Insect Physiol.,19,187-328.

DOWNING,N.(1980) The regulation of sodium, potassium, and chloride in an aphid subjected to ionic stress. J.exp.Biol., 87, 343-349.

FERREIRA,C., RIBIERO,A.F. & TERRA,W.R.(1981) Fine structure of thelarval midgut of the fly Rhynchosciara, and its physiological implications. J.Insect Physiol.,27,559-570.

FISHER,D.B., WRIGHT,J.P. & MITTLER,T.E.(1984) Osmoregulation by the aphis Myzus persicae: a physiological role for honeydew oligosaccharides. J.Insect Physiol.,30,387-393.

FLORKIN,M.& JEUNIAUX,C.(1974) Haemolymph: Composition. In The Physiology of Insects, 2nd.Edn. Ed.M.Rockstein, pp.255-307. Academic,N.Y.

FUTUYMA,D.J. & WASSERMAN,S.S.(1981) Food plant specialisation and feeding efficiency in the tent caterpillars Malacosoma disstria and M.americanum. Ent.exp. & appl.,30, 106-110.

GILBY,A.R. & WATERHOUSE,D.F.(1965) The composition of the scent of the green vegetable bug, Nezara viridula.Proc.Roy.Soc.B,162,105-120.

GOURANTON,J.(1968a) Observations histochimique et histoenzymologiques sur le tube digestif de quelques Homoptères cercopides et jassides. J.Insect Physiol.,14,569-579.

GOURANTON,J.(1968b) Ultrastructures en rapport avec un transit d'eau: Etude de la "chambre filtrante" de Cicadella viridis L. (Homoptera, Jassidae). J.Microscopie,7,559-574.

HORI,K.(1972) Comparative study of a property of salivary amylase among various Heteropterous insects. Comp.Biochem,Physiol.,42B,501-508

*HORI,K. & KURAMOCHI,K.(1986) Effects of temporal foods in early nymphal stages, on later growth of Palomena angulosa and Eurydema rugosum(Hemiptera:Pentatomidae).Appl.Entomol.Zool.,21,39-46.

*HORI,K, KURAMOCHI,K. & NAKABAYASHI,S.(1985) Effects of several different food plants on nymphal development of Palomena angulosa (Hemiptera:Pentatomidae). Res.Bull.Obihiro Univ. I,14,239-246.

*HORI,K., OKAMOTO,K. & KURAMOCHI,K.(1984) Distribution, feeding rate and development of the cabbage bug Eurydema rugosum (Hemiptera: Pentatomidae) on cruciferous plants. Appl.Entomol.Zool.,19,273-279.

KAYE,G.I., WHEELER,H.O., WHITLOCK,R.T. & LANE,N.(1966) Fluid transport in the rabbit gall-bladder. A combined physiological and electron microscopic study. J.Cell Biol.,30, 237-268.

*KOTAKI,T., HATA,K., GUNJI,M. & YAGI,S.(1983) Rearing of the brown-winged green bug, Plautia stali (Hemiptera-Heteroptera:Pentatomidae) on several diets. Jpn.J.Appl.Entomol.Zool.,27,63-68.

KUSKOP,M.(1923) Bakteriensymbiosen bei Wanzen. Arch.Protisenk.,47,350-385.

LINDSAY,K.L. & MARSHALL,A.T.(1980) Ultrastructure of the filter chamber complex in the alimentary canal of Eurymela distincta Sign. (Homoptera, Eurymelidae). Int.J.Insect Morph.& Embryol.,9, 179-198.

LINDSAY,K.L. & MARSHALL,A.T.(1981) The osmoregulatory role of the filter-chamber in relation to phloem feeding in Eurymela distincta (Cicadelloidea,Homoptera) Physiol.Entomol.,6, 413-419.

McNEILL,S.(1971) The energetics of a population of Leptopterna dolabrata (Heteroptera: Miridae) J.anim.Ecol.,40,127-140.

MARSHALL,A.T. & CHEUNG,W.W.K.(1970) Ultrastructure and cytochemistry of an extensive plexiform surface coat on the midgut cells of a fulgoroid insect. J.Ultrastructure Res.,33, 161-172.

MARSHALL,A.T. & CHEUNG,W.W.K.(1973) Studies on water and ion transport in Homopteran insects: Ultrastructure and cytochemistry of the cicadoid and cercopoid hindgut. Tissue & Cell,5,671-678.

MARSHALL,A.T. & CHEUNG,W.W.K.(1974) Studies on water and ion transport in Homopteran insects: Ultrastructure and cytochemistry of the cicadoid and cercopoid Malpighian tubules and filter chamber. Tissue & Cell,6,153-171.

MARSHALL,A.T. & CHEUNG,W.W.K.(1975) Ionic balance of Homoptera in relation to feeding site and plant sap composition. Ent.exp.et appl., 18, 117-120.

MARTOJA,R. & BALLAN-DUFRANCAIS,C.(1984) The ultrastructure of the digestive and excretory organs. In Insect Ultrastructure, Vol.2, pp.199-268.(Eds.King,R.C. & Akai,H.) Plenum, N.Y.

MEREDITH,J. & PHILLIPS,J.E.(1973) Rectal ultrastructure in salt- and freshwater mosquito larvae in relation to physiological state. Z.Zellforsch., 138, 1-22.

MILES,P.W.(1959) The salivary secretions of a plant-sucking bug, Oncopeltus fasciatus (Dall.)(Heteroptera: Lygaeidae). I. The types of secretion and their roles during feeding. J.Insect Physiol.,3, 243-255.

MILES,P.W.(1960) The salivary secretions of a plant-sucking bug, Oncopeltus fasciatus (Dall.)(Heteroptera:Lygaeidae). II. Physical and chemical properties. J.Insect Physiol.,4, 207-219.

MILES,P.W.(1964) Studies on the salivary physiology of plant bugs: oxidase activity in the salivary apparatus and saliva. J.Insect Physiol.,10, 121-129.

MISHRA,K.(1980) Filterchamber on the alimentary tract of Pyrilla perpusilla (Homoptera, Fulgoroidea, Lophopidae). Acta entomologica bohemoslovaca, 77, 196-200.

MITTLER,T.E.(1957) Studies on the feeding and nutrition of Tuberolachnus salignus(Gmelin). I. The uptake of phloem sap. J.exp.Biol., 34, 334-341.

MITTLER,T.E.(1958) Studies on the feeding and nutrition of Tuberolachnus salignus(Gmelin). II. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. J.exp.Biol., 35, 74-84.

*NARESH,J.S. & SMITH,C.M.(1983) Development and survival of the Rice Stink Bug (Oebalus pugnax)(Hemiptera:Pentatomidae) reared on different host plants at four temperatures. Environ.Entomol., 12, 1496-1499.

NICOLSON,S.W.(1976) Diuresis in the Cabbage White butterfly, Pieris brassicae: water and ion regulation and the role of the hindgut. J.Insect Physiol.,22,1623-1630.

NUORTEVA,P. & LAUREMA,S.(1961) Observations on the activity of salivary proteases and amylases in Dolycoris baccarum(L.)(Het., Pentatomidae) Ann.Ent.Fenn., 27, 93-97.

*PANIZZI,A.R. & HERZOG,D.C.(1984) Biology of Thyanta perditor(Hemiptera: Pentatomidae) Ann.Ent.Soc.Amer.,77, 646-650.

*PANIZZI,A.R. & SLANSKY,S.F.Jr.(1985) Legume host impact on performance of adult Piezodorus guildinii(Hemiptera:Pentatomidae) Environ.Entomol., 14, 237-242.

PAUL,A.A. & SOUTHGATE,D.A.T.(1978) McCance & Widdowson's The Composition of Foods, 4th.Revised Edn., H.M.S.O., London.

PHILLIPS,J.E.(1964) Rectal absorption in the Desert Locust, Schistocerca gregaria Forskal. I-III. J.exp.Biol., 41, 15-80.

PHILLIPS,J.E.,HANRAHAN,J.,CHAMBERLIN,M.,& THOMSON,B.(1986) Mechanisms and control of reabsorption in insect hindgut. Adv.Insect Physiol., 19, 329-422.

PONSEN,M.B.(1981) The digestive system of Eulachnus brevipilosus Börner (Homoptera:Aphididae) Mededelingen Landbouwhogeschool Wageningen, 81-3.

PONSEN,M.B.(1982a) The digestive system of Glyphina and Thelaxes (Homoptera:Aphidoidea). Mededelingen Landbouwhogeschool Wageningen, 82-9

PONSEN,M.B.(1982b) The digestive system of Phloeomyzus passerinii(Sign.) (Homoptera:Aphidoidea). Mededelingen Landbouwhogeschool Wageningen, 82-10

RAMSAY,J.A.(1950) Osmotic regulation in mosquito larvae. J.exp.Biol.,27, 145-157.

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

REYNOLDS,S.E.,NOTTINGHAM,S.F.,& STEPHENS,A.E.(1985) Food and water economy and its relation to growth in fifth-instar larvae of the Tobacco Hornworm, Manduca sexta. J.Insect Physiol.,31, 119-127.

RICHARDS,A.G.(1975) The ultrastructure of the midgut of haematophagous insects. Acta Tropica,32, 83-95.

SALKELD, E.(1959) Histochemical studies on localization and distribution of esterases in the salivary glands of the Large Milkweed Bug, Oncopeltus fasciatus(Dall.)(Hemiptera:Lygaeidae) Canad.J.Zool.,37, 113-115.

SAXENA,K.N.(1958) Digestion and absorption of carbohydrates in the alimentary canal of the Red Cotton Bug,Dysdercus koenigii Fabr.(Heteroptera:Pyrrhocoridae).Physiol.Zool.,31,129-138.

SAXENA,K.N.(1963) Mode of ingestion in a heteropterous insect, Dysdercus koenigii(F.)(Pyrrhocoridae).J.Insect Physiol.,9, 47-71.

SAXENA,K.N. & BHATNAGAR,P.(1961) Nature and characteristics of invertase in relation to utilization of sucrose in the gut of Oxycarenus hyalinipennis(Costa)(Heteroptera:Lygaeidae) J.Insect Physiol.,7, 109-126.

SCHNEIDER,G.(1940) Beitrage zur Kenntnis der symbiontischen Einrichtungen der Heteropteren. Z.Morph.Okol.Tiere.,36,595-644.

*SHIGA,M. & MORIYA,S.(1983) Utilization of food plants by Plautia stali (Hemiptera-Heteroptera:Pentatomidae): an experimental approach.Bull.Fruit Tree Res.Stn.A(Yatabe),0(11),107-121.

SLANSKY,F.(1985) Food utilization by insects: interpretation of observed differences between dry weight and energy efficiencies. Entomol.exp.appl.,39, 47-60.

SNODGRASS,R.E.(1935) Principles of Insect Morphology, McGraw-Hill, N.Y.

SRIVASTAVA,P.N. & ROUATT,J.W.(1963) Bacteria from the alimentary canal of the Pea Aphid Acyrthosiphon pisum(Harr.)(Homoptera, Aphididae). J.Insect Physiol.,9, 435-438.

WALDBAUER,G.P.(1968) The consumption and utilization of food by insects. Adv.Insect Physiol.,5, 229-288.

WATERHOUSE,D.F. & GILBY,A.R.(1964) The adult scent glands and scent of nine bugs of the superfamily Coreoidea. J.Insect Physiol.,10, 977-987.

WRIGHT,J.P., FISHER,D.B., & MITTLER,T.E.(1985) Measurement of aphid feeding rates on artificial diets using ^{3}H-inulin. Entomol.exp.appl.,37, 9-11

* *

STADDON,B.W.& I.J.EVERTON (1980) Haemolymph of the milkweed bug Oncopeltus fasciatus (Heteroptera: Lygaeidae): inorganic constituents and amino acids. Comp.Biochem.Physiol.,65A,371-374

APPENDIX 1

[*Reprinted from* PROC. ZOOL. SOC. LOND. Vol. 122, Part III. pp. 543–572.]

A study of the digestive system of the West African cacao capsid bugs (Hemiptera, Miridae).

By

A. J. P. GOODCHILD, B.Sc., D.I.C.

*West African Cacao Research Institute, Tafo, Gold Coast.**

[Communicated by Professor P. A. BUXTON, F.R.S.—Received December 17, 1951.]

(With 7 figures in the text.)

CONTENTS.

INTRODUCTION.

Investigations into the anatomy and physiology of the digestive system of the cacao capsid bugs were carried out by the writer between October 1947 and October 1950, as part of a research programme on these pests, and the present paper summarizes the results achieved. The aspects covered include anatomy and histology, the enzymes secreted by the intestine and the labial ("salivary") glands, and the microbe flora of the intestine.

LITERATURE.

The cacao capsids of West Africa (*Sahlbergella singularis* Hagl., *Distantiella theobroma* (Dist.), *Bryocoropsis laticollis* Schum. and *Helopeltis bergrothi* Reut., all of the sub-family Bryocorinae) have been known as pests since the early years of this century. The biology, economic status and methods of control of these insects have been fully discussed by Cotterell (1926), Squire (1947) and Voelcker (1949). Squire (*op. cit.*) describes briefly the gross anatomy of the salivary glands, and makes suggestions as to the possible nature of the

* Now at the London School of Hygiene and Tropical Medicine, Keppel Street, W.C.1.

secretions they produce. In the literature on the family Miridae* there are very few records of studies comparable with the present one. The very destructive nature of the saliva, which is often found in this family, has been the cause of some investigations, notably that of K. M. Smith (1920) on the Apple Capsid, *Plesiocoris rugicollis* Fall. Other papers which may be mentioned are the study by Awati (1914) of the mechanism of suction in *Lygus pabulinus* Linn., that of Painter (1930) on the anatomy of *Psallus seriatus* Reut. and the monograph by Kullenburg (1944) on the Swedish capsids. The latter deals mostly with feeding preferences and behaviour, and mentions the internal anatomy of the reproductive system only. There is an extensive literature on the many agriculturally important species of the family, among which are papers touching on the physiology of feeding in its relation to plant damage (*e.g.* Ewing, 1929; Roberts, 1930; Leach & Smee, 1933).

The literature on other families of phytophagous Hemiptera–Heteroptera contains several anatomical and histological studies (Woolley, 1949, and works referred to therein), general physiological studies (Bugnion & Popoff, 1908; Baptist, 1940) and studies on symbiotic bacteria and other organisms (Glasgow, 1914; Schneider, 1940). In each of these aspects the cacao capsids were found to have characteristics differing markedly from other phytophagous Hemiptera.

Anatomy: Technique.

In order to get a complete picture of the anatomy and histology of the intestine and its associated structures, three methods have been used, namely, serial sectioning, whole mounts, and the microscopic examination of teased fresh tissue. For the first method, organs or whole organ systems (*e.g.* the mid-gut and accessory salivary glands which remain bound together by fine tracheae when dissected) were dissected out and fixed immediately in Bouin's Fluid. Serial sections were prepared in the usual manner and stained with either Hansen's trioxyhaematin, Mann's methyl-blue-eosin, or Mayer's haemalum, of which the latter proved to be the most satisfactory and the most resistant to fading when mounted in Euparal. Canada balsam in xylol as a mounting medium was found unsatisfactory owing to the deterioration of the stocks of absolute alcohol in the humid climate. Where finer detail was required, Zenkers bichromate fixative and Hedenhain's iron haematoxylin gave useful results. Chitinous material was embedded in clove-oil celloidin and then in paraffin wax before sectioning. Sections were cut at 10 μ except where otherwise stated. Whole mounts were fixed in 70 per cent alcohol and stained with Mayer's haemalum. Stains other than those mentioned were used for special purposes and will be referred to at the appropriate place in the text. Drawings both from fixed and fresh material were done with the aid of a camera lucida.

Alimentary canal.

In their internal anatomy, the four species of cacao capsid bugs differ surprisingly little, though they are attributed to different genera, on points of external detail. The largest species, *Sahlbergella singularis*, is between 7 mm. (male) and 9 mm. (female) in body length, and the smallest, *Bryocoropsis laticollis*, 5 to 6 mm. It has been found convenient to pursue the investigations on the two largest species, *S. singularis* and *Distantiella theobroma*, but constant reference to the other species has shown that the following account can be taken as applying, in principle, to them all. The relative sizes of the internal organs differ slightly in different species, and also in the different instars and between the sexes of any one species. Where actual dimensions are quoted they refer to *S. singularis*, the illustrations being also of that species.

* Formerly Capsidae. The older nomenclature is retained in the common name.

Mouth-parts and fore-gut.

These organs are of an extremely constant pattern throughout the Hemiptera, and no attempt has been made to examine those of the cacao capsids in detail. They resemble those described by Awati (1914) and MacGill (1947), and in the more general account of Snodgrass (1935). There are two pairs of bristle-like stylets, of which the maxillary pair form between them two channels, for food and saliva, and are guided and enclosed by the mandibular pair. The combined stylet bundle is borne in a channel on the dorsal surface of the rostrum.

The food canal in the stylets opens posteriorly into the pharyngeal pump, which resembles that in other Hemiptera in which this organ has been described (Woolley, 1949; MacGill, 1947, etc.). It is regarded by MacGill as an external chamber and not part of the true fore-gut. The fore-gut proper consists of a narrow oesophagus posterior to, and about equal in length to, the pharyngeal pump. A gustatory organ is present, just anterior to the pharyngeal pump, at the base of the rostrum.

The pharyngeal pump is heavily sclerotized, with a V-shaped lumen which can be distended by a powerful set of dorsal muscles. It is not possible to distinguish any epithelial structure in this part of the alimentary canal, but in the oesophagus a cuboid epithelium of small (10 μ diameter) uninucleate cells can be seen. The oesophagus has a lumen about 45 μ wide, and rather wider in the middle of its length, so that it is spindle-shaped. There is a thin sheath of longitudinal muscle outside the epithelium, and outside that again are several stout bands of circular muscle. Inserted in these bands are four strong muscles which are attached to the body wall (in the "neck" region) dorso- and ventro-laterally on each side (fig. 2, mus.). On the inner surface of the cuboid epithelium there is a fine chitinous intima. This region of the intestine does not appear to secrete any digestive enzymes.

The oesophagus joins the mid-gut at the "oesophageal valve", in the prothoracic region of the insect. At this point the oesophagus is invaginated for about 0·1 mm. into the mid-gut. The inner epithelium of this invagination is similar to that of the rest of the oesophagus, but the nuclei stain more deeply. The cells on the outer surface of the fold are in the form of a dense columnar epithelium with dark staining, laterally compressed nuclei (fig. 2). The chitinous intima continues over the whole surface of the invagination. It is thicker on the outer side than in the lumen of the oesophagus and terminates in a distinct rim. The lumen of the valve is small and tricuspid in section. The folding and separation of the intima from the epithelium which is apparent in the illustration, is a fixation artefact.

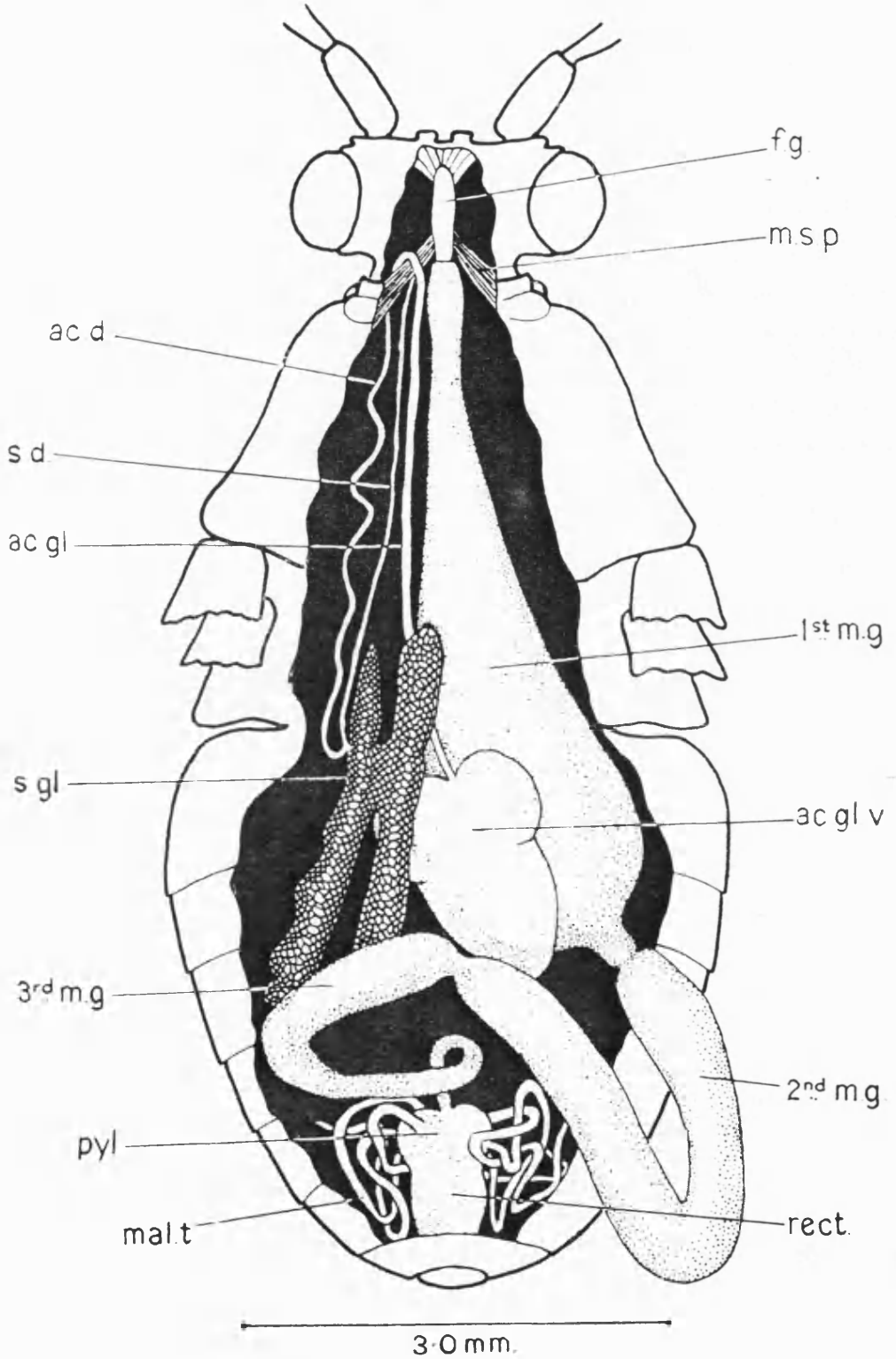
Mid-intestine.

In the cacao capsids, as in most Hemiptera, the mid-gut forms the major portion of the intestine, and by it the functions of storage, digestion, and absorption are performed. It is, therefore, with this region that the present paper is mostly concerned.

The length of the mid-gut is variable, on the average being some two and a half times the length of the insect. It consists of three regions, of nearly equal length, which are very distinct histologically and functionally, and will be referred to here as the first, second and third mid-guts.* They are divided one from another by constrictions which function as valves in controlling the flow of food and secretions through the alimentary canal. Common to the structure of the three regions are: a musculature consisting

* This rather rough terminology for the different regions of the one mid-gut is here adopted only for the sake of convenience, in the absence of a generally accepted terminology. It has the advantage of emphasizing the fact that in Hemiptera the subdivision of the intestine is almost all within the limits of the endodermal mid-gut, the fore- and hind-guts being comparatively insignificant.

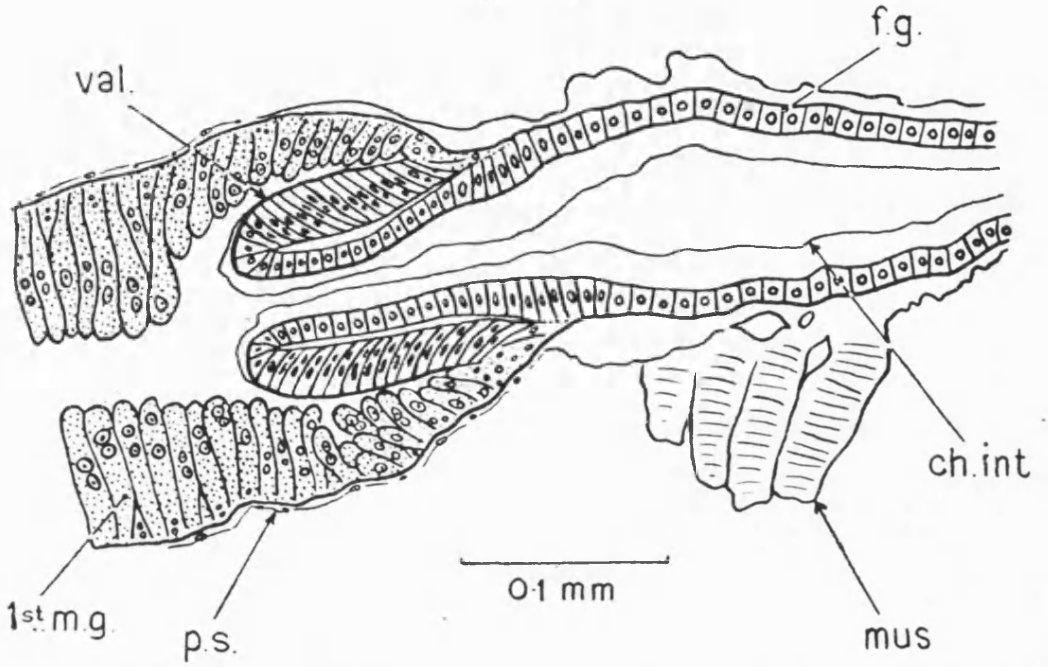
Figure 1.



Dorsal view of a capsid dissected to show alimentary canal and salivary glands. (Right-hand salivary glands removed). Key to lettering: f.g., fore-gut; 1st m.g., first mid-gut; 2nd m.g., second mid-gut; 3rd m.g., third mid-gut; pyl., pyloric region of hind-gut; rect., rectal region of hind-gut; mal.t., malpighian tubules; s.gl., main salivary gland; ac.gl., accessory salivary gland; ac.g.v., accessory gland vesicle; s.d., salivary duct; ac.d., accessory gland duct; m.s.p., muscles of salivary pump.

of a fine meshwork of longitudinal and circular strands, a delicate cellular sheath or basement membrane, and the almost complete absence of connective tissue. The nuclei of the epithelial cells are all of one type, pale in appearance when stained, but with a few scattered dark granules and a large central granule. In spite of the weak musculature, pronounced peristaltic or "mixing" movements can be observed in the first two regions. Gastric caeca such as commonly occur in phytophagous Hemiptera are absent, and there is no peritrophic membrane.

Figure 2.



Longitudinal section of oesophageal valve. Key to lettering : f.g., epithelium of fore-gut ; mus., dilator muscles of fore-gut ; p.s., peritoneal sheath ; 1st m.g., epithelium of first mid-gut ; val., invaginated part of fore-gut ; ch.int., chitinous intima.

First mid-gut.

The first mid-gut begins at the oesophageal valve with a short tubular portion which passes through the narrow space between the flight muscles in the thorax, and becomes, in the abdomen, a bulbous sac. This sac tapers gently forwards towards the oesophageal valve, and rather sharply behind, to join, in the region of the third to fourth abdominal segments, the valve which terminates it posteriorly. Scattered over the outer surface, and partly embedded in the ensheathing membrane, small crystalline spherules may quite often be found. The spherules are 15 to 50 μ in diameter, fibrous, and with concentric and radial striations. Similar crystalline bodies have very occasionally been found in the lumen of the intestine, and in two instances, single large concretions with this structure, were found distending the third mid-gut. In the same situation on the outer surface of the first mid-gut, small groups of fat globules 3 to 12 μ in diameter may be found along the paths of the tracheoles.

The epithelium of which the first mid-gut is composed consists of a single layer of large cells which are extremely elastic, and which vary between a thin pavement epithelium and a tall columnar epithelium according to the state of distension of the organ. The first condition is especially well seen when the first mid-gut is distended with air bubbles, as it frequently is, not

only when this distension is required by the moulting process, but as a normal condition when starved. The first mid-gut is only found in a collapsed state in the early stages of feeding, when the air bubbles have been passed along the intestine and out through the anus, and the distension of a full meal is not yet acquired. Material fixed and sectioned in the early stages of feeding shows a tall columnar epithelium with an irregular border formed by the bulbous tips of individual cells crowded together under lateral pressure (fig. 2). Dissection of fresh material reveals that the cells are separate for most of their length, of inverted flask shape, and it can be seen that they are subjected to lateral pressure, from the tendency of the epithelium to turn " inside-out ".

The cells of this epithelium are all binucleate, this fact being verified from whole mounts, since the large size of the cells renders it difficult to decide from sections. Very small regenerative cells are scattered singly or in small groups throughout the epithelium, these also being binucleate. Cells of all sizes between these and the largest are present in the epithelium, but they are all seen to take part in the secreting cycle, and there is no evidence to show that they are not of the one type. Multiplication of the regenerative cells seems to involve first the division of the cytoplasm into two uninucleate cells and then the division of the nuclei. Quadrinucleate cells are only occasionally seen, and then among the older, degenerating cells, whereas small groups of uninucleate cells can always be found. No mitotic division of nuclei has been detected in the digestive system, and multiplication of cells is presumed to be by amitosis. The limits of size observed for cells and nuclei in the first mid-gut are 25 μ to 70 μ and 8 μ to 22 μ respectively.

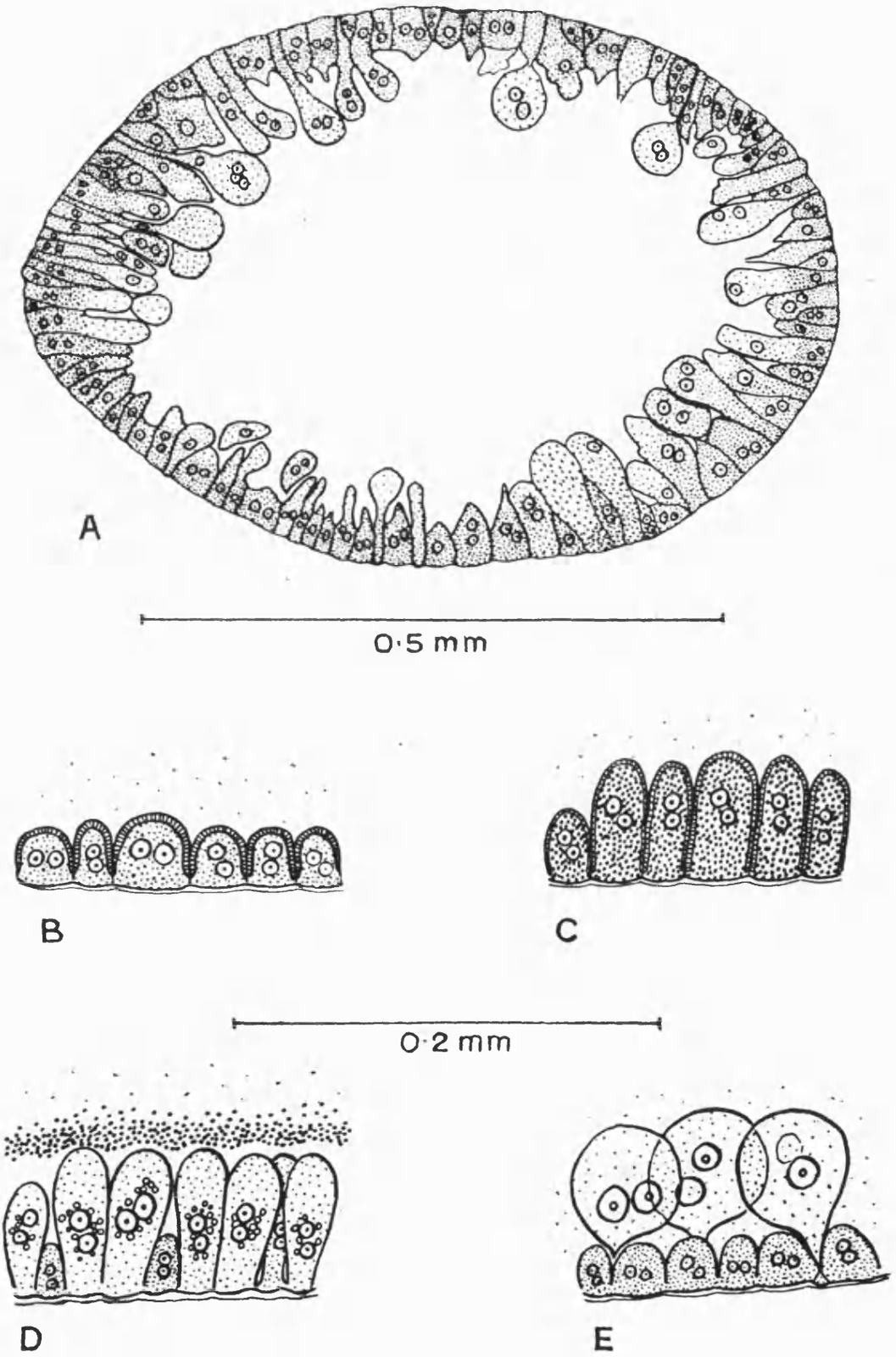
The epithelium of the tubular anterior portion of the first mid-gut, which joins the oesophageal valve, is of the type described. There is seen, however, in sections of this region a greater variability in density of staining of both cytoplasm and nuclei than in the sac-like region, and it would appear that the secretion and regeneration of the cells is not synchronized to the extent that it is in the latter region.

The phases through which the cells of the first mid-gut pass are strikingly distinct in the living insect. Some of these phases are, however, very susceptible to distortion in the fixative, and it is not easy to follow the secreting cycle of the cells from sectioned material alone. An intensive study has therefore been made on fresh material. For this purpose batches of capsids were starved overnight, so as to bring them into as nearly uniform a condition as possible, and then allowed to begin feeding on freshly cut twigs of cacao. A few capsids from each batch were dissected at intervals as feeding progressed, and the mid-gut teased out and examined. Detailed notes were kept of the first hundred capsids examined but many more than this have been examined to confirm the impressions originally formed.

After a short period of starvation, the first mid-gut epithelium is seen as a thin translucent sheet distended by a mass of air bubbles. On dissection the cells contract slightly and become small button-like bodies (fig. 3 B) presenting a hemispherical surface to the intestinal lumen. This surface has sometimes, but not always, at this stage, a distinct striated border, with a sharp outer boundary showing that it is formed in the cell wall itself and not from protoplasmic filaments extended from the cell (*i.e.* it is a "honeycomb" border). Perhaps because of this, this striated border is not easily seen in sections of fixed material. The presence or absence of this striated border may be connected with the secretion or non-secretion of the digestive enzymes which have been detected in this region (for which *vide infra*), but proof of this has not been obtained. At this stage the cytoplasm of the cell is dense and finely granular, and the nuclei small.

During the first three or four feeds after the starvation period the air bubbles in the lumen of the first mid-gut diminish in size and number as fluid

Figure 3.



(A) Transverse section of first mid-gut in actively-secreting phase. (B), (C), (D) and (E) Phases in the secretory cycle of first mid-gut cells, as seen in fresh tissues. For details, see text.

contents begin to accumulate. These contents are at this stage watery and contain coarse granules of irregular shape and size. Under high-power magnification, there may also be seen a few small crystals of a characteristic octahedral shape (fig. 4 E). The coarse granules presumably originate in the plant, and the crystals certainly do, as they have been seen forming a " tide-line " at the edge of a section through a fresh lesion. These granules and crystals may be products of the reaction between saliva and plant tissue, since they do not seem to correspond with any part of the cell structure of undamaged cacao tissue.

The epithelial cells at this stage are more swollen than at first, and from this point onwards they cease to be translucent but become strongly charged with opaque granular matter (fig. 3 C). They then appear almost black by transmitted light, the boundaries and nuclei showing up as light areas. The first mid-gut now appears to the naked eye as a dense white organ. This change occurs in scattered cells and groups of cells at first, particularly in the most posterior part of this region of the intestine, but it rapidly spreads throughout the whole epithelium. As far as the individual cell is concerned, the accumulation of this dense mass of granules seems to be very rapid, since few cells are seen in an intermediate stage. The striated border is always present during this phase, but as the cells have increased in size, the cell wall has become stretched, and so it appears narrower.

By about three hours after beginning to feed, that is, after about six or seven feeds, there is a change in the nature of the contents of the first mid-gut. They become thick, viscous, opaque, and finely granular (as opposed to their previous state of coarse granulation), owing to the release of the granular matter in the epithelial cells. At first, the secretion clings to the epithelium in a mucus-like coat, but later the contents become a homogeneous mass which falls away easily from the epithelial wall. Sections stained with Mann's stain show the coarse granules in the centre of the lumen staining with eosin, while the surrounding secretion granules stain with methyl blue. At this stage, the mass of secretion and food material begins to pass into the second mid-gut. The cells of the first mid-gut do not seem to be in any way disrupted by release of their accumulated secretion, which is therefore of the merocrine type They remain the same size, or increase slightly in size, with a clear watery cytoplasm and a clump of granular matter and fat globules around the nuclei, while the cell wall is so distended that its striations are obliterated (fig. 3 D). Also visible in the post-secretion cytoplasm of the epithelial cells are small numbers of the bacteria which will be mentioned later. The merocrine secretion of a granular material is at variance with the conclusions of many published works on insect digestion, but the processes observed in cacao capsids are constantly and unequivocally as described. A possible mechanism by which the granules could be transmitted across an intact cell wall is suggested by the observation that they are rapidly soluble in dilute acid or alkali. The secretion is precipitated again (as an opalescent suspension) on neutralization. Thus, the secretion might be dissolved in the cell wall and then precipitated into the intestinal lumen. On the other hand, the striations in the cell border may mark the site of pores through which the granules might pass in the solid state. The fate and possible function of the granular secretion of the first mid-gut will be discussed in the section dealing with the digestive enzymes.

At the time when the above-mentioned change takes place, the insect is by no means replete and continues to feed for another two or three hours. The condition then is that both first and second mid-guts are filled with food and secretions, and the insect ceases to feed and becomes quiescent. The digestion of this meal (actually, of course, a series of feeds) takes about twelve hours before the intestine is again empty, but feeding may recommence after a shorter period than this.

By the time the insect is ready to feed again regeneration of the epithelium has taken place, together with the casting off of large numbers of degenerate cells. At the base of the epithelium there can be seen large numbers of comparatively small cells. There is a certain degree of size variation amongst them, and it is probable that they are derived not only from regenerative cells but also from young secreting cells which have reconstituted themselves. This conclusion is arrived at from the consideration that the observed frequency of true regenerative cells seems insufficient to replace the epithelium entirely at such short intervals. A process has been observed which is apparently one of rejuvenation of secreting cells. It consists of a sudden release of the watery cytoplasm of a post-secretion cell, the nuclei and cell wall remaining intact. When a piece of the first mid-gut tissue at this stage is examined as a fresh preparation, cells here and there may suddenly shrink, while a stream of fine granules issues from an indefinite point between that cell and its neighbours.

The growth of a new epithelium greatly constricts the bases of the senescent cells (which have remained swollen) so that they become spherical, and, attached very precariously on the surface of the new epithelium (fig. 3 E), they break off easily and float freely in the intestinal lumen. The contents of the first mid-gut in the last stages of a feeding cycle thus consists of a mass of large spherical cells, with nuclei in various stages of degeneration. The cell walls usually remain intact until the mass passes into the second mid-gut and is digested. The intensity of regeneration is not constant throughout the epithelium, being most vigorous in two lateral patches which lie beneath the accessory salivary gland vesicles. In other parts of the first mid-gut, it would seem that cells are able to pass through several cycles of secretion before they are ejected from the epithelium.

The question arises—does the epithelial cycle govern the feeding of the insect or vice versa? In capsids which are starved for longer than a few hours, the epithelial cells become charged with the dark secretion, although remaining small in size, but the impression gained from intensive study is that the presence of food in the first mid-gut accelerates the process. Discharge of the secretion has never been seen to occur in a starved insect.

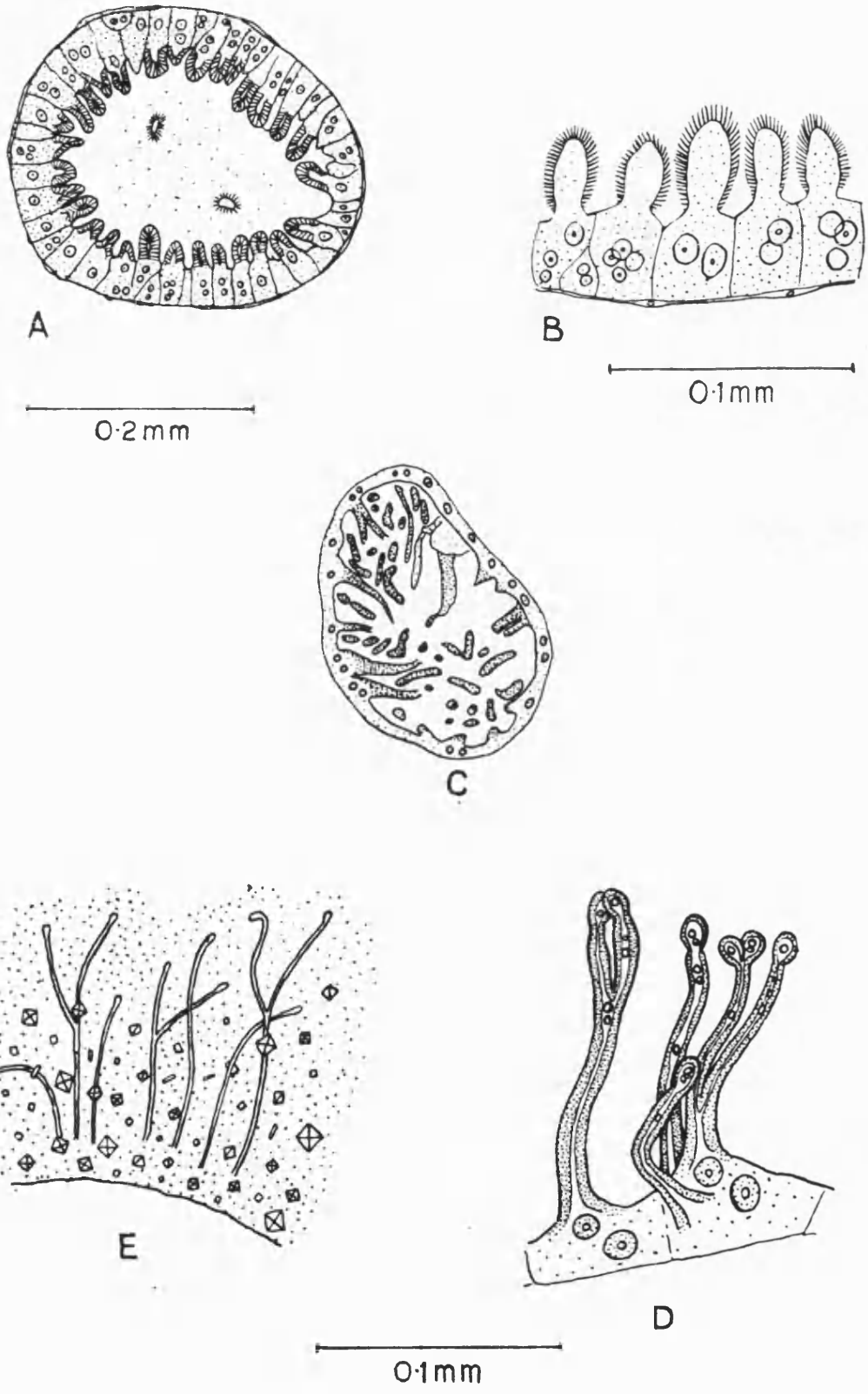
This cycle of secretion and regeneration has been elucidated under conditions in which it shows most clearly. However, in dissections of capsids which have had constant access to food, the appearance of the first mid-gut cells has always been recognizable as belonging to one or other of the above stages.

Second mid-gut.

The second mid-gut is a tubular region (fig. 1) with a constant width of about 0·5 mm. throughout its length, the latter dimension varying between 33 per cent and 45 per cent of the total mid-gut. It is joined to the first mid-gut by a distinctly constricted valve which has a columnar epithelium of binucleate cells resembling, in shape, those of the first mid-gut. There is at this point a slight thickening of the circular muscle layer.

As with the first mid-gut, there is a single layer of epithelial cells, which are of only one type. They are smaller and more uniform in size than those of the former region, size limits observed being: cells, 10 to 30 μ diameter, nuclei, 7 to 10 μ. As far as can be determined, the cells are all binucleate, though cells which appear to be quadrinucleate are occasionally visible (fig. 4 B). In section, the basal part of each cell is approximately cuboid, and from this base a large lobe projects into the intestinal lumen. This lobe is about equal in height to the cell base. It is slightly constricted where it joins the basal region, and its surface is thickly covered with long protoplasmic strands, forming a brush-like border. These strands can be seen in fresh tissue

Figure 4.



(A) Transverse section of second mid-gut. (B) Detail of cells in (A). (C) Transverse section of third mid-gut, to same scale as (A). (D) Filamentous cells in third mid-gut, drawn from a whole mount. (E) Filamentous cells in disintegrating phase drawn from fresh tissues.

to be separate from one another and to move freely with the passage of fluid past them. This border remains conspicuous in fixed material.

The cytoplasm of these cells is dense and very finely granular. During secreting activity it becomes slightly less dense and the lobes swell to a nearly spherical shape, forming a continuous layer. Apart from this there are no regular cyclical changes, although there are periods of (presumably) rejuvenation, when some of the lobes are nipped off, sometimes with a nucleus, and degenerate in the lumen. The periodicity of this occurrence is not known.

In the second mid-gut, the thick granular material from the first mid-gut is reduced to a clear, yellowish, though still viscous, fluid. The enzymes of this region act progressively as the contents move down its length, the clear digested material forming an involucrum which is thin at the anterior end, and forms the whole of the intestinal contents at the posterior end.

The second mid-gut joins the third mid-gut by a valve very similar to that at its anterior end.

Third mid-gut.

This region is also tubular, and usually slightly shorter and narrower than the preceding region, but increasing in width towards the middle of its length. It forms between 18 per cent and 30 per cent of the total mid-gut. In gross appearance it is distinguished from the rest of the alimentary canal by being of an opaque orange colour for the anterior two-thirds of its length. The epithelium of the third mid-gut is composed of binucleate cells, with size variation intermediate between the two preceding regions, the limits being cells. 15 to 45 μ, nuclei 6 to 12 μ. The cell border is a thin cuticle without visible striations. Its exact nature is not known.

At the anterior and posterior limits of this region the cells project into the lumen of the gut as tall domes, but there is a gradation from each end in which the base of the cell becomes more distinguishable from a finger-like outgrowth (fig. 4 C, D, E). At the extreme, in the widest part of the third mid-gut, the cell is wide and flat, and the outgrowth filamentous, its average dimensions being 150 $\mu \times 10\,\mu$ (fig. 4 E). In this part of the third mid-gut there is more variability in size to the outgrowths than there is towards either end. These filamentous outgrowths are terminated by small knob-like swellings, and many of them are dichotomously branched at some point in their length (fig. 4 E). Filaments which branch twice are not uncommon, each of the four branches ending in a small knob. In fixed material there appears to be a constriction below the terminal swelling, and in both fixed and fresh material the cuticle of the outgrowths is very rough and wrinkled, and takes up stains very strongly. It is encrusted with fine crystals from the insoluble food residues which accumulate in the third mid-gut. In the centre of each filament there is a distinct endoplasmic strand about one-third of the total thickness (fig. 4 E). When a meal is being digested, this strand contains groups of small shining spheres, each group causing a local swelling. The terminal knob almost invariably contains one or more such spherules. At the junction with the cell, it can be seen that the thick cuticle of the filament merges gradually into the thinner cuticle of the cell itself. The cytoplasmic strand of the filament likewise merges gradually into the less densely staining cytoplasm of the cell.

The cell outgrowths almost occlude the lumen of the gut, and a section of this region cuts them at all angles and is thus somewhat difficult to interpret (fig. 4 C). Sections cut at 20 μ, and very lightly stained, showed the structure more clearly. Since accumulation of insoluble food residues eventually completes the blockage of the intestinal lumen, some mechanism for dealing with this event is to be expected. This seems to be found in the observed fact that these cell outgrowths undergo a periodic disintegration. At these

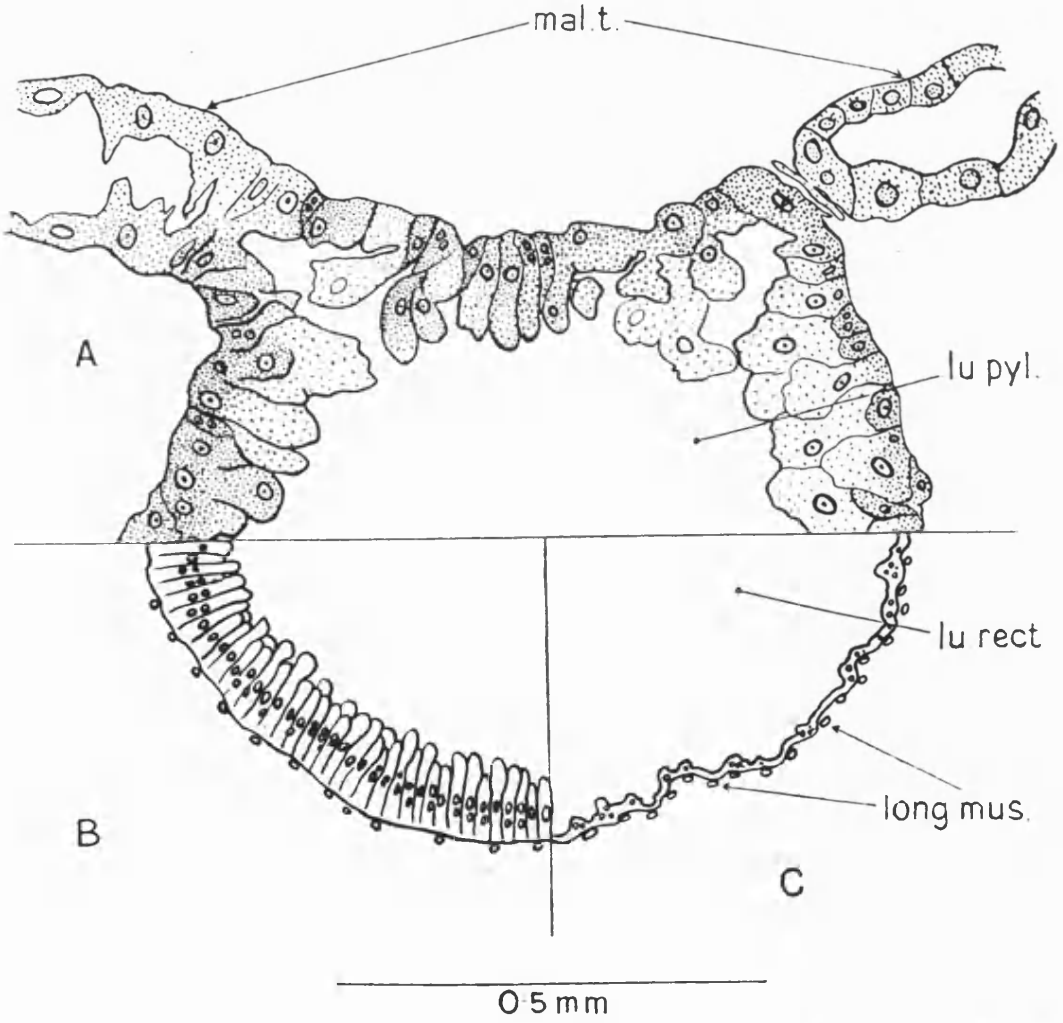
times the central cytoplasmic strands can be seen to remain amidst a mass of cuticular debris (fig. 4 D), but whether they also disintegrate or whether they can secrete a new cuticle, is not known.

The contents of the third mid-gut, which cause its orange colour, are a mass of crystalline residues, mostly consisting of the octahedral crystals found sparsely elsewhere in the intestine. Bacteria are more numerous and varied in the contents of this region than in those of the first and second mid-guts.

Hind gut.

This part of the intestine joins the third mid-gut by a valve of columnar cells similar to the other intestinal valves. It is a thin walled sac, only faintly constricted posterior to the junction with the malpighian tubules, and in effect one large chamber rather than a separate rectum and pylorus (fig. 1).

Figure 5.



Transverse sections of hind-gut at three different levels. (A) At level of malpighian tubules. (B) At recto-pyloric constriction. (C) Through rectal region. Key to lettering: mal.t., malpighian tubules; lu.pyl., lumen of pyloric region; lu.rect., lumen of rectal region; long.mus., longitudinal muscles of rectum.

When the hind-gut is distended even this slight indication of the recto-pyloric junction disappears. The hind-gut is then pear-shaped, with the third mid-gut joining it at the blunt end, and the malpighian tubule openings distributed, nearly evenly, around the widest part. Continuing from the third mid-gut

over the broad end of the hind-gut is a cap of large bulbous cells which resemble those of the posterior end of the third mid-gut and the valve joining mid- to hind-gut. This cap extends to, and includes, the openings of the malpighian tubules (fig. 5 A). From that point to the rectopyloric constriction the epithelium is columnar, the cells being equal in height to those of the more anterior part, but of about one-third the width (fig. 5 B). More posteriorly still, the cells are small and flattened, and appear somewhat irregular in section (fig. 5 C). The junctions between these different types of epithelium are not sharp, and lie in planes uniformly perpendicular to the long axis of the hind-gut. From the region just posterior to the openings of the malpighian tubules to the anus the longitudinal muscle fibres are more conspicuous than elsewhere in the alimentary canal, and only in this region can any definite indication of a chitinous intima in the hind-gut be seen.

There are no rectal glands or specialized regions of any sort in the hind-gut of cacao capsids. The anal musculature consists of a pair of powerful muscle bands, dorsal and ventral. The contents of the hind-gut are a clear yellowish fluid with a small proportion of solid debris.

Malpighian tubules.

These organs, of which there are four as is usual in Heteroptera, are each about one and one-third times the length of the insect. They are coiled in the last two segments of the abdomen, and the bulk of them lies posterior to their point of junction with the intestine. They are slightly constricted at this latter point, and, as has been mentioned, the openings are not significantly associated into two pairs. Their proximal eighth is about 0·25 mm. to 0·27 mm. in diameter and is colourless, while the remainder tapers to a terminal width of 0·12 mm. to 0·14 mm. and is coloured green or brown by granules in the cytoplasm of the cells. While the proximal half of each tubule is curved in a few large radii, the distal half is strongly convoluted. It is the coloured region which can be seen to absorb dyes, either injected or *in vitro*. Indigo-carmine added to the saline in which dissection took place appeared in the lumen within five minutes. The contents of this region of the malpighian tubules are fluid, while those of the wider, colourless, proximal region contain a high proportion of very small crystals.

The cells of the tubules are large (90–100 μ) and flattened, with large, uniformly staining nuclei. No striated border has been detected, but, as seen in sections, the nucleus appears to rest on a base of dense, apparently perpendicularly striated cytoplasm, while a large vacuole occupies the apical part of the cell. The size of the cells does not vary with the changing diameter of the tubule.

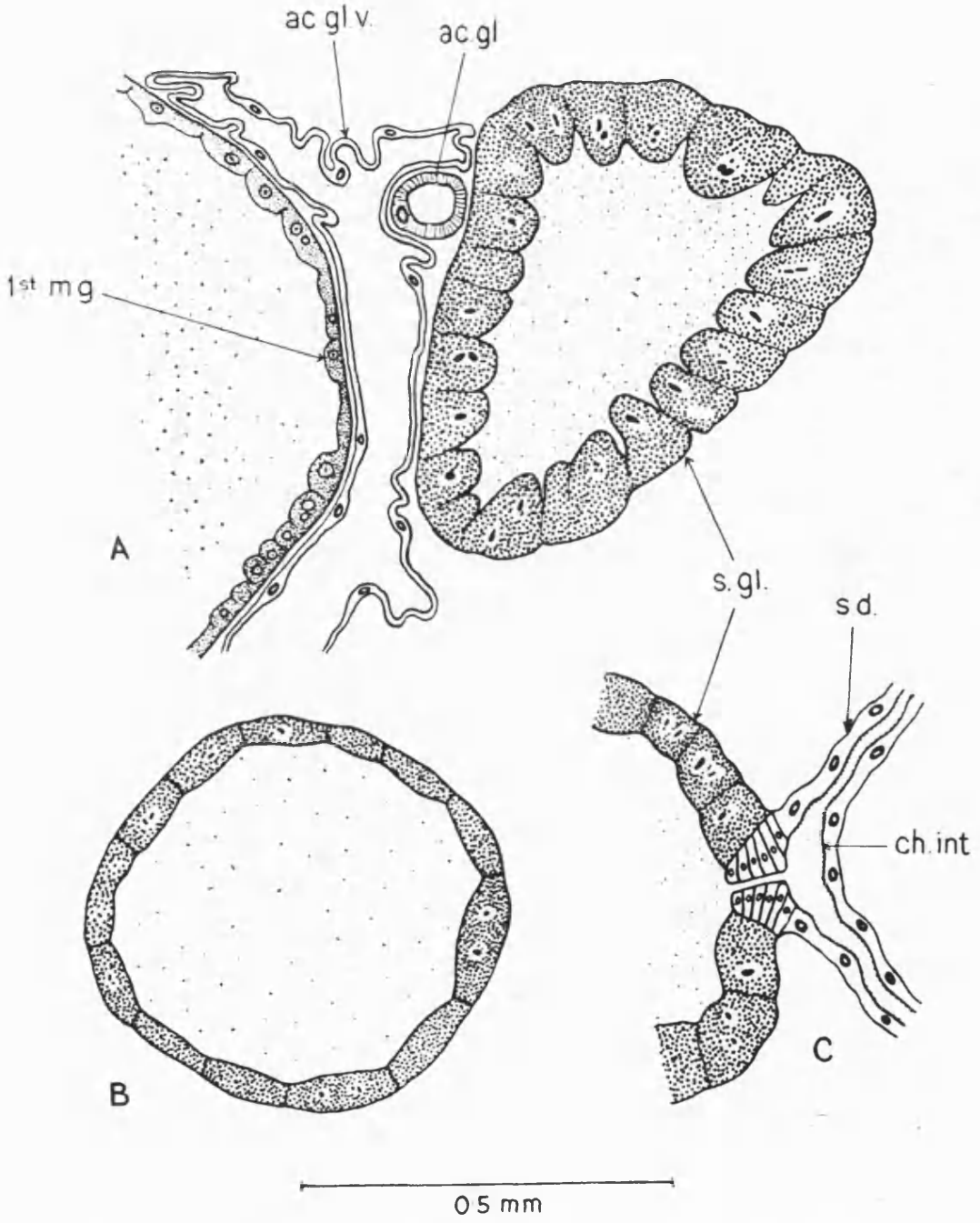
Salivary (labial) gland system.

This consists of a pair of very large main glands and a pair of accessory glands, the whole being situated lateral to the first mid-gut. As described by Squire (1947), the main glands are four lobed, with two long posterior lobes situated in the abdomen, and two shorter anterior lobes situated partly in the abdomen and partly in the thorax (fig. 1). The whole gland about equals in length the first mid-gut. The dorsal lobes, anterior and posterior, are of simple finger-like shape (the anterior about half the length of the posterior), with an irregular outline. The posterior ventral lobe also has an irregular outline, but the simple shape is modified by the presence of three ventral swellings, while the anterior ventral lobe has a smooth outline and a narrow conical shape. This latter lobe is slightly constricted where it joins the rest of the gland, and the junction with the main gland of the salivary and accessory gland ducts is situated at its base.

All four lobes of the main salivary gland are alike in the histological detail of their cells. The cells of the anterior ventral lobe (the distinct shape of

which has been mentioned) are more flattened than those of the other lobes (fig. 6 B), and the secretion of this lobe contracts into a granular clump under the influence of a fixative, while that of the other lobes remains a uniform

Figure 6.



(A) Transverse section showing spatial relationship between first mid-gut, accessory gland, and main salivary gland. (B) Transverse section of anterior ventral lobe of main salivary gland. (C) Transverse section, junction of salivary duct with salivary gland. Key to lettering: 1st m.g., epithelium of first mid-gut; ac.gl., accessory gland tube; ac.gl.v., accessory gland vesicle; s.gl., main salivary gland; s.d., salivary duct; ch.int., chitinous intima of salivary duct.

structureless mass. The anterior ventral lobe sometimes showed a slightly stronger uptake of methylene blue *in vitro*, but in the fresh state the difference in nature of the secretion was not detectable. The lobes join in a large central

chamber, and in this and the lobes themselves there is only one type of cell. The cells (fig. 6 A) are large (60 μ in diameter) and uniform in size, strongly domed internally, but never, as in the first mid-gut for instance, laterally compressed into a columnar form. There is no separate basement membrane or connective tissue detectable. The cytoplasm of the cells stains very deeply with basic stains, so that the nuclei show as pale areas with irregular dark central granules. In fresh tissue the cytoplasm is very finely granular with a few small vacuoles. The nuclei are ovoid and usually lie perpendicular to the base at the widest point of the cell, and all the cells are binucleate.

The secretion shows as a dense mass in the lumen of the gland, staining with eosin or (with Mann's stain) methyl blue. With the latter stain, the origin of the secretion as minute droplets can be seen, the droplets being most distinct and most densely stained near the surface of the cells, and merging inwardly into the structureless mass of secretion. Secretion is merocrine and no striated border is visible in either fixed or fresh material. No rejunative changes have been observed, nor do true regenerative cells appear to be present. When the gland is dissected into distilled water or hypotonic saline, the contents soon become an opaque white mass owing to the formation of a flocculent precipitate. Glands from moribund individuals contained a small amount of this precipitate even when freshly dissected. The salivary gland secretion, even when this precipitate was present, showed an enzyme activity not much less than normal.

The accessory salivary gland consists of a large thin-walled vesicle and a thick-walled glandular tube. The vesicle is sagittate in shape with the point posteriorly directed, and the tube joining it at its anterior end between the two basal cusps. When the vesicle is distended the points become rounded into a heart shape (fig. 1). These vesicles are closely applied at either side of the widest part of the first mid-gut, and are bound to that organ by fine tracheae. Their epithelium is thin, in relation to the size of its nuclei, and the cell boundaries are indistinct. The rather flattened nuclei are spaced singly, 40 to 50 μ apart, and are 15 to 20 μ in diameter (fig. 6 A). In the cytoplasm, which is dense and finely granular, many small vacuoles may be seen, of 5 to 8 μ diameter. In sections (*i.e.* fixed material) both the inner and outer borders of the cells appear thick in proportion to the thickness of the whole, and a finely ciliated fringe may sometimes be seen on the internal surface. No such structure is visible in fresh tissue, nor is there any form of striated cell border.

The tubular part of the accessory salivary gland has a cellular structure not very different from the vesicle. In it, however, there are more distinct cell boundaries (the size and distribution of the nuclei being similar), the cytoplasm in fixed material appears denser than that in the vesicle epithelium, with fine striations radial to the axis of the tube, and there is a definite, circularly ridged, chitinous intima. The intima has, at irregular but frequent intervals, small funnel-like excrescences projecting into the cytoplasm, which may mark the points at which the secretion of these cells is released. This tubular gland is about 75 μ in diameter with a lumen of about 50 μ. It extends forward to the head of the insect, where it loops around the dilator muscles of the salivary pump and joins the narrower (*circa* 45 μ) and non-glandular accessory gland duct.

The salivary and accessory gland ducts are narrow thin-walled tubes with a circulatory thickened chitinous intima. Their cytoplasm has no apparent cell boundaries, the nuclei being uniformly distributed about 30 μ apart. In the nuclei, the dark-staining matter is in the form of small scattered granules. The cytoplasmic wall of the duct is distended where the nuclei occur (fig. 6 C) and they are spaced around the lumen so that the latter follows a helical path. The two ducts named above are in effect one continuous

duct, joining the salivary gland tangentially, and being connected to its lumen by a short collar of small, uninucleate, cuneiform cells (fig. 6 C) which is inserted between the cells of the main gland. The accessory gland duct is about 25 per cent longer than the salivary duct, and follows a more convoluted path through the thorax. The salivary duct travels forward from the main gland to the salivary pump, which is of the usual Hemipterous type (*cf.* Woolley, 1949).

Function of the intestine and salivary glands.

Evidence concerning the function of the digestive system has been gathered from observations of the feeding habits of the insects and of the appearance of the gut contents at different stages. As has been mentioned, the feeding cycle is conditioned by changes in the mid-gut, the quiescent state being associated with complete filling of the mid-gut and the subsequent digestion of its contents. It has also been observed that the air-filled starvation-condition of the first mid-gut is common in insects in constant contact with food, and that it is associated with a noticeably greater irritability and readiness to take flight. This would seem to be of biological value in its effect on the ovipositing female, in that successive egg batches will be fairly widely distributed.

With regard to the function of the salivary glands, it will be necessary to describe the exact sequence of events during feeding. First of all the stylet-like mouth-parts are inserted into the cacao pod or stem, usually without much hesitation or prolonged exploration. The insect raises its head so that the rostrum can be brought into a vertical position, and then gently sinks while the stylets penetrate and the rostrum folds backwards at its first (*i.e.* lowest) joint. Not uncommonly, very deep penetration takes place, when the rostrum folds again at its third joint, the second joint retaining a grip on the stylets, and the rostrum being W-shaped in side view.

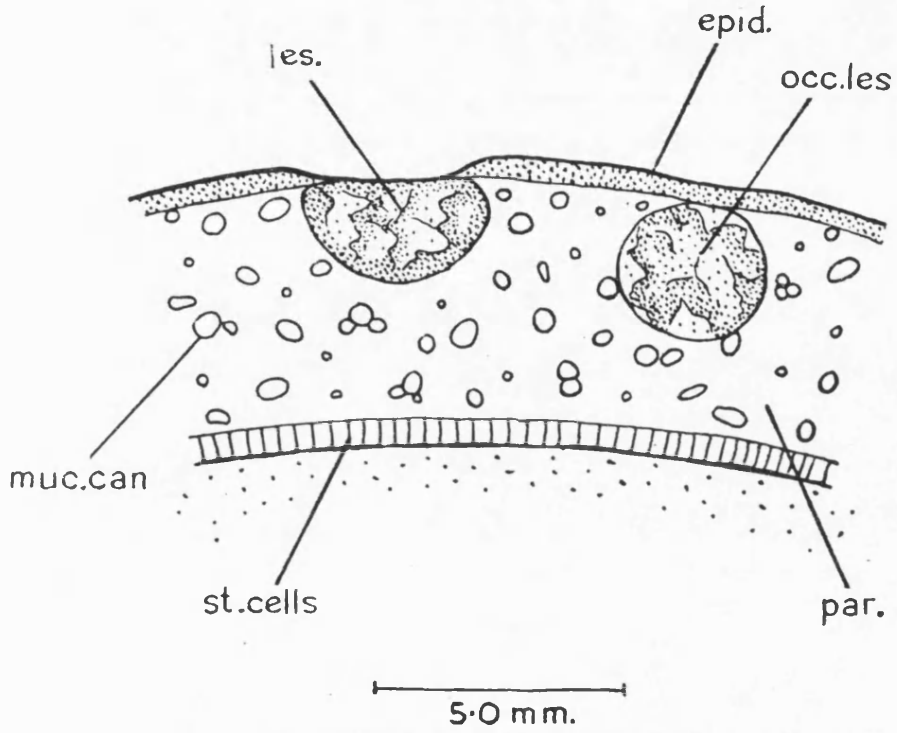
The appearance of the " water-soaked area " (*vide* Squire, 1947) is the next visible occurrence, this area being that which will later become a brown sunken lesion. Leach & Smee (1933) working with *Helopeltis bergrothi*, as a pest of tea in East Africa, describe the water-soaked area as appearing subsequent to the insect's feeding, but on cacao pods it definitely appears between two and five minutes after the beginning of a feed which may last for thirty minutes or more. The length of time taken by one feed varies inversely with the temperature, from about thirty minutes at 75° F. to ten minutes or less at 85° F., and is remarkably constant at a given temperature. At the end of a feed, the insect withdraws its stylets and, after moving a short distance, almost immediately inserts them again. The area of the next feed may overlap the previous lesion, and is never very far away from it. The lesions formed by these feeds are, on pod tissue, circular spots about 3 mm. in diameter, sometimes as large as 4 mm. This area, about the same as that of the insect's thorax, is beyond the possibility of being merely mechanical damage by the stylets.

In section, a fresh lesion (or " water-soaked area ") on cacao pod cortex is a brownish hemisphere contrasting with the greeny-white of the undamaged tissue. The cellular structure is softened, but if cut very carefully remains intact, and there is a brown staining of the cell walls. Disruption of the tissue is at this stage very slight, but the affected cells are killed by the saliva and eventually shrivel, leaving a cavity (fig. 7). The lesion does not exude any fluid on to the surface of the plant. The first feed after a period of starvation may be prolonged, and the stylets may penetrate so deeply that there is no external damage and the lesion is completely occluded within the pod cortex. It is this fact which undoubtedly gives rise to the apparently lower number of lesions per instar with pod feeding as compared with stem feeding.

Defecation, in the form of a single globule of about 0·5 cubic mm. of a golden-brown fluid, first occurs after two or three feeds, in a previously starved

insect. By this time the abdomen, from being flattened, has regained its normal biconvex shape. At about the same time as defecation begins, the insect begins to regurgitate small drops of fluid from the mouth-parts. During this act, the rostrum is held vertically as if about to feed, but instead of the usual gentle sinking, this position is maintained while a small drop of clear fluid appears around the tip. The rostrum, still extended, may then be moved a millimetre or so and another drop deposited, and not infrequently a third. The insertion of the stylets into the plant tissues has never been seen to take place through one of these drops (*cf.* K. M. Smith's observations on *Psallus ambiguus*), the insect moving away before settling down to feed again.

Figure 7.



Section through capsid lesions in cacao pod. Key to lettering: epid., epidermis of pod; les., normal capsid lesion; occ.les., occluded capsid lesion; muc.can., mucilage canals; par., parenchyma of pod cortex; st.cells, layer containing many calcified cells.

This regurgitation is a regular feature of the feeding behaviour of cacao capsids. Apart from observation of the behaviour, a study of the excretory droplets themselves indicates a dual origin. These droplets (their actual source not having been observed) can be easily divided into two classes by their appearance and behaviour when taken up into strips of litmus paper. One class (the faecal droplets) are large, almost always single, golden brown, sticky, and distinctly acid to litmus. The other class, equally abundant in number though not in volume, is the exact opposite, namely, smaller, usually two or three together, clear, watery and alkaline to litmus.

The source of these regurgitated droplets could be the salivary glands or the intestine. The latter seems to be ruled out, since the intestinal contents in the early stages of feeding are granular, not clear, and quite strongly acid, rather than alkaline. Furthermore, the oesophageal valve exists for the very purpose of preventing regurgitation when suction is generated by the pharyngeal pump. Further evidence comes from experiments with the injection of solutions of neutral red. This dye, when injected into the

haemocoele, very rapidly passes into the lumen of the intestine throughout its length, staining the tissues only faintly. It was expected, therefore, that excretions originating in the alimentary canal, including faeces, would be coloured red. What was actually found to happen was that both clear and coloured droplets were being deposited simultaneously, from a few minutes after injection to the end of six hours observation. The source of each droplet was not actually observed, but since it had been found that neutral red entered the first mid-gut as readily as it did the rectum and malpighian tubules, the presence of uncoloured droplets was regarded as evidence against their origin in the intestine.

Supposing the origin of the regurgitation to be the salivary glands, the evidence points to the accessory glands since their contents are watery and slightly alkaline, while those of the main gland are thick and syrupy and about neutral in reaction.

Function of the accessory salivary gland.

The presence of a vesicle or reservoir on the accessory salivary gland has been described in the predominantly carnivorous families of Hemiptera-Heteroptera. Opinion as to its function has varied, with different authors, between that of a reservoir and that of a secreting organ (*e.g.* Poisson, 1924 ; Breakey, 1936). The vesicular type of accessory gland has not been found in the predominantly phytophagous families Pentatomidae, Coreidae, Pyrrhocoridae, Lygaeidae, and their allies (Baptist, 1941).

In the cacao capsids, the close anatomical relationship between the accessory gland vesicles and the first mid-gut has been mentioned, and can be seen in fig. 6 A. It seems likely that there is a filtration of water from the ingested food into these vesicles, rather in the manner of the filter-chamber in the mid-gut of some Homoptera (Weber, 1930). The reservoir function, as far as cacao capsids are concerned, can be discounted, since, firstly, the main gland lumen itself is actually more capacious than the accessory gland vesicle, and secondly, the main secretion is distinct in both fresh and stained material, and has never been detected in any other part of the salivary system. On the other hand, though the accessory gland does secrete enzymes in detectable amounts, the hypothesis of a purely secretory function offers no explanation of the anatomical relation with the first mid-gut.

The following observations provide evidence supporting the hypothesis of a water absorbing function. First, the walls of both the first mid-gut and the accessory gland vesicle are osmotically permeable, and imbibe large amounts of water when dissected into hypotonic solutions, and secondly, that water passes out of the first mid-gut during feeding can be reasonably inferred from the observation that the coarse granular matter becomes more concentrated as feeding progresses (*before* the release of secretion, described above). Finally, the accessory gland vesicles show distension and contraction appropriate to the hypothesis, in that they are distended during feeding and digestion, and contracted during starvation, while the main gland, as far as it varies at all, tends to be distended with accumulated secretion during starvation. The slightly greater activity of the first mid-gut epithelium beneath the accessory gland vesicles may possibly be due to a localized outflow of water.

It seems that there is a restricted water cycle encompassing the first mid-gut, accessory gland, saliva, feeding site, and so back to the first mid-gut. Water would be added to this system from the plant tissues, and by its accumulation bring about the necessity for regurgitation of the excess. The impression has been gained from a careful study of these insects, that the saliva as injected into the plant is mostly the watery secretion of the accessory gland, with which a small quantity of the thick main gland secretion is mixed, and the regurgitations are therefore surplus saliva.

Experiments with injected dyes.

Some evidence concerning the function of the intestine has been supplied by the behaviour of certain vital dyes, notably methylene blue, when injected into the haemocoele. Many dyes other than methylene blue have been used, and the action of neutral red has already been referred to above. Others were either rapidly excreted by the malpighian tubules, and did not stain any other tissue appreciably (*e.g.* indigo-carmine, trypan blue, gentian violet), or were absorbed by the pericardial cells and other scattered groups of phagocytic cells (*e.g.* ammonia carmine, congo red).

The larger capsids (*Sahlbergella singularis* and *Distantiella theobroma*) were used for the injection experiments, and proved to be very amenable to such treatment. The method used was to inject the dye with a fine glass syringe into the midst of the thoracic muscles. The pronotum could be moved aside for this purpose without injuring the insect, thus exposing an area of thin, soft, cuticle. Between 0·1 and 0·2 cubic cm. of a dilute solution of the dye was injected into each insect.

Methylene blue was found to be consistently taken up, not only into the malpighian tubules and thence into the rectum, but also into the tissues of the second mid-gut and accessory gland vesicle. The posterior part of the first mid-gut and the rectum-mid-gut valve were sometimes stained, as well as parts of the ovarian system, while the main salivary gland and third mid-gut (the orange coloured region) only very rarely took up the dye. The point of interest is that the uptake of methylene blue coincided with those parts which other evidence suggested were probable sites of translocation of water out of the haemocoele. Apart from the malpighian tubules, which actively and selectively absorb substances from the blood, there is to be considered the constant secretion of enzymes by the second mid-gut, and the cyclical secretion of the first mid-gut, which processes will necessitate the withdrawal of water and nutritive material from the blood. There is likewise a strong flow into the accessory gland vesicle, but presumably the main gland secretes too slowly to have an appreciable inflow from the haemocoele.

The third mid-gut, which did not stain with methylene blue, is almost certainly a region of absorption from the lumen of the intestine, since it contains so high a concentration of the insoluble crystalline constituents of the food. The flow of soluble matter through the intestinal wall is here in the opposite direction to that occurring in the secreting regions. In the first mid-gut there is a varying balance between outward flow of water and inward flow of secretion, and, correspondingly, this organ is variable in its uptake of methylene blue.

When the internal surface of the intestinal epithelium is presented to the dye solution, as when the intestine is teased apart in such a solution, the reaction to the dye is inverted. The filaments of the third mid-gut epithelium absorb the dye strongly and rapidly, while the secreting lobes of the second mid-gut only absorb it weakly. The intense absorption of dyes by the third mid-gut filaments was found to be constant for a very wide range of dye-stuffs, both acidic and basic, including the dyes quoted above, and also basic fuchsin, safranin, and the indicators used for pH determinations. This great affinity of the third mid-gut cells for these large organic molecules must be in some way related to the absorptive function of this region. Since the mid-gut possesses this specialized absorptive region, it is of interest to recall that there are no areas in the hind-gut obviously specialized for this purpose. Such rectal glands occur in many other Hemiptera, and even in the not very distantly related Mirid, *Proboscidiocoris fuliginosus* Reut., there are conspicuous organs of this nature. According to the observations of the present author the occurrence of rectal glands in Hemiptera is associated with the absence from the mid-gut of any obviously absorptive region, and the occurrence of a

thick food mass in the rectum, rather than a watery excrement. This does not exclude the probability that a certain amount of diffusion of digestion products into the haemolymph occurs in all regions of the intestine.

Hydrogen ion concentration of the intestine and its contents.

For the determination of pH of the different parts of the gut and their contents, only simple methods were available. The first work on this subject was the dissection and teasing of the intestine in a solution of B.D.H. Universal Indicator under the low power of a binocular microscope. Having obtained some idea of the ranges of pH involved, the appropriate indicators for those ranges were used, the technique being to place the dissected parts of the gut into small drops of the indicator on waxed glass tiles. Where possible, a double check was obtained by repeating with another indicator having a range overlapping that of the first one. In all cases the observations were repeated until their veracity was beyond reasonable doubt. Table I gives the results obtained, the values for pH being as near as the method permitted. It will be seen that the food when first ingested is strongly acid, and that this acidity is somewhat reduced by the secretion of the first mid-gut. From that stage the processes of digestion, and perhaps bacterial action, cause a progressive increase in acidity as the food passes down the intestine. When the second mid-gut contains much undigested matter the pH found by using the technique described was apparently about 6·0 but the digested matter could be seen, under magnification, to be more acid than the bulk of the gut contents.

A determination was made of the pH of cacao pod cortex tissue, since no figures were available for the pH of this or any other organ of the cacao plant. The following method was used. About 4 cubic cm. of distilled water was put into each of two clean test-tubes and a few drops of indicator added. In one tube was placed a slice of pod cortex with the epidermis peeled off. The two tubes were left overnight and the colours compared. The pod cortex produces mucilage which converts the contents of the test-tube into a jelly. It was

Table I.

Hydrogen ion concentration in the digestive system.

Organ tested.	pH.	Indicator used.
Saliva	7·5 to 8·0	Litmus paper Phenol red
Salivary gland (main)	7·0 to 7·2	Bromothymol blue
Salivary gland (accessory)	7·0 to 7·5	Phenol red
First mid-gut (contents at beginning of feeding).	5·2	Bromocresol purple Bromocresol green
First mid-gut (secretion)	7·0	Bromothymol blue
First mid-gut (contents at end of feeding).	6·0	Bromocresol purple
Second mid-gut (contents undigested)	6·0	Bromocresol purple
Second mid-gut (contents digested)	5·2 to 5·5	Bromocresol purple
Third mid-gut (during feeding)	4·5	Bromocresol green
Third mid-gut (after short starvation)	3·6 to 3·8	Congo red
Faeces	4·5 to 5·5	Bromocresol green Bromocresol purple

found that it changed the colour of the indicator to a moderately acid point, estimated with bromocresol purple at pH 5·8. An attempt was made to compare the pH produced by pod cortex bearing many capsid lesions with that produced by cortex undamaged by capsids. It was found that oxidative changes in the lesion caused a move in the alkaline direction, the same change occurring at the surface of undamaged tissue when the epidermis is peeled off and the mucilage is exposed to the air. Thus, it was not possible to obtain clear evidence for the pH of capsid lesions.

Enzymes of the saliva and alimentary canal.

A certain amount of work on the salivary glands of the family Miridae has been done using simple methods, for instance, placing dissected glands on to host plant tissue or on to slices of potato, cucumber, etc. (Smith, K. M., 1920). There is little in the literature concerning the intestinal enzymes of phytophagous Hemiptera. Although a certain amount has been achieved in this direction, the main object of the present work has not been merely to make a list of enzymes detected. It is a sufficiently well established principle that the enzyme complement of an animal's digestive system becomes adapted to its diet, to make such a list of little interest. What is of interest is the mechanism of action of the saliva, the extreme phytotoxicity of which is the reason for the economic importance of cacao capsids and of capsid bugs in general, and the relation of the salivary to the subsequent intestinal digestion.

Squire (1947) inferred the presence of what he called a histolytic enzyme, from the nature of the damage done to cacao tissues, and performed a simple experiment with salivary glands placed on thin slices of cucumber, which, he claimed, demonstrated a pectinase. Repetition of this sort of experiment by the present writer gave very indefinite results. Dissected glands had, as Squire records, no effect whatever on cacao tissue, owing to its mucilage protecting the living cells. Smith (1920) produced damage on apple leaves similar to that of *Plesiocoris rugicollis*, by injection of toxic chemicals such as ammonia. An attempt was made to produce damage on cacao by such means. A two-foot length of quarter-inch glass tubing was drawn to a point, so that, when full of water, a flow at the rate of about one drop in ten seconds occurred. It was then filled with 5 per cent ammonia solution and clamped vertically in a burette stand. A fresh cacao pod was placed beneath the stand, and the glass point lowered gently so as to just penetrate the epidermis. No fluid leaked out from around this puncture. The apparatus was left overnight, but only a pinhead spot of brown, dead, cells was found, insignificant compared with the 3 mm. diameter of a capsid lesion. It seems probable that a much higher hydrostatic pressure is generated by the capsid when injecting its saliva.

A series of experiments was embarked upon, using techniques based on those of Roy (1937) and Hawk, Oser & Summerson (1947). The principle of the techniques used is that the desired parts of the insect are dissected out, crushed or ground, and the tissue suspension allowed to react with a pure substrate, the mixture being subsequently tested for the presence of digestion products or the disappearance of the substrate. In the present work great attention was paid to the hydrogen ion concentration. Solid watch glasses, if used as vessels for grinding the tissues with sand, proved to release too great an amount of alkali, and all grinding was done with an agate pestle and mortar, using carborundum if an abrasive was thought necessary. A refinement which greatly assisted in some of the subsequent tests was to centrifuge the tissue suspension, and use only the clear enzyme solution. The reaction vessels were, for convenience, six by half-inch test-tubes, and the volume of enzyme and substrate was between one and two cubic centimetres according to the particular experiment. A few drops of toluene were added to each tube to suppress bacteria.

Owing to the small size of the insect and the fact that in all cases only part of the intestine was being used, about twenty individuals were dissected for each experiment. The descriptions of the experiments and results will be given under the headings of the different enzymes investigated.

Amylase.—Simple preliminary experiments showed a strong salivary and intestinal amylase. This easily detectable enzyme was used to investigate the optimum hydrogen ion concentration for the functioning of the salivary and first mid-gut secretions. Five pH values were tested, the stock of B.D.H. buffer solutions being limited to pH 4 to pH 8 inclusive. The suspensions of first mid-gut and salivary gland tissue were diluted so as to give about 1·0 cubic cm. for each pH value, and two drops of buffer solution were added. Four drops of 1 per cent starch solution were then put in each tube, toluene added, and the tube closed with a cotton-wool plug. They were then left for twenty-four hours at the laboratory temperature of 80° F., since no incubator was available. At the end of this time, five drops of iodine solution (Lugol's) were added to each tube, and the presence or absence of starch noted. The pH was also checked at this time.

A fairly constant result was obtained over twelve replications, and the optimum pH of the salivary amylase was found to be in the region of pH 5, with some action of pH 6, but very little at pH 4, 7 or 8. With the first mid-gut amylase, there was a two-fold optimum, one optimum point being at pH 5 and possibly due to ingested saliva, the other optimum being at pH 7 to pH 8. The rate of action and optimum pH of salivary amylase were not significantly altered by the omission of the accessory gland from the tissue suspension. On the other hand, an amylase was found in the accessory gland, which, when the small volume of tissue in the accessory gland is taken into account, must be regarded as not much less powerful than the main gland enzyme.

For the comparisons of main gland and accessory gland amylase, the reacting mixture was buffered to pH 6 and controls were used which were boiled for five minutes before the starch substrate was added. Three drops of 1 per cent starch were used in 0·5 cubic cm. of buffered tissue suspension. Comparisons of the three sub-divisions of the mid-gut with regard to amylase content showed that the second and third mid-guts did not secrete this enzyme. A slight action, capable of being produced by enzymes carried from the first mid-gut, was sometimes found in the second mid-gut, but the third mid-gut was consistently negative. Boiled controls were used in these tests, and showed no action on the starch.

Invertase.—Next to the amylase in ease of detection and strong action was the invertase of the capsid intestine. In tests for this enzyme, 1·0 cubic cm. of diluted tissue suspension, buffered to pH 6, had ten drops of 1 per cent sucrose added to it, and was allowed to react for twenty-four hours. At the end of that time, sufficient Fehling's solution to give a faint blue tint was added, the boiled control being treated similarly and both were warmed in boiling water for a few minutes. A copious orange precipitate, absent in the control, showed that the tissue suspension was capable of inverting sucrose. This was found to occur mainly in the first and second mid-guts. The salivary glands had no action at all and the slight amount of invertase found in the third mid-gut was probably not secreted there. In addition to boiled controls in these experiments, unboiled controls to which no substrate had been added were used. This was to check on the amount of reducing sugars present in the intestinal contents themselves. In all controls, action on Fehling's solution was negligible. In an experiment on the optimum pH of the first mid-gut invertase, it was established that the effective range was pH 5 to pH 7, but there was a slight indication of a bimodal optimum, with less activity at pH 6.

Maltase.—The methods used were similar to those described for invertase, but substituting maltose for sucrose, and testing with either phenylhydrazine

acetate (for osazone formation) or Barfoed's cupric acetate reagent. The latter reagent was found to be the most convenient. Only a slight reaction was obtained, perhaps owing to the absence of incubation and the short duration (twenty-four to forty-eight hours) of the experiments. The reaction was weaker with the salivary gland than with the first and second mid-guts.

Lactase.—In experiments exactly similar to the above, using lactose instead of maltose, no reaction with Barfoed's reagent could be detected.

Pectinases.—The mucilage which permeates cacao tissues in the micro- and macroscopic mucilage canals is evidently related to the pectins. Its jelly-like nature is destroyed by boiling for thirty minutes with dilute acid or alkali, and the resulting fluid reacts with phloroglucinol and hydrochloric acid in a manner suggesting the presence of pentoses. Mucilage for digestion experiments was prepared by soaking small pieces of pod cortex in about three times their volume of water. After soaking overnight the mucilage was scraped off the pod fragment and the latter discarded. About 2 cubic cm. of mucilage in a test-tube was shaken up with about 0·5 cubic cm. of tissue suspension, and about a quarter of an inch of toluene added as an air-excluding layer. A duplicate was prepared with boiled tissue suspension. For subsequent tests the following were used :

(*a*) visual assessment of change of viscosity,

(*b*) test with Fehling's solution, and

(*c*) test with phloroglucinol and hydrochloric acid.

Owing to the fact that the mucilage contained soluble carbohydrates leached from the pod, test (*b*) was neither reliable nor consistent. No part of the salivary gland or intestine of the capsids was found which produced any change in the mucilage as judged by the other two tests, and it was concluded that the mucilage was not utilized by these insects to any appreciable extent. It was not found that the bacterial flora of the capsid intestine had any action on cacao mucilage and the sticky nature of the faeces is therefore possibly due to unchanged mucilage.

Proteases.—Investigations into the proteases were somewhat hampered by the fact that suitable substrata and test chemicals were not available. Samples of carmine- and congo red-fibrin were prepared, but no digestion of these could be obtained with any part of the intestine or salivary glands. The following substances, used as substrata, gave slight indications of protease action, and consistent distribution of such action among the different organs tested.

(*a*) Diluted tinned milk (fresh milk not being available) calcified with with 10 per cent of a 5·5 per cent solution of calcium chloride. The criterion of protease action was the coagulation of the milk.

(*b*) Small fragments of coagulated egg-white, in acid and alkaline solutions, subsequently tested for peptones by the biuret test (see Hawk, Oser & Summerson, 1947).

(*c*) Dilute egg albumen solution, which was turned brown in colour by the action of certain parts of the intestine.

The distribution of protease action was as follows :

Salivary glands.—With test (*a*) a very slight action was observed, but no action on (*b*) or (*c*).

First mid-gut.—Detectable action on all three types of substrata.

Second mid-gut.—Detectable action, in some replicates distinctly stronger than first mid-gut, on all three types of substrata.

Third mid-gut.—As second mid-gut, the digestion initiated in the second mid-gut continuing in the contents of this region (there is no histological evidence of secretion in the third mid-gut).

In test (*b*) the action in acid solution was stronger than in alkaline solution, as would be expected from the acid reation found in the intestinal contents. There was no action in boiled controls, but it was found necessary to boil the control tubes for fifteen to twenty minutes to destroy the enzyme. A comparison of the whole salivary glands and the whole mid-gut, using reconstituted powdered milk as a substrate, showed no action by the salivary glands, but coagulation and discoloration was caused by the intestinal secretions. It would appear that the mid-gut, particularly the middle portion ot it, contains a rather weak protease with an optimum on the acid side of neutral. There is no evidence for a histolytic protease in the saliva.

Peptidases.—The techniques used proved to be insufficiently sensitive to detect any peptidases. A substrate of "Difco Bacto-Peptone" was used, tested for disappearance of peptone by the biuret test.

Lipase.—Two kinds of substrata were used to detect lipolytic action. These were (*a*) undiluted tinned (evaporated) milk, and (*b*) an aqueous emulsion of "salad oil" (probably cotton-seed oil). Indicators were incorporated into these substrata and the action of the lipase was estimated by the degree of acidity produced. Because of the prevailing acidity of the gut contents, the indicator used was bromocresol purple (range pH 5·2 to 6·8) and the substratum was made alkaline to this indicator before the experiment began.

The result from tests on substratum (*a*) was that after a long period, forty-two to forty-eight hours, all tubes (except controls) had become acid to the indicator, but after shorter periods there was a gradation in rate of action, from the salivary glands with the weakest lipase, to the second mid-gut with the strongest. With substratum (*b*) the salivary glands showed no action even after forty hours, and the first and second mid-guts were about equal in action.

Esterase.—Using the technique of the above lipase tests, with an aqueous emulsion of amyl acetate as a substratum, all parts of the digestive system were found to produce strong acidity, while boiled controls remained alkaline to bromocresol purple. The action was equally strong in the salivary glands as in the first and second mid-guts, and it was found that the omission of the accessory glands did not affect the result.

Summary of enzymes detected.—In table II the presence and relative strength (in terms of ease of detection) of the enzymes found in the digestive system of cacao capsids are summarized.

TABLE II.

The enzymes of the digestive system.

Organ tested.	Enzymes present. —not detectable. + detectable. ++ action powerful.							
	Amylase.	Invertase.	Maltase.	Lactase.	Pectinase.	Protease.	Lipase.	Esterase.
Main salivary gland.....	+ +	—	—	—	—	—	+	+ +
Accessory salivary gland	— +	—	*	*	*	*	*	*
First mid-gut	+ +	— —	—	—	—	+	— +	— +
Second mid-gut	—	— —	—	—	—	+	— +	— +
Third mid-gut.....	—	—	—	—	—	+	+	+

* Not investigated.

Physico-chemical characteristics of the secretions.

There are certain observations on the nature of the salivary and intestinal secretions which are of interest. The salivary secretion, in the form of a centrifuged tissue suspension, is cloudy in appearance. When acidified with buffer solutions of pH 4 and 5, the cloudy matter becomes a flocculent precipitate, with a clear supernatant liquid, but, as has been mentioned, there is strong amylase action at pH 5. Conversely, the amylase is quite easily destroyed by boiling, although the cloudy matter is not coagulated by this. It is of interest to note that the precipitate is, in microscopic appearance, very similar to the granular material found in the first mid-gut, but without the more coarsely granular fraction.

The first mid-gut tissue suspension gives a clear supernatant liquid when centrifuged, which contains most of the enzyme activity. This clear fluid throws down a white coagulum when boiled, and the enzymes are destroyed by the process. The centrifuged residue contains tissue fragments to which clings the mucus-like secretion. This can be dissolved by dilute acid and reprecipitated as an opalescent solution on neutralization. The opalescent solution is not noticeably altered by boiling. Centrifuging of the second and third mid-gut tissues also gives an opalescent solution which is unaffected by boiling, unless there is an appreciable amount of first mid-gut secretion present.

The change which takes place in the food mass in the second mid-gut, that is, the change from a granular mass to a clear fluid, can be simulated by adding a drop of dilute hydrochloric acid to the first mid-gut contents. In this, as in the living insect, a small amount of crystalline residue remains. It is possible, therefore, that the apparent digestion is really only a dissolving of the solid matter, the true digestion taking place subsequently. In this case the granular first mid-gut secretion may be an enzyme precursor. The origin of the acidity of the mid-gut contents and faeces may be solely the digestion of lipoids and esters, or it may be bacterial action, or it may be a property of the second mid-gut secretion. No other substrata but lipoids and esters have consistently shown increased acidity on digestion. As regards the second possibility, bacteria are only abundant in the third mid-gut whereas the acidity undoubtedly begins in the second mid-gut.

Whatever the cause of the acidity, and no clear evidence has been obtained which would distinguish between the alternatives, the enzymes of the digestive system appear to be adapted to such conditions. Notable in this respect is the divergence between the optimum pH for the salivary amylase and the pH of the saliva. Although the saliva is slightly alkaline, the enzyme is well suited to the pH of cacao tissue, which will be reduced below its normal figure of 5·8 by the action of salivary esterase.

Microbe flora of the intestine.

Investigations into the micro-organisms of the intestine were begun mainly with the idea, later discarded, that the peculiar filaments of the third mid-gut might be of this nature. The technique which was arrived at after some preliminary trials, and with which more than 120 capsid intestines were cultured, was as follows.

For convenience of dissection, fifth instar nymphs were used, and they were first sterilized by immersion for five minutes in each of (*a*) 90 per cent alcohol, (*b*) 0·1 per cent mercuric chloride, and (*c*) 90 per cent alcohol. They were then washed twice with sterile water and rapidly dissected. The abdomen could be easily separated from the thorax by gently pulling with needles and the intestine exposed. Organs for culturing were rinsed in a watch-glass of sterile water and their contents expressed over a flamed slide. From this the inoculum was picked up on a platinum wire and inoculated into tubes of media.

Abundant growth occurred on a starch-dextrose-agar slope, and the organisms which regularly appeared included yeasts. In an attempt to separate

the yeasts from the bacteria, Hansen's Medium (maltose-sucrose with acid phosphate) was used, but it was found that most of the bacteria also grew on this medium, being acid-resistant types. Addition of a few drops of lactic acid to the Hansen's Medium produced a medium on to which the yeasts could be sub-cultured and would grow, free from bacteria, but fresh inoculum would not grow on this medium. These yeast cultures were closely examined for the occurrence of filamentous cells, but none were found. Small numbers of elongated cells, connected in chains, were seen, but these did not in any way resemble the third mid-gut filaments. The first and second mid-gut flora were *Micrococcus* sp., which are very small and inconspicuous. They can be seen in small numbers in the gut contents and in the post-secretory cells of the first mid-gut, but in stained sections even when cut at 5 μ thickness, they were not distinguishable from the mass of granules in the fixed cell cytoplasm. Attempts to culture bacteria from the first mid-gut tissues of starved capsids, by grinding with sand under sterile conditions, were very variable in their results, perhaps due to excessively alkaline conditions in the grinding.

The cacao capsids have, in the male, an elaborate accessory genital gland system. There are two pairs of glandular sacs, the anterior pair of which secrete a clear fluid, while the posterior pair are filled with an opaque milky secretion. Woodward (1949) describes structures similar in principle, if not in detail, from the Reduviid genus *Nabis* Latreille. He quotes works on the authority of which he supposes the opaque mass to consist of bacteria, passed on in this way from generation to generation, and having a symbiotic association with the insect. On the other hand, Carayon (1951) claims that this is erroneous, and that this opaque mass consists of bacteriform secretions. As far as the work on cacao capsids goes (and Carayon's work was unknown to the writer when this work was in progress), the writer would be inclined to take a middle view, namely, that bacteria of the Micrococcus type can undoubtedly be cultured from these glands, but the number of colonies produced are very much less than one would expect were the opaque mass to consist of bacteria and nothing else. The issue is further complicated by the fact that in *Bryocoropsis laticollis* the posterior lobes of the accessory genital gland contain a secretion which simulates, not bacteria, but yeast cells. It is hoped that further work will clear up this aspect of the biology of cacao capsids.

In the third mid-gut and hind-gut there are, in addition to the Micrococci, several types of rod-shaped forms. There is a possibility that one of these, which forms dark red colonies, may be pathogenic to the insect, since a high proportion of capsids dying in the laboratory became red in colour after death and this organism was isolated from them.

Discussion.

One of the most interesting features of the digestive system of cacao capsids, and as far as is known, of the family Miridae as a whole, is the absence of the rows of gastric caeca on the posterior mid-gut, which are so conspicuous in other phytophagous Hemiptera-Heteroptera. While Glasgow (1914), having studied a wide range of hemipterous families, describes such caeca as confined to the Pentatomidae, Thyrecoridae, Pyrrhocoridae, Lygaeidae and Coreidae, Elson (1937) regards them as " structures which may be considered criteria for distinguishing the phytosuccivorous group ". There are, it is true, good examples of non-caeca-bearing members of caeca-bearing families having wholly or partly carnivorous habits (*e.g.* the Asopidine Pentatomids; *Leptocoris trivittatus* (Say), among the Coreidae). On the other hand, though a few capsids are partly or completely carnivorous (Kullenberg (1944) quotes 25 partly and 5 wholly so, out of 100 Swedish capsids), the majority, including the cacao capsids, are completely phytophagous. Descriptions

of the digestive systems of caeca-bearing Hemiptera are given in the literature as follows: Pentatomidae, Sarel-Whitfield (1929), Malouf (1933), Harris (1938); Coreidae, Breakey (1936). Those of non-caeca-bearing species are: Lygaeidae Hood (1937); Coreidae, Woolley (1949), Miridae, Painter (1930). In order to make the comparison more fruitful, in addition to information from these works, the writer has dissected and sectioned the following species: *Oncopeltus famileus* (Lygaeidae), *Dysdercus nigrofasciatus* (Pyrrhocoridae), and *Cletomorpha lancigera* (Coreidae), using the same techniques as for cacao capsids.

In caeca-bearing Hemiptera, the caeca are usually borne on the most posterior region of a four-fold mid-gut. In such species as *Oncopeltus famileus* and *Dysdercus nigrofasciatus*, the intestine consists of three regions which compare exactly with the first three regions of their caeca-bearing relatives. There is a large sac-like first region followed by a tubular region, as in cacao capsids, but the third region is a small rather thick-walled sac, which cannot be homologized with the third region of the capsids. Throughout these intestines there is a nearly uniform columnar epithelium, the cells of which are smaller than those found in cacao capsids (15 to 20 μ compared with 25 to 70 μ) in spite of the much larger size of the insect, and are uninucleate. In the third region this epithelium is not absorptive, but produces an abundant eosinophile secretion, which can be seen to surround the food mass and infiltrate into it. There is thus no simple way of homologizing the regions of the mid-gut of cacao capsids with those of non-caeca-bearing members of normally caeca-bearing families. Painter (1930) describes the mid-gut of the capsid *Psallus seriatus* as a single sac-like chamber lined with large generalized cells, uninuclear and bearing a striated hem. He describes a structure which seems to resemble the filaments found in the third mid-gut of the cacao capsids. This is just inside the termination of the malpighian tubules in the rectum, and consists of two circles of cells with long cuticular projections which stain deeply with haematoxylin. They are long enough to extend into the hind-gut. The present writer (unpublished observations) has found a very similar structure in the common tropical capsid *Proboscidiocoris fuliginosus*, which has a mid-gut comparable to the first two regions of that of cacao capsids, though the cell types are less distinct from one another in the two regions. The presence of filamentous processes on the cells at the bases of the malpighian tubules resembles to a remarkable degree the cells of the basal ampullae of these organs in the Reduviid, *Rhodnius prolixus* (Wigglesworth, 1931). The writer has been able to examine these structures in *R. prolixus*, and finds that they do not give the very rapid reaction to vital stains which occurs in the third mid-gut of cacao capsids. Even so, the morphological resemblance remains, which at least means that the filamentous cells in cacao capsids are less of an isolated and inexplicable characteristic. The direct homology of cells which in one insect are clearly part of the mid-gut, with those in the malpighian tubules of another, depends on the production of evidence to show that the malpighian tubules are in reality endodermal in origin. Complete proof of this cannot here be given, but it must be mentioned, first, that the chitinous intima of the hind-gut of cacao capsids does not extend further forward than the recto-pyloric constriction, as far as can be detected, thus suggesting that this is also the limit of the ectodermal hind-gut, and secondly, that Harris (1938) also queried the ectodermal origin of the malpighian tubules in the Pentatomid *Murgantia histrionica*.

The possession of a vesicular accessory salivary gland has already been mentioned as a feature of the carnivorous Hemiptera. Such a structure has been found to be present in all members of the family Miridae of which the internal anatomy is known. It would appear that in the matter of intestinal structure as well, the capsids can most usefully be compared with such forms as the Reduviidae, where the mid-gut is a simple affair having only a sac-like stomach and a tubular intestine composed of one region.

The function of gastric caeca is generally supposed to be that of a harbourage for specialized symbiotic bacteria. These bacteria may provide some accessory nutritional factor needed by insects living on a restricted plant diet. No diet could be more restricted than that of the cacao capsids, which can feed successfully on only a few species of Sterculiaceous plants and which in practice feed almost entirely on *Theobroma cacao*, yet, if symbiotic bacteria are essential, manage to accommodate them in a caeca-less intestine.

The structure of the salivary glands of cacao capsids falls easily within the wide limits of variation of this organ in the Hemiptera. A large number of hemipterous salivary glands were described by Baptist (1941), with which those of the capsids may be compared. The accessory gland of these capsids approximates to the condition in the typically phytophagous families, in that it possesses a tubular gland in addition to its vesicle. This may be related to some special need of phytophagous feeding. In none of those which Baptist describes is there the extraordinary degree of cytoplasmic basophilia which is found in cacao capsids. Painter (1930), however, found this feature in *Psallus seriatus* (though the cells were uninuclear). He also found a histological distinction between the two lobes of the main gland, which agrees with the findings of Breakey (1936). Baptist (1941) was quite unable to find any consistent histological or enzymological distinction between different salivary gland lobes in any of the species with which he worked. The cacao capsids have rather larger salivary glands than is usual, which are unlike the previously described hemipterous salivary glands in that, though having more than two lobes, the lobes are not associated together and bound with a common peritoneal sheath.

As far as enzymes are concerned, information in the literature on Hemiptera refers only to salivary enzymes. Painter (1930), although he simulated flea hopper damage to cotton with diastase solutions, could not detect any amylase in the salivary glands. In *Psallus seriatus*, however, the oesophageal valve is much reduced, and perhaps mid-gut enzymes are regurgitated. Baptist (1941) found both an amylase and an invertase in the salivary glands of the capsid, *Lygus pratensis*. An enormous destruction of plant tissue is characteristic of the feeding of capsid bugs. This is produced by quite small numbers of bugs, by means of what is usually called the toxic saliva, in contrast to plant feeding bugs of other families, which seem to feed from the vascular systems of their hosts, and cause damage by interrupting this system when large numbers of bugs are present (e.g. *Blissus leucopterus*, *vide* Painter (1928)). Small Homoptera such as aphids may suck out the contents of individual plant cells. The feeding of capsids may be regarded as similar, in many ways, to that of the predatory Hemiptera to which they appear to be allied. Just as those bugs wash out the internal tissues of their prey with a stream of saliva, so the capsids kill the plant cells within a limited area and wash out their contents. In these processes the accessory salivary gland vesicles seem to be responsible for the maintenance of salivary circulation. In the past, the toxicity of capsid saliva has not been traced to any distinct constituent. From what has been found in the cacao capsids, we may conjecture that the following processes take place. First of all, saliva is injected under high pressure, filling all intercellular spaces, and showing the external appearance of a "water-soaked area". Then, a strong acidity is produced, possibly by the action of esterases, which has a toxic action on the plant cells. This acidity we see in the strong acidity of freshly ingested food. With the death of the cells, their walls become permeable and soluble contents are leached out, while the salivary amylase penetrates and dissolves any insoluble carbohydrates. It will be noted that the absence of an invertase in the saliva is no disadvantage, since disaccharides are readily soluble. The brown discoloration of the lesion is a post-mortem reaction in no way connected with the direct salivary action, and can be produced by mechanical wounding of the cacao tissues.

Summary.

1. A description is given of the anatomy and physiology of the digestive system of the cacao capsid bugs, which is valid for all four species. The salivary glands are also described, as are, briefly, the fore- and hind-gut and malpighian tubules, although they are not involved in the digestive processes.

2. The cyclical changes occurring in the intestine during feeding and digestion are described. There are three divisions to the mid-gut, the first being a sac-like region in which ingested food becomes more concentrated and is mixed with a granular secretion, the second is a tubular region in which most of the digestion occurs, and where the food mass becomes a clear fluid, and the third is specialized for absorption.

3. Evidence is given for supposing the accessory salivary gland to function as an organ for maintaining a copious flow of saliva by absorbing water from the first region of the mid-gut.

4. The evidence for the functions of the regions of the mid-gut and the salivary glands is supported by experiments with injected dyes, especially methylene blue.

5. Investigations into the enzymes of the mid-gut and salivary glands are described. The pH of these regions and their secretions has been measured.

6. The bacterial flora of the mid-gut has been investigated and found to consist mostly of *Micrococcus* spp. There are also a variety of rod-like bacteria in the third region of the mid-gut.

7. In comparing the cacao capsids with other Hemiptera, reasons are given for regarding them as more closely allied to predatory types than to the other phytophagous families.

8. A theory is put forward to explain the destructive action of capsid saliva on plant tissues.

Acknowledgments.

This paper is published by permission of Mr. J. West, Director of the West African Cacao Research Institute, to whom I am indebted for his kind encouragement during the course of the work. I also wish to acknowledge the ready assistance and advice of all my colleagues on the staff of the Institute, and especially Mr. G. Williams for reading through the text of this paper, and Mr. H. Owen for bacteriological help.

References.

Awati, P. R. (1914). The mechanism of suction in the Potato Bug, *Lygus pabulinus* Linn. *Proc. zool. Soc. Lond.* **1914**, 685–733.

Baptist, B. A. (1941). The morphology and physiology of the salivary glands of Hemiptera-Heteroptera. *Quart. J. micr. Sci.* **83**, 91–139.

Breakey, E. P. (1936). Histological studies of the digestive system of the Squash Bug (*Anasa tristis* De G., Hemiptera, Coreidae). *Ann. ent. Soc. Amer.* **26**, 561–577.

Bugnion, E., & Popoff, N. (1908). L'appareil Salivaire des Hemipteres. *Arch. Anat. micr.* **10**, 227–268.

Carayon, J. (1951). Les organes genitaux males des Hemipteres Nabidae ; absence de Symbiontes dans ces organes. *Proc. R. ent. Soc. Lond.* (A) **26**, 1–10.

Cotterell, G. S. (1926). Preliminary study of the life-history and habits of *Sahlbergella singularis* Hagl. and *Sahlbergella theobroma* Dist. *Dept. Agric. Gold Coast Bull.* **3**, 1–26.

Elson, J. A. (1937). A comparative study of Hemiptera. *Ann. ent. Soc. Amer.* **30**, 579–597.

Ewing, K. P. (1929). Effects on the Cotton Plant of the feeding of certain Hemiptera of the family Miridae. *J. econ. Ent.* **22**, 761–765.

Glasgow, H. (1914). The gastric caeca and caecal bacteria of Heteroptera. *Biol. Bull. Wood's Hole* **26**, 101–170.

Harris, C. S. (1938). The anatomy and histology of the alimentary system of the Harlequin Cabbage Bug, *Murgantia histrionica* Hahn. (Hemiptera, Pentatomidae). *Ohio J. Sci.* **38**, 316–331.

HAWK, P. B., OSER, B. L., and SUMMERSON, W. H. (1947). *Practical physiological chemistry*, 12th Edn. London : Churchill.

HOOD, C. W. (1937). The anatomy of the digestive system of *Oncopeltus fasciatus* Dall. (Hemiptera, Lygaeidae). *Ohio J. Sci.* **37**, 151–160.

KULLENBERG, B. (1944). *Studien uber die Biologie der Capsiden*. Uppsala.

LEACH, R., & SMEE, C. (1933). Gnarled stem canker of tea caused by the Capsid Bug (*Helopeltis bergrothi* Reut.). *Ann. app. Biol.* **20**, 691–706.

MACGILL, E. I. (1947). The anatomy of the head and mouth-parts of *Dysdercus intermedius* Dist. *Proc. zool. Soc. Lond.* **117**, 115–128.

MALOUF, N. S. R. (1933). Studies on the internal anatomy of the Stink Bug, *Nezara viridula* L. *Bull. Soc. ent. Egypte* **1923**, 91–119.

PAINTER, R. H. (1928). Notes on the injury to plant cells by Chinch Bug feeding. *Ann. ent. Soc. Amer.* **21**, 232–241.

PAINTER, R. H. (1930). A study of the Cotton Flea Hopper, *Psallus seriatus* Reut., with especial reference to its effect on Cotton Plant tissues. *J. agric. Res.* **40**, 485–516.

POISSON, R. (1924). Contribution a l'etude des Hemipteres Aquatiques. *Bull. biol. Fr. et Belg.* **58**, 49–305.

ROBERTS, J. I. (1930). The Tobacco Capsid (*Engytatus volucer* Kirk.) in Rhodesia. *Bull. ent. Res.* **21**, 169–183.

ROY, D. N. (1937). The physiology of digestion in larvae of *Gastrophilus equi*. *Parasitology* **29**, 150–162.

SAREL-WHITFIELD, F. G. (1929). The Sudan Millet Bug, *Agnoscelis diversicolor* F. *Bull. ent. Res.* **20**, 209–224.

SCHNEIDER, G. (1940). Beitrage zur Kenntnis der symbiontischen Einrichtungen der Heteropteren. *Z. Morph. Ökol. Tiere* **36**, 595–644.

SMITH, K. M. (1920). Investigations of the nature and cause of the damage to plant tissue resulting from the feeding of Capsid Bugs. *Ann. appl. Biol.* **7**, 40–55.

SNODGRASS, R. E. (1935). *Principles of insect morphology*. 1st Edn. New York : McGraw-Hill.

SQUIRE, F. A. (1947). On the economic importance of the Capsidae in the Guinean Region. *Rev. Ent. S. Paulo* (*Rio de J.*) **18**, 219–247.

VOELCKER, O. J. (1949). *W.A.C.R.I.*, 1944–49. London : Crown Agents for the Colonies.

WEBER, H. (1930). *Biologie der Hemipteren*. Berlin : Julius Springer.

WOODWARD, T. E. (1949). The internal male reproductive organs in the genus *Nabis* Latreille (Nabidae ; Hemiptera-Heteroptera). *Proc. R. ent. Soc. Lond.* (A) **24**, 111–118.

WOOLLEY, T. A. (1949). Studies on the internal anatomy of the Box-Elder Bug (*Leptocoris trivittatus* (Say), Hemiptera, Coreidae). *Ann. ent. Soc. Amer.* **42**, 203–226.

WIGGLESWORTH, V. B. (1931). The physiology of excretion in a blood-sucking insect, *Rhodnius prolixus* (Hemiptera, Reduviidae). II—Anatomy and histology of the excretory system. *J. exp. Biol.* **8**, 428–442.

APPENDIX 2

SOME NEW OBSERVATIONS ON THE INTESTINAL STRUCTURES CONCERNED WITH WATER DISPOSAL IN SAP-SUCKING HEMIPTERA

By A. J. P. Goodchild

(*Makerere University College, Kampala, Uganda*)

Manuscript received 13th February, 1963

(Read 1st May, 1963)

With 7 Text-figures

Contents

I. Introduction

All members of the Homoptera and many members of the Heteroptera feed by sucking plant juices. This mode of life has brought about modifications of the alimentary canal for dealing with an excess of water in the diet. In the Homoptera this has been effected in the Cicadoidea, for instance, by the development of the so-called " filter chamber ", characteristic of that superfamily, which has been described in a number of papers (*vide* bibliographies in Snodgrass, 1935 and Pesson, 1951). This highly complex organ brings the anterior and posterior ends of the midgut into very intimate contact, the coiled posterior end and the proximal parts of the Malpighian tubules fitting into labyrinthine folds in the wall of the sac-like anterior end, the whole being enclosed within the peritoneal membrane investing the intestine. A filter chamber is found in all Cicadoidea except the most primitive Jassidae (of the subfamily Typhlocybinae), in which there is simple contact between the anterior and posterior ends of the midgut, not enclosed within the peritoneum (Willis, 1949 ; Saxena, 1955). In many Sternorrhyncha also, there is an approximation of the extremities of the midgut, which allows them to function as a water-transferring device comparable to that of the filter chamber. In certain Aphidae (Knowlton, 1925) and Aleyrodidae (Weber, 1930), the peritoneal membrane encloses a fairly simple region of contact, but in Psyllidae (Brittain, 1923) and in Coccidae (Pesson, 1944) the approximated parts are twisted together in a helical manner. In the Coccidae, this region of contact is invaginated into the anterior end of the rectum.

In comparison with the volume of literature on the above mentioned families, references to the alimentary structure and function of the superfamily Fulgoroidea are exceedingly scanty, probably on account of their much smaller economic importance.

Kershaw (1913) gave an account of the intestine of *Flata* sp. (Flatidae), from which it is clear that there is no approximation of the extremities of the intestine in this insect. Several papers have been published (Muller, 1940, 1949; Ermisch, 1960) on the internal anatomy of Fulgoroidea, with special reference to the complicated system of organs harbouring symbiotic micro-organisms. Apart from the fact that one of these organs is situated in the wall of the rectum, these authors do not concern themselves with intestinal anatomy, but such figures as are given suggest that the structure of the alimentary canal is similar to that of *Flata*. Text-book accounts are not unanimous, for while Imms (1957) states clearly that a filter chamber is not found in Fulgoroidea, Pesson (1951) describes the Cercopid filter complex and remarks, " On retrouve les dispositifs tout à fait comparable chez les Cicadides, Fulgorides, et Membracides." Snodgrass (1935) states simply that a filter chamber is present " in most Homoptera ".

As a result of a fortunate opportunity to examine in detail a Tettigometrid of convenient size and availability, and dissections of Fulgoroids belonging to several other families, the writer has gathered sufficient evidence to justify the conclusion that the alimentary canal of Fulgoroidea is in fact constructed on an entirely different pattern from that of Cicadoidea, and to suggest the mechanism by which water disposal is effected in this group.

In the Heteroptera, since no organ as complex and specialised as the filter chamber has hitherto been described, little attention has been paid to the problem of water disposal in the sap-sucking forms. The situation is more complicated than in the Homoptera, because of the variety of feeding habits and habitats (including aquatic) that may be found in different families of Heteroptera. Phytophagous species of some families (Miridae, Tingidae) are derived from a predominantly zoophagous line (the Cimicomorpha), and feed by a destructive method on plant tissues, while members of some other families (Lygaeidae, Pyrrhocoridae) feed upon ripe seeds, or the tissues of the developing ovule. None of these species is faced with a problem of osmotic regulation. There are, however, in the families Plataspidae, Pentatomidae, and Coreidae, a large number of species which are undoubtedly sap-sucking. If the intestinal arrangements of these forms are examined as possible mechanisms of water disposal, certain approximations of organs may be seen that suggest likely routes for water transfer. Unfortunately, these conjunctions are usually only loosely bound by a few fine tracheal branches, and are easily broken when the intestine is dissected. Thus a number of possibly significant features may have been overlooked in earlier descriptions. The present writer has drawn attention (Goodchild, 1952) to the intimate contact between the anterior midgut and the expanded accessory salivary gland vesicles in the Bryocorine Miridae, in which it was observed that drops of clear fluid were exuded from the mouthparts on completion of a feed. In the truly sap-sucking Heteroptera, belonging to the division Pentatomomorpha, however, the accessory salivary glands are not of this vesicular type. In many Coreidae there is a tubular anterior diverticulum of the rectum inserted among the intestinal coils, and it has been suggested by Huber-Schneider (1957) that this may function in the manner of a filter chamber. The intestinal structures that occur in the essentially phytophagous Pentatomomorpha, but not in the Cimicomorpha or Hydrocorisae (which are mainly zoophagous), are a sac-like expansion at the extreme posterior end of the midgut and the gastric caeca. The former has been termed the ileum, although it is definitely mesenteric, and not equivalent to the ileum in other Orders, which is a tubular hind gut region. It is the region into which the Malpighian tubules open. The gastric caeca are a series of thin walled pouches that open into the midgut region anterior to the ileum. In

certain Lygaeidae (of the subfamilies Blissinae and Rhyparochrominae), the caeca are long, and closely enfold the expanded anterior end of the midgut. A case has been made out (Goodchild, 1963) for considering the gastric caeca as organs of water excretion, based on this anatomical evidence and a number of other features, such as the histology of the caeca and their absence from zoophagous and seed-sucking forms, which support this interpretation. The case for the gastric caeca is confused, however, by their modification, in the most highly specialised sap-sucking species, into mycetome-like organs for the harbourage of symbiotic micro-organisms, the intestinal lumen being restricted or obliterated both anteriorly and posteriorly to the caecal region. In these insects, it was found (Goodchild, *loc. cit.*) that there is anatomical and histological evidence that the cells of the ileum take over the task of water excretion. For instance, in certain large Plataspidae (*Libyaspis flavosparsus* Mont.), the ileum was found to be completely invaginated into the posterior end of the anterior sac of the midgut. In spite of this, the parts separated without much difficulty in dissections, and the relationship was revealed with certainty only in sections of the whole undisturbed alimentary canal. It was therefore with great interest that the writer discovered, while dissecting a phyllocephaline Pentatomid (*Gellia* sp.) for the purpose of culturing caecal bacteria, that there was evidently a more complete binding together of the parts of the intestine than had previously been found in any Heteropteran. Unfortunately, before this was realised, too much destruction had taken place for that particular specimen to be described. Prolonged search failed to reveal any further specimens of that species, but a specimen of another phyllocephaline species, *Dalsira bohndorffi* Dist., was collected recently, and careful dissection and sectioning of the entire gut has shown that the structure of the alimentary canal is unlike that of any previously described Heteropteran, and fully deserves to share with the Cicadoidea the term " filter chamber ". Although only one specimen, a male adult, has been available, the remarkable nature of its anatomy makes it desirable to publish a description.

II. The Alimentary Canal of Fulgoroidea (Homoptera)

(*a*) *Material and Methods*

The main subject of this investigation has been *Phalix titan* Fennah (Tettigometridae), a moderate sized (8–9 mm.), green to mottled greenish-brown insect of Cercopid-like appearance. A fairly dense infestation has been present for the past few years, with seasonal fluctuations, on an isolated group of small trees of *Acacia decurrens* var. *mollis* (Black Wattle) in the grounds of Makerere College. The infestation was remarkable for the conspicuous incrustation of eggs, both fresh and empty shells, on the bark of twigs and branches, while the nymphs and adults inhabited the petioles of the finely divided bipinnate leaves, concealed by their cryptic colour among the pinnules. A sooty growth of moulds on older leaves resulted from contamination by the honeydew excreted by the insects.

The insects were studied by dissection and by serial sections of whole intestines, cut at 10 μ thickness and stained with haemalum and eosin. Serial sections were also made of the whole intestine of a single specimen, caught at a light trap, of the giant lantern fly, *Pyrops tenebrosus* Fab. (Fulgoridae), and of the intestines of an adult and three mature nymphs of the moth-bug, *Gyarina nigritarsis* Karsch (Flatidae). The intestines of specimens of *Ptyelus flavescens* Fab. (Cercopidae) and *Tettigoniella mitrata* Gerst. (Jassidae) were sectioned for comparison, as representing typical Cicadoid species, with well-developed filter chambers. Other species of Fulgoroidea that were dissected for a check on the uniformity of the intestinal pattern of this superfamily were *Dictyopharina serena* Stål (Dictyophoridae), *Elasmoscelis stali* Dist. (Lophopidae) and *Kelisia* sp. (Delphacidae).

(*b*) *The Alimentary Canal of* Phalix titan *Fennah*

On removal from the insect, the alimentary canal (fig. 1) presents superficially the appearance of two consecutive sac-like structures, with the four Malpighian tubules joining it at the constriction between the two. From the more anterior sac, a narrow diverticulum, filled with air bubbles, extends forward into the thorax of the insect, parallel to a delicate oesophagus. On closer inspection, it can be seen that the anterior sac encloses a coiled tubular intestine in which vigorous twisting

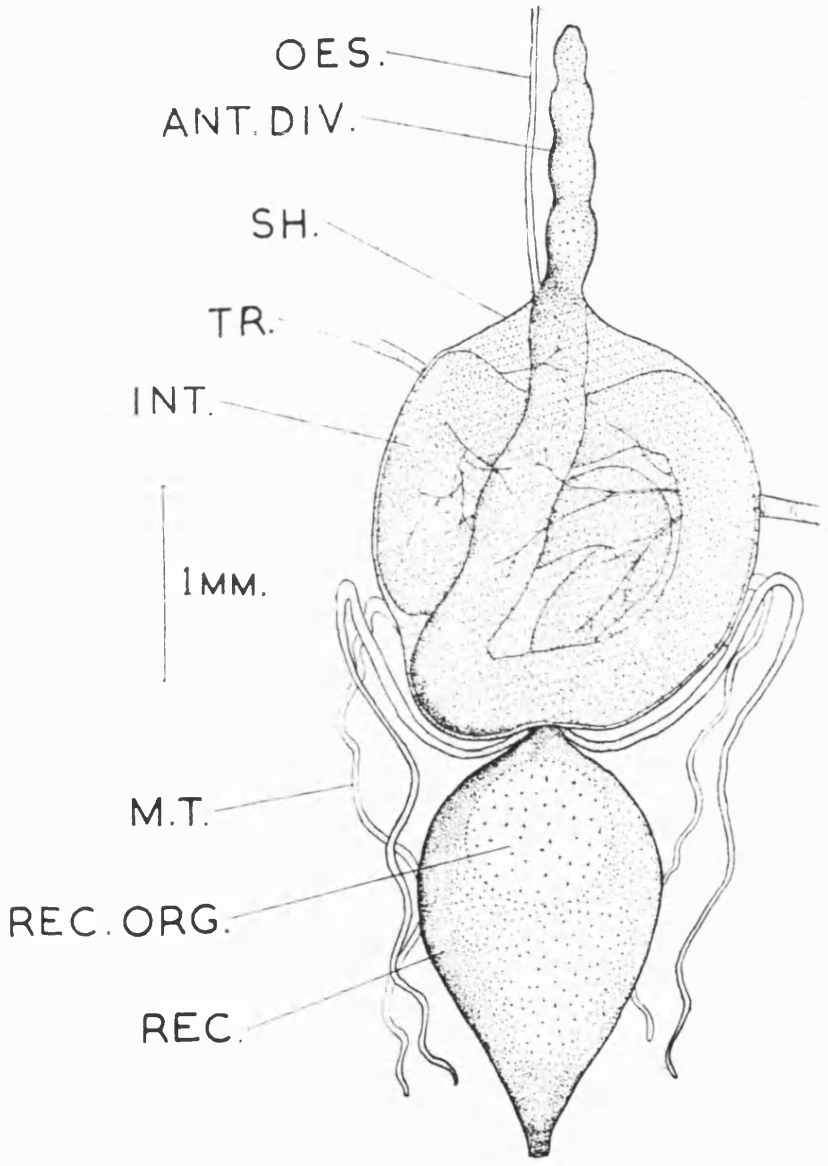


FIG. 1.—The alimentary canal of an adult female *Phalix titan*, ventral view.

and peristaltic movements are taking place, although no intrinsic movement can be seen in the wall of the sac. Dissection of the sac shows that its lumen is not a continuation of that of any part of the alimentary canal, so that the structure is not comparable to the invagination of the twisted midgut (filter complex) into the rectum that occurs in Coccidae. The wall of this anterior sac may best be regarded as a separated peritoneal membrane, as was observed in *Flata* sp. by Kershaw (1913), but was omitted from his figures. As will be made clear from the description and analysis given below, this membrane should not have been so lightly dismissed, as it may be of the greatest physiological importance.

Beginning from the anterior end of the alimentary canal, the detailed anatomy and histology is as follows. The oesophagus, 75 μ in diameter, is lined with a chitinous intima secreted by small cuboid cells (fig. 2) and joins the midgut just anterior to the sac (which will be referred to hereafter as the *intestine sheath*), at the level of the anterior end of the abdomen. There is a well-developed valve-like invagination at the junction (fig. 2), with cells of midgut type, and no trace of a peritrophic membrane. The air-filled anterior diverticulum arises from the midgut immediately posterior to the oesophageal valve, and is lined with small rounded cells with large, sometimes paired, nuclei. As these cells are mesenteric, it is not impossible that enzymes are secreted here, to pass backwards into the main intestine, but no fluid

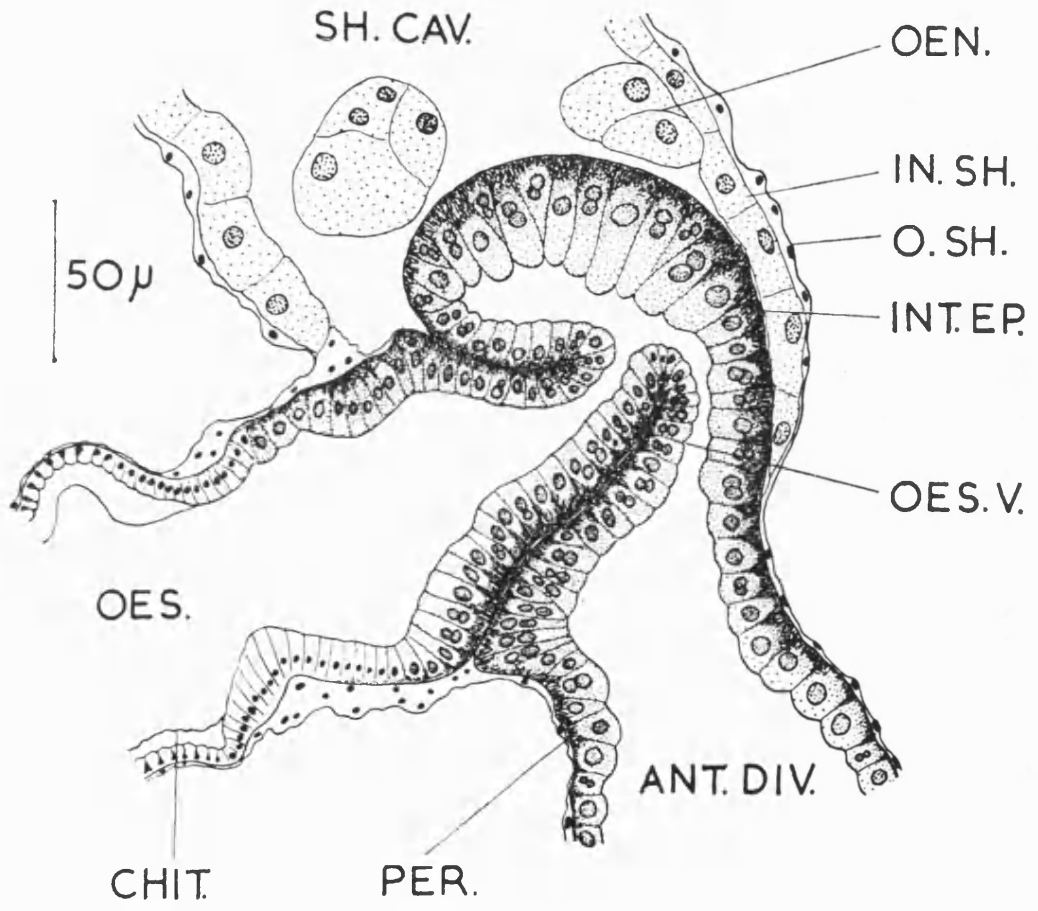


Fig. 2.—Transverse section at junction of oesophagus, mid-intestine, and anterior diverticulum of *Phalix titan*.

or solid contents accumulate in the diverticulum. The intestine posterior to the valve is within the sheath. It is entirely tubular, and lacks the sac-like expansions characteristic of Cicadoidea and Heteroptera. From the oesophageal valve, the intestine passes ventrally and slightly to the right to the posterior end of the sheath, then turns to the left side and passes forward again. A dorsal transverse loop at the anterior end of the sheath carries the intestine to the right, where it forms a small loop and returns to the dorsal midline and thence posteriorly to its point of emergence from the sheath. As a result of these convolutions, a midgut 10 mm. in length is accommodated in a sac 2 mm. long. The junction with the hind gut is coincident with the emergence of the intestine from the sheath. Just anterior to the point of emergence, the intestine is joined by the narrow proximal regions of the Malpighian tubules, which have entered the sheath from the rear, close to the intestine. Their openings are evenly spaced across the dorsal side of the intestine, and are simple,

with typical tubule cells joining directly to typical midgut cells (as in other Hemiptera, the Malpighian tubules appear to be endodermal). There are four tubules, of the usual Hemipterous type (Wigglesworth, 1931), the cells having large granular nuclei, and an eosinophil brush-like border. They have no remarkable features, and run forward to about half-way along the sheath, then backward to terminate near the rectum, but are not inserted into the rectal wall, as are those of many Cicadoidea.

Histologically, the first half of the intestine is composed of large, frequently binucleate, bulbous cells (fig. 2), with the very finely reticulate nuclei situated towards the cell base, resting on a basal zone of fibrous, rather basophil, cytoplasm. The bulbous tip contains little stainable material, and the cell border is a thin membrane. Towards the posterior end of the intestine, these cells grade into cells of a different type. In the latter, a cuboid or low columnar cell base, in which the basal zone is poorly developed, gives rise to a long lobe projecting into the gut lumen (fig. 3*A*). This lobe has a broad brush-like border, in which there are, in addition to the main mass of border filaments, smaller numbers of much longer filaments, extending to about double the width of the border proper. Wigglesworth (1931) observed similar long filaments in the brush-like border lining the Malpighian tubules of *Rhodnius prolixus* Stål, and found that they, together with the shorter filaments, were destroyed by the process of fixation. This also happens with the intestine cells of *Phalix*, so that figure 3*A*, being drawn from a stained section, does not show adequately the filamentous nature of the cell border. It may be remarked that very similar lobed cells with a deep brush-like border are found in absorptive regions of the gut of other Hemiptera, particularly Miridae and Tingidae (Goodchild, 1952, 1963).

Throughout the intestine, very small cells with clear cytoplasm and dark staining nuclei are scattered among the bases of the intestinal cells. These are presumably replacement cells. There appears to be a cellular basement membrane around the intestinal tube, with widely separated, very small dark staining nuclei. Large tracheal trunks pass through the sheath on each side, and branch abundantly over the intestine. Among the intestinal coils, and apparently adherent to tracheal branches or to the intestine, are many rounded cells of moderate size (25–35 μ). These have single large granular nuclei and strongly eosinophil cytoplasm, and fall into the category of oenocytes, on histological characteristics. Although the space enclosed by the sheath is not in communication with the haemocoele, the progenitors of this oenocyte population could have entered along with the tracheal branches.

The intestinal sheath itself, in the living state, appears to be a sheet of moderately flattened rhomboid cells, uninuclear, and with clear refractive cytoplasm. The junctions between the cells seem to be slightly indented. In stained sections (fig. 2), the sheath can be seen to consist of two layers, the inner one composed of cells about 12 μ thick and 33 μ wide, with granular nuclei and eosinophil cytoplasm of the same type as the oenocytes, and the outer one being a very thin membrane similar to a normal peritoneal membrane, with non-staining cytoplasm and very small dark staining nuclei. No muscle fibres or other tissues could be detected in the sheath, which grades smoothly into the normal peritoneal membrane where the intestine passes through it.

The hind gut is an expanded, thin walled sac, like that of Heteroptera, and is the posterior of the two sac-like structures seen when the insect is first dissected. The junction of this rectum with the intestine is by a narrow, slit-like, dorsoventrally orientated valve, the edges of which are composed of small cuboid cells with dark staining nuclei. This valve, and the rectum itself, are lined with a chitinous intima. The dorsal and ventral walls of the rectum have epithelium of true hind gut type, as found in Heteroptera (Goodchild, 1963), which is a thin syncytial layer with scattered small nuclei, whereas the lateral walls are composed of larger, domed, gland-like cells, with large granular nuclei, and dense eosinophil cytoplasm. The gland cells do not form a continuous layer, but have small nuclei irregularly distributed amongst

them. In the adult female, a large hemispherical mass of yeast-like symbionts is found between the ventral rectal epithelium and its peritoneal covering. This is the " rectal organ ", well known to students of Fulgoroid symbiosis.

(*c*) *Comparison with other Fulgoroidea*

In all other Fulgoroidea that have been examined, the intestine is essentially similar to that of *Phalix*. In the stained sections of *Pyrops tenebrosus*, it was apparent that the sheath was much thinner than in *Phalix*, and consisted of one kind of cell

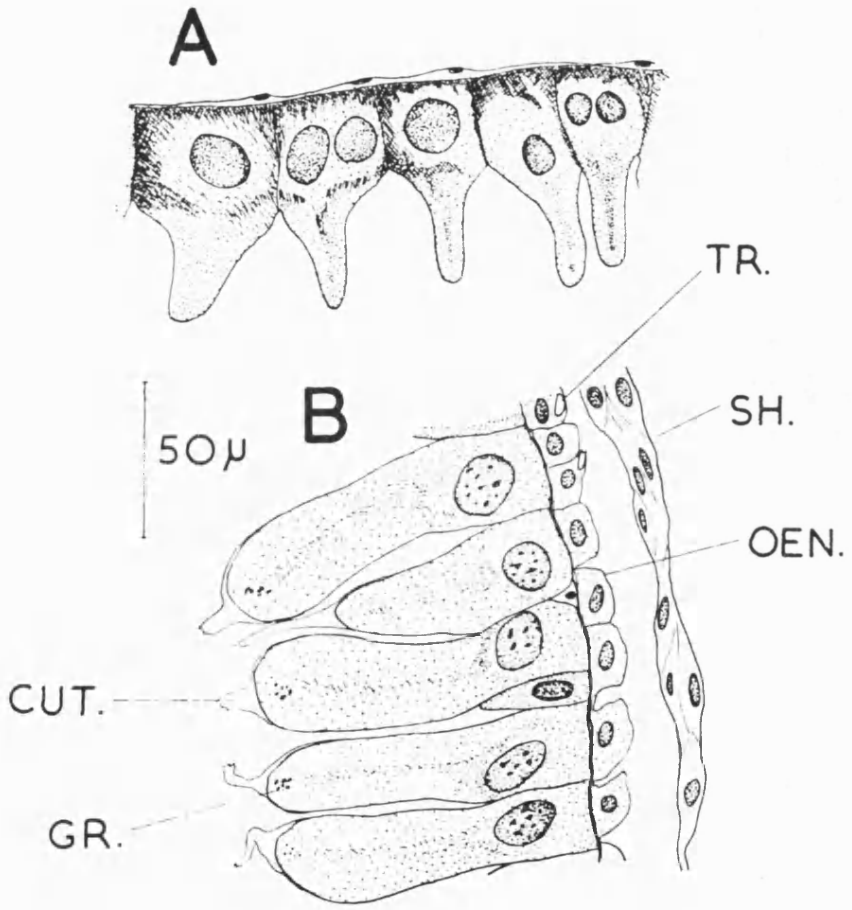


FIG. 3.—(*A*) Cells from posterior mid-intestine of *Phalix titan*. (*B*) Intestinal lining cells of *Pyrops tenebrosus*.

only. These cells seem to be intermediate between the inner and outer layers found in *Phalix*, in that, although they are much flattened and are not eosinophil, they have relatively large granular nuclei (fig. 3*B*). They overlap so as to form a double layer in places. In the fresh state, the sheath of *Pyrops* is tenuous and almost transparent. The absence of an oenocyte-like layer in the sheath wall of *Pyrops* is, apparently, compensated for by the existence of a dense layer of such cells around the intestinal tube (fig. 3*B*). This tube is much more convoluted than in *Phalix*, and for most of its length consists of cells with tall lobes projecting into the lumen. It was not possible to examine these in the fresh state, but in sections it would seem that the tip of each lobe gives rise to a finger-like outgrowth of structureless cuticle. Within the tip of the cytoplasmic lobe, there is a group of large basophilic granules, from which a core of dense cytoplasm leads down to the basal nucleus. A structureless cuticular border, rather than a brush-like border, invites comparison with the

posterior part of the midgut in the Miridae Bryocorinae (Goodchild, 1952), in which intense absorption takes place. The rectal lining in *Pyrops* is composed entirely of a thin gland-like epithelium, thrown into deep folds in the fixed material, and having a great resemblance to the rectal lining of certain sap-sucking Pentatomidae (Goodchild, 1963). This type of epithelium has large, widely spaced, granular nuclei, with a thin striated eosinophil zone beneath the chitinous intima that forms the cell border. Corresponding to the occupation of the whole rectal surface by gland-like epithelium, the rectal organ was found to be suspended in the lumen from a narrow attachment around the valve leading to the midgut.

In the specimens of Flatidae (*Gyarina nigritarsis*) that were examined, a situation much closer to the earlier description by Kershaw (1913) could be discerned. Unfortunately, this material was dealt with rather hurriedly, just before the writer's return to Britain from Uganda, and the sections were not entirely satisfactory. The sheath in these insects seemed to be fragmentary and degenerate, a thin layer remaining only at the anterior and posterior ends of the midgut. At these points also, a covering of strongly eosinophil cells was present around the intestinal tube, but such cells were absent from the greater part of the midgut. Oenocyte-like cells among the intestinal coils were few, and those that were seen appeared to be abnormal in that the eosinophil material was not uniformly spread through the cytoplasm but was formed into coarse granules. The midgut epithelium appeared to be composed of simple rounded, mostly binucleate, cells throughout its length. The anterior diverticulum contained a structureless eosinophil secretion (resembling that frequently seen in sections of Hemipteran salivary glands), and the tips of the bulbous cells of this region were distended by similar material. This appearance is similar to that noted by Kershaw, who interpreted the anterior diverticulum as a reservoir of digestive secretion. The rectum was lined entirely with gland-like epithelium, the cells being flattened and strongly eosinophil. The rectal organ appeared to have lost its limiting membrane, the mass of symbiotic micro-organisms lying free in the lumen. A point of some interest is that the lumen of the midgut contained masses of basophil material similar to the rectal contents. Nothing in the nature of a peritrophic membrane, such as was described by Kershaw in *Flata* sp., could be detected. There were no obvious differences in intestinal anatomy and histology between the adult specimen and the mature nymphs. In view of the discussion that follows, it is worth mentioning that Kershaw observed that the anterior midgut diverticulum was distended with air bubbles at the time of moulting.

(*d*) *Discussion*

Those species of phytophagous Hemiptera that feed mainly on sap, rather than on plant tissues, need to pass large quantities of this material through their intestines in order to extract sufficient nourishment. Insects of other Orders that feed upon nectar, namely Lepidoptera, Hymenoptera (Apoidea) and many species of Diptera, do so only in the adult stage, their food requirements for larval growth having been met by feeding on solid or highly concentrated fluid materials. In most of these forms, reserves built up during larval life also suffice for reproduction, so that nectar is only needed to maintain life in the adult. In the apoid Hymenoptera, it must be remembered that the nectar is concentrated to form honey by exposure upon the mouthparts of the workers, and that the diet is supplemented by highly nutritious pollen as a protein source. In the Orders named above, as compared with Hemiptera, relatively small amounts of liquid food are needed. It is therefore significant that in such insects a chitin-lined impermeable crop is developed in the fore gut region, in which the nectar is stored, and from which it is transferred to the midgut slowly, so as to keep within the limits of the relatively low power of osmotic regulation of

the Malpighian tubules (Ramsay, 1950, 1958). In the sap-sucking Hemiptera, growth throughout all stages of the life history, and the needs of reproduction, must be met from a diet of plant juices alone, and so special adaptations have been evolved for increasing the rate of ingestion while avoiding a fatal degree of dilution of the haemolymph. The Hemiptera all lack a large chitin-lined crop, but have a capacious rectum in which hypotonic excreta can be safely retained. Their physiological problem may be stated in terms of getting the excess water in the ingesta passed into the rectum with the least possible delay.

Before attempting to form any conclusions about the physiology of the fulgoroid intestine from the anatomy and histology that have been described, it will be useful to relate histology to probable function in the Cicadoidea. This will give some measure of the validity of a purely histological approach to this physiological problem.

The filter chamber of Cicadoidea is an extremely complicated structure, and it would seem that its function has been regarded as self-evident, without the need for reasoned analysis. The significant features of this organ system are, firstly, the enormous area of intimate contact between the anterior end of the midgut sac and the convolutions of the posterior end of the midgut and the proximal parts of the Malpighian tubules. The wall of the midgut sac is pushed out among these convoluted tubes in a series of labyrinthine folds. Secondly, the epithelia of all parts concerned, both extremities of the midgut and the Malpighian tubules, are identical in histological appearance, being extremely thin and structureless, with non-staining cytoplasm and widely spaced flattened nuclei. Thirdly, the rectum, which begins at the point where the midgut emerges from the peritoneal covering of the filter complex, is long, narrow and lined with domed gland-like cells. Finally, there is a well-developed muscle strand running from the body wall in the neck region to the junction of the filter chamber with the main part of the anterior midgut sac ; this is a feature not found in other Hemiptera and is possibly connected with the functioning of the filter chamber. It is generally agreed that the excess water in the ingesta passes directly from the anterior end of the midgut to the posterior end of that region, through the filter chamber, and thence rapidly to the rectum. The accumulation of ingested dyes found by Pesson (1944) in the intermediate part of the midgut, and the observation by the present writer during the course of these studies that the midgut of the Cercopid, *Ptyelus flavescens*, is occluded by enormously hypertrophied lining cells (presumably acting as storage cells), show that there is no significant flow of material through the midgut.

In order to effect a simple filtration of water through to the hind gut against an osmotic gradient, it would be necessary for a hydrostatic pressure to be generated in the filter chamber. It seems possible that such a pressure could be built up if the muscle strand referred to above was capable of closing off the filter chamber from the remainder of the anterior midgut sac, the pharyngeal pump being the source of pressure. This view of the mechanism of filtration is, however, upset by the difficulty that the pressure would be applied to the *outside* of a system of delicate tubes, which would collapse under such circumstances. Other mechanisms that could bring about the transfer of water across a membrane would be, firstly, active secretion by the cells lining the receiving organ, and secondly, passive osmosis. The first of these alternatives is unlikely in the Cicadoid filter chamber, as its epithelia are degenerate, and present the appearance of a passive membrane. It is therefore necessary to examine the possibility of passive osmosis. We know that in sap-sucking Hemiptera the excreta discharged by the anus must be hypotonic to the ingesta, since the insect absorbs part of the dissolved material as its source of nourishment. In the sac-like rectum of most Hemiptera, in which the contents are only discharged at intervals, it will follow that the whole contents of the rectum are hypotonic, but in the narrow tubular rectum of Cicadoidea, with a steady flow along its length, this need not be so. Ramsay (1958) has shown that Malpighian

tubules usually pass a fluid more or less isotonic with the haemolymph, and that an important function of rectal glands is to re-absorb useful molecules such as amino acids and sugars. In the Cicadoidea, the Malpighian tubule fluid passing through the filter chamber would subject the ingesta to the same osmotic gradient as it would encounter in the anterior midgut, but over a very large area, and (to judge from histological appearances) through highly permeable epithelia. Thus the ingesta would give up most of their excess water to the Malpighian tubules before passing into the anterior midgut sac, while the long glandular rectum is admirably adapted to removing solutes, so that hypotonic excreta eventually reached the anus. An anatomical feature of Cicadoidea which, while not actually involved in this process, emphasises the efficiency with which water is prevented from diluting the haemolymph, is the insertion of the distal ends of the Malpighian tubules beneath the peritoneal membrane of the rectum. This is a feature found usually in insects in which water must be conserved.

The alimentary canal of Fulgoroidea seems to follow a different pattern from that of Cicadoidea, and there is no trace of a filter chamber, nor even of an approximation of the anterior and posterior ends of the midgut. Instead, we find a collection of structural features, which are as constant in their occurrence and as characteristic of Fulgoroidea as the filter chamber is in Cicadoidea. These features are: the sheath-like membrane enclosing the midgut; the absence of an expanded sac at the anterior end of the midgut; the blind diverticulum of the midgut, outside the sheath and usually air-filled; the sac-like rectum; and the Malpighian tubules not being inserted into the wall of the rectum.

The above analysis of the filtration process, which has been made in order to show that a mechanism consistent with the observed anatomy and histology can be postulated, is obviously inapplicable to Fulgoroidea, in which there is no filter chamber. It is a fact that some Cicadoidea also lack the filter chamber and it may be asked whether their ability to dispense with this organ may apply equally to Fulgoroidea. The Cicadoidea in which the filter chamber is missing are all very small forms (*e.g.* certain Jassidae), which can lose a relatively large amount of water by cuticular and tracheal transpiration, but the lack of a filter chamber is more likely (since many small Sternorrhyncha have filter mechanisms) to be due to their feeding on whole contents of plant cells rather than on sap alone. As their ingesta contain large molecules of proteins and carbohydrates, the breakdown of these will tend to raise the osmotic pressure, and will balance the lowering due to absorption by the insect's tissues. Many of the smallest phytophagous Hemiptera feed in this way, and it is characteristic of them that they leave distinct lesions on the plant, do not feed for long periods in one place, and have solid residues in the gut. Their intestines are simple, lacking any specialised water excreting structures, and in fact there is a superficial similarity between the intestines of such insects in different subdivisions of the Order, in such families as Jassidae, Peloridiidae (Pendergrast, 1962), Piesmidae, certain Lygaeidae, Tingidae and Miridae. On the other hand, large species of Hemiptera with simple intestines are either zoophagous (Reduviidae, Belostomatidae, *etc.*) or seed-sucking (Lygaeidae, Pyrrhocoridae), whereas large plant-sucking forms seem to be sap-suckers (since tissue feeding would involved too frequent a change of feeding site), and in the Cicadoidea and pentatomorph Heteroptera have complex intestines in which water disposal systems can be discerned.

From observation of its feeding habits, there can be little doubt that *Phalix* is as much a sap-sucker as a Cicadoid of equal size (such as many Membracidae and Cercopidae with well-developed filter chambers). The feeding of *Pyrops* has not been observed, but from its size, and the consideration that no other Homoptera are known to be zoophagous or seed-sucking, it may reasonably be assumed to be a sap-sucker. Both *Phalix* and *Pyrops* have, to a greater degree than the writer has observed in any other Hemipteran, the sap-sucking characteristic of absence

of solid matter in the gut. It may also be noted that, for the usual physical considerations arising from the relation of area to volume, the filter chamber becomes more complex with increasing size of the insect. Since *Pyrops*, at 20 mm. body length (excluding the head extension), is one of the largest Fulgoroidea, any mechanism of water control must be correspondingly more efficient than in a smaller species.

Since the Fulgoroidea do not possess any obvious means of shunting water from the anterior to the posterior end of the midgut, nor have they any organs that might pump water out of the haemolymph (such as the gastric caeca of Heteroptera might be (Goodchild, 1963)), attention must be concentrated upon the structural features of the alimentary canal that are unique to the superfamily. One of these is the intestinal sheath. It is clear from its structure that it is more than merely a detached peritoneal membrane (though that may be its origin), since there still is a cellular membrane surrounding the intestine within it. The presence of oenocyte-like cells, usually associated with the development of the external cuticle (Kramer and Wigglesworth, 1950), makes it possible that the sheath has some power of resisting the passage of water. At the same time, the sheath cells must be active in absorbing and transferring the dissolved substances needed by the insect. In these respects, it would not seem to be important whether the active cells are lining the sheath, as in *Phalix*, or surrounding the intestine, as in *Pyrops*. If the food materials in sap are in the form of simple molecules that do not require a prolonged digestive process, then the activity of the intestinal epithelium will be entirely absorptive. The intestinal contents will become progressively more dilute as they pass along its length, but dilution of the haemolymph will be prevented by the oenocytic cells of the sheath. Further extraction of solutes may take place in the rectum, through the gland cells. The fluid within the thin sheath of *Pyrops* is probably isotonic with the haemolymph, but in *Phalix* the osmotic barrier would be the sheath wall itself, and the fluid within the sheath cavity would be isotonic with the ingesta. In so far as the sheath wall must transfer nutrients into the haemolymph, there will be a loss of solutes from the sheath cavity, which would be made good by simple diffusion at the anterior end of the midgut, and by active absorption at the posterior end. This corresponds with the observed gradation in cell pattern down the intestine. The efficiency of extraction, in both species, and therefore the osmotic pressure of the material reaching the rectum, will depend upon the rate of flow through the intestine in relation to the absorptive ability of the lining cells, and the extent of the difference in osmotic pressure that the barrier cells can withstand. In a process of this kind, the material entering the rectum must necessarily be hypotonic to the ingesta, whereas in Cicadoidea it must be, at least slightly, hypertonic. This would account for the sac-like, less intensely glandular rectum of the Fulgoroidea, since it does not have the heavy burden of solute absorption.

The situation in *Gyarina nigritarsis*, in which the sheath cells appeared to be degenerate, may be related to a cessation of active sap ingestion on reaching maturity. Observation of the living insects supports this, and the occurrence of solid matter in the intestine suggests that at maturity the source of nourishment does not involve osmotic stress.

The anterior diverticulum of the midgut, which extends into the head prolongation in Fulgoridae, and may in this family act as a kind of buoyancy chamber, comparable with the expanded abdominal tracheae of Pentatomidae and Plataspidae, can also be regarded in a more general way as an essential complement to the specialised intestine. It can be inflated with air to assist ecdysis in the nymphal stages, whereas such inflation of the ensheathed intestine would probably be impossible without damage to its structure. It may therefore be concluded that the alimentary canal of Fulgoroidea is adapted for sap-sucking in a manner that differs from either the Cicadoidea (and the somewhat similar Sternorrhyncha) or the pentatomomorph Heteroptera, and must have evolved independently.

III. The Alimentary Canal of *Dalsira bohndorffi* Dist.

(*a*) *Material and Methods*

Dalsira bohndorffi is a moderately large insect, 20 mm. in length (excluding antennae), of a deep maroon colour and typical pentatomid appearance. Its preferred host plant is not known, as it was caught in a sweep net sample in an overgrown

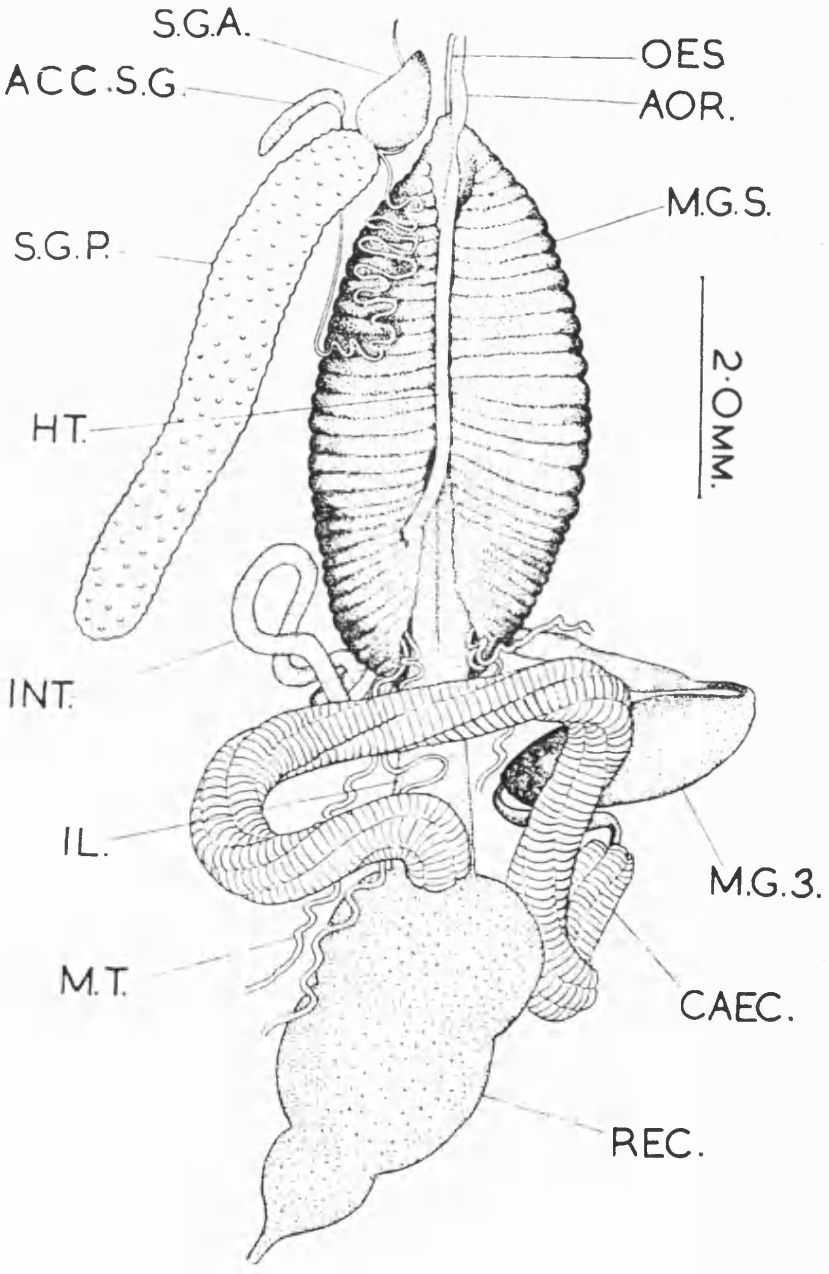


Fig. 4.—Whole alimentary canal of adult male *Dalsira bohndorffi*, dorsal view, drawn from fixed specimen. The right-hand salivary glands are omitted for clarity, and the Malpighian tubes curtailed.

vegetable garden plot at the University Farm. It may be significant that it was caught in the vicinity of a plant of the vegetable marrow (*Cucurbita pepo* L.) since plants of this family seem to be favoured by several species of sap-sucking Heteroptera, possibly because of their well-developed vascular tissues. The specimen was dissected and the whole gut was fixed in Bouin's Fluid, after which sketches were

made from which figure 4 was prepared. The gut was then embedded, serially sectioned at a thickness of 10 μ and stained with haemalum and eosin.

(*b*) *The Alimentary Canal and Associated Structures*

(i) *Gross Anatomy*

When the dissection was carried out with the minimum of disturbance in the relative positions of the parts, the remarkable features of the anatomy were not immediately apparent. A description will be given of the gut as it appeared in that state, before proceeding to the full details revealed by the sections. Anteriorly, lightly bound to the alimentary system by fine tracheae and dissected out along with it, were the very large salivary glands (fig. 4). Apart from their size and rearward extension into the abdominal cavity, they are unusual in having only two lobes, for Hemiptera of this size tend to have multi-lobed glands. Indeed, those of Cercopidae and scutellerine Pentatomidae appear as a dense cluster of narrow tubular lobes, and those of Hydrocorisae as a grape-like bunch. The anterior lobe of *Dalsira* is relatively small and smooth in outline, whereas the long posterior lobe has a rugose surface. The accessory gland is more normal, being a small sausage-shaped organ just ventral to the junction of the lobes of the main gland. The duct of the accessory gland runs backward for a short distance, and then returns in a sinuous path to the junction of the main lobes, from which point the main duct runs forward to the head. Between the anterior lobes of the salivary glands, the delicate oesophagus joins the midgut, the anterior end of which forms an expanded sac, as is usual in Heteroptera. This sac is brown in colour, and has strongly marked transverse ridges upon its surface. There appeared to be a dorsal groove, in which the heart was situated, bound to the edges of the groove by fine tracheae. Anteriorly, the heart expands into a sinus-like aorta over the dorsal side of the oesophageal junction. On either side of the midgut sac, the coils of the accessory salivary duct are conspicuous by their shining white colour. In order to make out the structures of the more posterior parts of the alimentary canal, it was necessary to sever the heart at the posterior end of the midgut sac. The gastric caecal region is well developed, of the type normal in Pentatomidae, having four longitudinal rows of densely clustered caeca, and passing dorsally across the posterior end of the midgut sac. It then turns backward to join the broad anterior end of the pear-shaped, thin-walled rectum. Just ventral to this junction, a wide, thin-walled tube was seen, extending forward from the rectum and entering a dorsally situated cavity in the posterior end of the midgut sac, from which gentle traction failed to dislodge it. At first glance, this seemed to be an anterior rectal diverticulum, recalling Huber-Schneider's suggestion, but on further consideration it was realised (even before the sections confirmed this) that it must represent the ileum. This segment of the midgut is present in all other Pentatomomorpha, in its more primitive form (*e.g.* in Lygaeidae) lying in the main axis of the intestine, but in Pentatomidae becoming a diverticulum receiving only the contents of the Malpighian tubules, as a result of the dorsal and backward migration of the opening of the caecal region of the midgut. Thus *Dalsira* represents an extreme case, with the caecal region apparently opening into the rectum directly (though in fact still within the folds of the ileo-rectal valve). Since the Malpighian tubules, the distal parts of which coiled extensively around the rectum, were seen to pass into the cavity in the midgut sac, together with this tube extending from the rectum, it was assumed that their junction with the ileum took place in the obscurity of that cavity. The remaining parts of the midgut are of the normal Pentatomid pattern, the second, tubular, region of the midgut arising from the anterior sac just ventrally to the above mentioned cavity, forming two short loops beneath the sac and joining a smaller expanded region (the third midgut region) on the right side of the abdomen, from which a short tube leads to the gastric caecal region.

(ii) *Microscopical Anatomy and Histology*

The structures revealed by sectioning are even more remarkable than those seen in the dissection. From a study of the serial sections, it became apparent that the cavity in the midgut sac into which the ileum disappears is, in fact, a deep channel formed by the upward and inward reflection of the edges of the sac, so that, in cross-section, its lumen is shaped like a C lying on its back (fig. 5*A*). The dorsal " groove " is formed by the approach of these edges to each other above the ileum. The edges are joined by a delicate septum, probably derived from the dorsal abdominal septum,

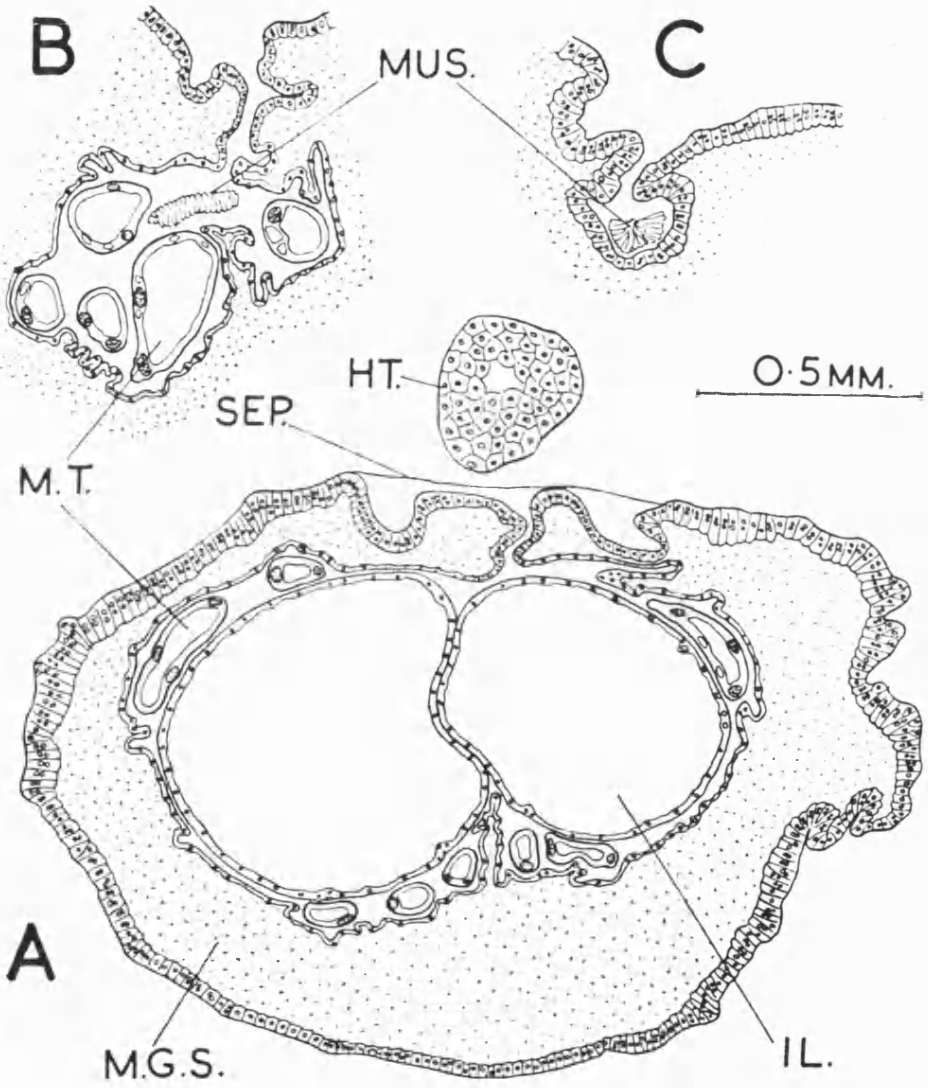


FIG. 5.—Filter chamber of *Dalsira bohndorffi* : (*A*) transverse section through middle ; (*B*) detail of chamber just anterior to ileum ; (*C*) detail of region anterior to Malpighian tubules.

above which is the heart. The heart, in this region, is unusually densely packed with pericardial cells, but presents a more normal appearance at its anterior and posterior extremities. The ileum is enormously inflated (at a rough guess at least ten times the volume in proportion to the size of the insect, compared with any other known Heteropteran) and it is almost entirely enclosed in the folded midgut sac. It will be most convenient, in referring to this cavity in which the ileum is situated, to anticipate the conclusions of this paper and use the term filter chamber.

The ileum leaves the rectum in the form of a thin-walled tube of circular cross-section, but this becomes bifurcated just before it enters the filter chamber, each branch rapidly swelling to a diameter equal to that of the common stem (fig. 6).

This diameter is about 0·65 mm. The ileum branches are flask-shaped, and contract slightly in diameter at their anterior ends, where they merge into the inflated Malpighian tubules, two tubules arising from each branch. The tubules turn posteriorly from their junction with the ileum (which is very far forward, being almost at the anterior end of the midgut sac) and follow a sinuous course backwards between the ileum and the wall of the midgut sac, on the ventral and lateral sides of the filter

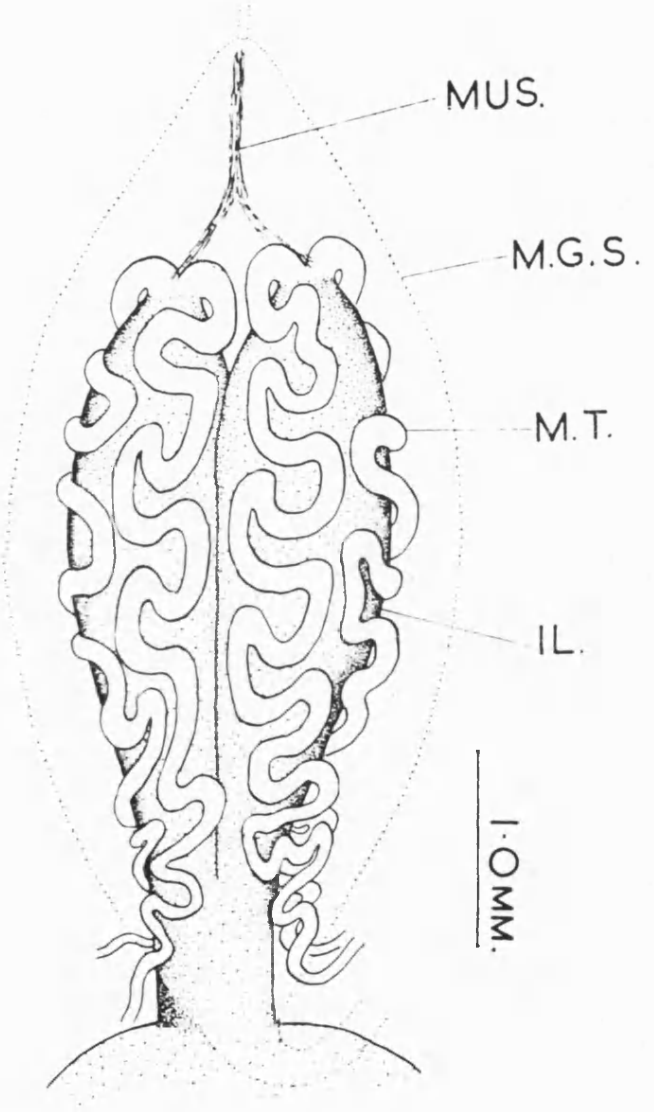


FIG. 6.—Reconstruction of ileum and Malpighian tubules of *Dalsira bohndorffi* from ventral side, midgut sac shown as if transparent.

chamber. At the anterior end of each branch of the ileum, a well-defined muscle strand arises between the junctions of the Malpighian tubule and travels forwards (fig. 5*B*, *C*), joining its fellow from the opposite side and emerging from the anterior end of the filter chamber to be inserted into the dorsal wall of the midgut near the oesophageal junction. This muscle is clearly the reason why the parts do not separate during dissection, and is among the highly unusual features of this intestine. A similar muscle, inserted into the ventral side of the oesophageal junction, arises from widely spaced strands on the ventral side of the midgut sac. These strands are much thicker than the usual delicate network found around the gut of Hemiptera.

The histology of the alimentary canal is as follows. The C-shaped lumen of the midgut sac tapers to a narrow circular section at its anterior and posterior ends. At the anterior end, the oesophagus joins the midgut in a moderately well-developed,

but widely open, valve, a short collar of small cuboid fore gut cells extending into the midgut lumen and giving rise to a valve-like fold of the chitinous intima. The outer wall of the midgut sac consists of low columnar, slightly bulbous-tipped cells, frequently binucleate and strongly charged with brown granules, and with a thin, not striated, border. The whole length of the tubular intestine is uniform in cell structure, the cells being columnar, rather taller than those of the sac, with the lumen-ward border produced into small lobes having a slightly eosinophil striated zone within the border. The distal part of these cells is full of brown granular material, as in the sac cells, but this is not present below the central nuclei, where the cytoplasm is precipitated in coarse strands. The expanded third region of the midgut is lined with low columnar cells having an irregularly lobed brush-like border, which did not take up stains. The depth of this border is variable, and in places there appeared to be secretory droplets passing through it. The cytoplasm contains no granular inclusions, and is moderately eosinophil. The entrance of the intestine to this third-region sac is widely open, but the exit, leading to the gastric caecal region, is a moderately constricted tube of small columnar cells. This tube becomes wider and lined with low columnar cells with a lobed, non-staining, striated border, for the short distance between the third-region sac and the beginning of the gastric caecal rows. In the gastric caecal region, the central tube is narrow, and formed of low columnar cells with pale cytoplasm and dark staining nuclei. The junction of each caecum with this tube is a capillary-like tube consisting of six to eight irregular-shaped cells, which is inserted among the cells of the central tube. At the posterior end of the caecal region, the central tube is invaginated for a short distance into the posterior dorsal part of the ileum, largely obstructing the passage between the ileum and the ileo-rectal valve. The end of the intestinal tube is blunt, and it is covered by a dome of columnar cells which represent a modified area of the ileum lining. The opening of the intestine into the ileum is an extremely restricted pore (minimum diameter 3–4 μ) which penetrates both epithelia at the tip of the dome. The ileo-rectal valve is typically pentatomid, with complex folds of very small pale cells with dark staining nuclei, and lined with a chitinous intima. The rectal lining resembles that found in other sap-sucking Pentatomidae (Goodchild, 1963), in being composed entirely of large, thin, gland-like cells, with large granular nuclei and a narrow striated eosinophil zone beneath the chitinous border.

The histology of all parts described above is not significantly different from that of other sap-sucking Heteroptera. On the other hand, the filter chamber components, namely the inner wall of the anterior midgut, the enclosed parts of the Malpighian tubules, and the ileum, have a histological appearance that is highly modified from the normal one for these organs, and there is, moreover, a remarkable similarity between these layers of differing origin. In general, the cells are very flattened, so that the nuclei cause a distinct bulge in the thickness, their cytoplasm is uniform, very finely granular and mildly eosinophil, and contains large brown inclusions of size comparable with the nuclei (fig. 7). The nuclei are coarsely granular, those of midgut and ileum being of moderate size and those of the Malpighian tubules much larger. The slight differences between the layers are as follows. The midgut epithelium is thinner between the nuclei than the others, and appears like a string of beads, and its cytoplasm is darker staining than the others. The ileum lining is of more even thickness, little constricted between the nuclei, and its brown inclusions are not more than about half the size of its nuclei. In the spaces between these epithelia there is evidence of tenuous cellular basement or peritoneal membranes around each organ, and numerous fine tracheal branches. Where the ileum cells come close to midgut cells, they often seem to be inflated, and the cytoplasm stretched into strands perpendicular to the cell border. This may be a fixation artefact, but since it is not seen in the part where the two halves of the ileum are in contact, it may indicate osmotic activity in the cell. The cell borders of the ileum seem to

bear very fine, short, refractile cilia, though this appearance may be the result of precipitation (during fixation) from the contents of the organ. The cell border of the Malpighian tubules in the filter chamber is simple, but the more distal parts of the tubules, in the main body cavity, have a normal histological structure with a deep eosinophil brush border. In the common stem of the ileum, and extending a short way up the branches, the lining cells are folded into a number of narrow ridges (11 in the specimen examined) of varying height. The highest was about one-fifth of the diameter of the lumen.

The nature of the contents of the alimentary canal was as follows. In the midgut sac and tubular intestine there was a mildly basophil flocculent precipitate ; in the third midgut region, a fibrous hyaline non-staining mass with an outer zone of eosino-

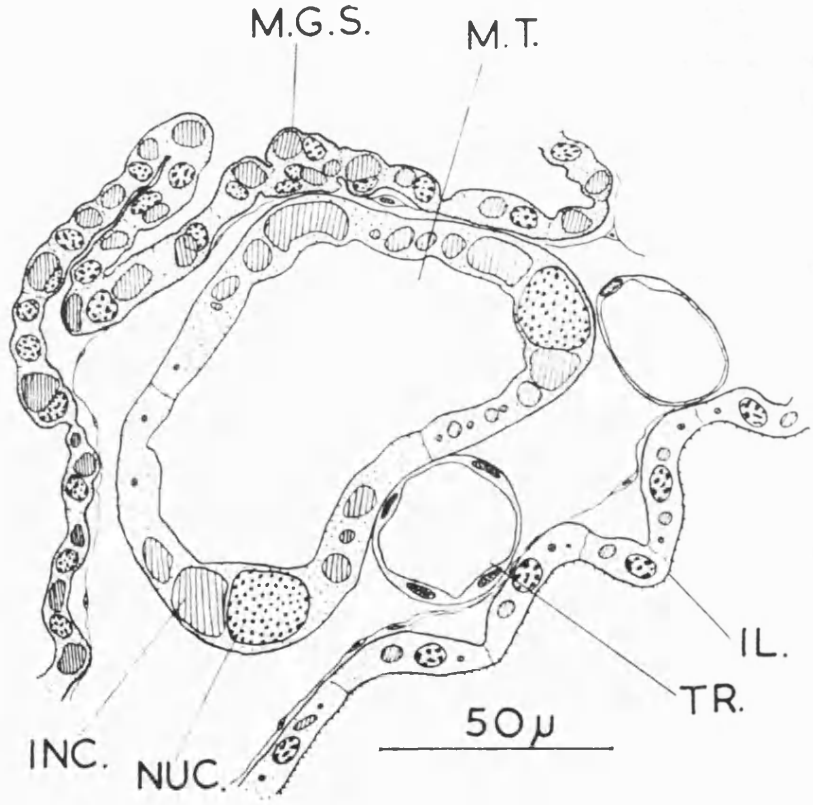


FIG. 7.—Histological detail of tissues involved in filter chamber of *Dalsira bohndorffi*.

phil flocculent material ; in the tube connecting to the gastric caeca, a finely granular basophil mass with a number of clearly recognisable nuclei embedded in it ; in the gastric caeca, dense masses of thread-like basophil micro-organisms ; in the ileum, and Malpighian tubules in the filter chamber, a small amount of finely granular non-staining refractile precipitate clumped against the cells. In the rectum and central tube of the caecal region there were no significant contents at all.

Although it is not immediately relevant to the subject of this study, it may be remarked that the unusually shaped salivary glands did not show any abnormalities as regards histology. The main gland cells are domed and very strongly basophil, while the accessory glands are lined with a deep columnar, mildly basophil, epithelium, the very narrow lumen having apparently a structureless cuticular lining.

(*c*) *Discussion*

It may reasonably be assumed that so elaborate an arrangement must have some important physiological significance. Since the existence in the sap-sucking cicadoid

Homoptera of a complex filter chamber, and the general need of insects with such a diet for rapid through-put of dilute watery food is known, it is no long step to regard the midgut-Malpighian tubule-ileum complex of *Dalsira* as being adapted for this purpose. Structures not very different exist also in *Gellia* sp., and probably in other species of Phyllocephalinae. The processes involved may be further investigated by considering the possible mechanisms of water transfer referred to earlier. Firstly, simple osmosis, as postulated in the cicadoid filter chamber, would attract water from the midgut to the Malpighian tubules and ileum. Indeed, this must happen if the tubules pass a fluid more or less isotonic with the haemolymph, as Ramsay (1958) has shown. However, if such a mechanism is to yield eventually hypotonic excreta, the rectal epithelium must be active in absorbing solutes. As stated earlier (p. 226), the narrow tubular, highly glandular, rectum of Cicadoidea is well adapted to perform this task, but in the sac-like, rather weakly glandular, rectum of Heteroptera, it would be a slow process. Although the internal area of the ileum is increased by the formation of ridges, it is not easy to see how this organ could selectively absorb solutes. It does not have an impermeable chitinous lining, and unless some active process were continually rejecting water, the transfer of solutes across the cell border would be accompanied by an osmotic flow of water. This would be aggravated by the very confined haemocoel space between the cell layers of the filter chamber, which would prevent a rapid assimilation of solutes into the main volume of haemolymph. In the Cicadoidea, the rectum, as well as having an impermeable lining, is situated in the main stream of the haemolymph, and so the solutes absorbed by its cells do not remain for long in a local concentration.

It has been suggested (Goodchild, 1963) that active secretion of hypotonic solutions by the cells lining the ileum occurs in other Heteroptera. In these forms, the ileum cells have large bulbous tips lacking any visible cytoplasmic structure. In living tissues, these tips occasionally become detached and pass into the rectum, where they degenerate. The cause of this seems to be pressure from similarly expanded neighbouring cells, and if it is a means of excreting hypotonic fluid, then it is clear that the rate of detachment of cell tips will vary according to the degree of distension of the ileum lining as a whole, that is, according to the need for such an excretory process. In stained sections such an epithelium usually shows many broken and distorted cell tips, but nevertheless the general pattern is recognisable, and is not like that seen in *Dalsira*. In the gastric caeca, on the other hand, the apical vacuoles seen in living cells, which are clear evidence of a secretory process, are totally destroyed by fixation, and in sections the cells present the appearance of thin pavement epithelium. As the ileum cells of *Dalsira* have so far not been observed in the living state, it is not possible to say whether any vacuoles are present but do not survive fixation. Nor is it possible to say, from purely histological evidence, whether active water secretion could take place without the formation of intracellular vacuoles. The significance of the similarity between the cell layers is not readily apparent, nor is that of the brown inclusions. In the Cicadoid filter chamber, one may suppose that all the cell layers involved have become similarly degenerate in order to offer the least possible hindrance to the osmotic transfer of water.

The possibility of filtration by hydrostatic pressure need not be considered; not only would such pressure tend to destroy the filter chamber by pushing the C-shaped midgut lumen into a circular section, but it would force water through the outer wall into the haemolymph as much as inwards to the ileum. It would thus appear that, of the mechanisms available, that of active water secretion by the cells of the ileum is most likely.

The picture of water disposal in phytophagous Heteroptera that emerges from the studies of the present writer is one of a steady pumping of water out of the haemolymph by the action of cells in the gastric caeca and ileum, rather than the Homopteran method of excluding water from entry into the haemolymph either by

shunting it directly from the impermeable oesophagus to the equally impermeable rectum, or, in Fulgoroidea, by the development of a water resistant sheath around the midgut. The arrangement of a few long caeca enclosing the anterior midgut sac, found in certain Lygaeidae, seems to have given way, in the evolution of the more advanced sap-suckers in the Coreidae and Pentatomidae, to caecal regions with many short caeca, offering a greater surface area to the haemolymph. The ileum in these Heteroptera is always in close proximity to, often in contact with, the posterior end of the anterior midgut sac, but at best the amount of direct transfer of water, compared with that passing into the haemolymph, must be small. The suggestion that, in those species in which the gastric caecal region is not in open connection with the ileum and rectum, the ileum is able to take over water excretion has been based on anatomical and histological observations, as it has not been possible to make the delicate measurements of osmotic pressure in cell tips or lumen contents which would be needed to substantiate the argument. In a similar way, final proof of this kind is lacking in the argument in favour of gastric caeca. It could be held that, if gastric caeca do not excrete water, then when they are closed off posteriorly, there is no need to postulate a taking over of this function by the ileum. In spite of the existence of a minute pore connecting the caecal region of *Dalsira* to the ileum and rectum, the caecal region is certainly of the specialised mycetome type, because of the evidence (explained below) of digestion of bacteria ; and by analogy with many Pentatominae in which the posterior closure of the caecal region is found only in the nymphal stages, it may be that even this restricted opening was not present earlier in the insect's life. Thus, the evidence from the remarkable anatomy of *Dalsira* as to the association of the ileum with water disposal serves to support this interpretation of other, less specialised, cases and, in turn, to support the idea of the gastric caeca having this function.

In sap-sucking Heteroptera with discontinuous intestines, it has been found (Goodchild, 1963) that a bulbous expansion is developed at the anterior end of the caecal region. This may be as large as the expanded third midgut region, which lies immediately anterior to it. Between these two sacs the intestinal lumen is interrupted or severely restricted (as well as being interrupted posteriorly to the caecal region). The third midgut contains a pasty mass of the food residues that form even in sap-suckers by precipitation of soluble proteins, *etc.* In stained sections this mass appears as granular material of mixed staining reactions. In the bulbous expansion at the anterior end of the caecal region, which together with the caecal region itself forms an isolated mycetome system, the contents have an entirely different appearance in sections, being fibrous, hyaline and non-staining. It has been inferred that this material represented the debris of digested bacteria, because there is a smooth gradation towards unchanged bacteria at the end of the bulb nearest to the caeca. In *Dalsira* no bulb is developed at the anterior end of the caecal region, but the contents of the third midgut region are of the fibrous hyaline kind. There is no interruption of the gut between the third and caecal regions, and so it would appear that the food source of this insect leaves little residue, thus freeing the third midgut region for digestion of the bacterial symbionts. The ability of sap-sucking Heteroptera to avoid the accumulation of solid residues is not unique to *Dalsira*, because in *Piezosternum calidum* Fab. (Tessaratominae) both the third midgut region and its contents are absent. In Homoptera, in which no intestinal restrictions occur, such residues are presumably discharged from the intestine as fast as they form.

It may be said in conclusion that *Dalsira bohndorffi* represents a peak of evolutionary development, following the predominant tendency in the Pentatomidae to divert the gastric caeca into functioning solely as a harbourage for symbiotic bacteria. It may be worth recording that the bacteria from the related *Gellia* were found to be less specialised than those usually found in Pentatomidae, being long thread-like

organisms that grew easily in culture, the culture forms being short rods like those obtained from Coreidae.

IV. Summary

1. The alimentary canal of a fulgoroid Homopteran, *Phalix titan* Fennah (Tettigometridae) is described. In this species, and in other Fulgoroidea that have been examined, the midgut is tubular, and is coiled within a membranous sheath. The only previous description of a Fulgoroid alimentary canal (Kershaw, 1913) dismisses this sheath as consisting of the detached peritoneal membrane, and omits it from his figures. In *Phalix*, the sheath is two-layered, the inner layer of cells being relatively thick, and with dense, strongly eosinophil cytoplasm, whereas the outer layer is thin and more like a normal peritoneal layer. There do not appear to be any muscle fibres, and the sheath seems to be resistant to stretching. The Fulgoroidea do not possess a filter chamber as is found in Cicadoidea, and it is suggested that the sheath cells have an active role in limiting the dilution of the haemolymph with water from the ingested sap. This would seem to indicate that sap-sucking habits have been evolved independently in Fulgoroidea and Cicadoidea. An analysis is also made of the probable mechanism of the Cicadoid filter chamber, based on the anatomical and histological features that it presents.

2. The alimentary canal of a phyllocephaline Pentatomid, *Dalsira bohndorffi* Dist., is described. It is remarkable for the fact that the ileum region is enormously enlarged, and, together with the proximal parts of the Malpighian tubules, is enclosed in a chamber formed by the dorsalward reflection of the edges of the sac-like anterior midgut. The parts are in very close contact, and have a similar histological structure of an unusual kind. It is suggested that the function of this association of organs is to facilitate the rapid elimination of excess water from the plant sap on which the insect feeds, the mechanism being probably an active excretory process.

V. References

Brittain, W. H., 1923, The morphology and synonymy of *Psylla mali* Schmidt. *Proc. Acadian ent. Soc.* **8** : 16 pp.

Ermisch, A., 1960, Vergleichend-anatomische Untersuchungen uber die Endosymbiose der Fulgoroiden mit besonderer Berucksichtigung der Araeopiden. *Z. Morph. Ökol. Tiere* **49** : 47–138.

Goodchild, A. J. P., 1952, A study of the digestive system of the West African Cacao Capsid Bugs (Hemiptera, Miridae). *Proc. zool. Soc. Lond.* **122** : 543–72.

—— 1963, Studies on the functional anatomy of the intestine in the Heteroptera. I. The histology of the rectum and posterior mid-intestine. II. Discontinuity of the intestine. *Ibid.* **141**.

Huber-Schneider, L., 1957, Morphologische und physiologische Untersuchungen an der Wanze *Mesocerus marginatis* L. und ihren Symbionten (Heteroptera). *Z. Morph. Ökol. Tiere* **46** : 433–80.

Imms, A. D., 1958, *A General Textbook of Entomology*, 9th Edition, revised by O. W Richards and R. G. Davies. London.

Kershaw, J. C. W., 1913, The alimentary canal of *Flata* and other Homoptera. *Psyche, Camb., Mass.* **20** : 175–88.

Knowlton, G. F., 1925, The digestive tract of *Longistigma caryae*. *Ohio J. Sci.* **25** : 244–50.

Kramer, S. and Wigglesworth, V. B., 1950, The outer layers of the cuticle in the cockroach *Periplaneta americana* and the function of the oenocytes. *Quart. J. micr. Sci.* **91** : 63–72.

Muller, H. J., 1940, Die Symbiose der Fulgoroiden (Homoptera-Cicadina). *Zoologica* **98** : 1–220.

—— 1949, Zur Systematik und Phylogenie der Zikaden-Endosymbiosen. *Biol. Zbl.* **68** : 343–68.

PENDERGRAST, J. G., 1962, The internal anatomy of the Peloridiidae (Homoptera : Coleorrhyncha). *Trans. R. ent. Soc. Lond.* **114** : 49–65.

PESSON, P., 1944, Contribution à l'étude morphologique et fonctionelle de la tête, de l'appareil buccale et du tube digestif des femelles de Coccides. *Monogr. Sta. Lab. rech. agron. Paris* **1944**, 266 pp.

—— 1951, Ordre des Homoptères, *in* Grassé, P.-P., *Traité de Zoologie* **10** : 1390–1656.

RAMSAY, J. A., 1950, Osmotic regulation in mosquito larvae. *J. exp. Biol.* **27** : 145–57.

—— 1958, Excretion by the Malpighian tubules of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae) : Amino acids, sugars, and urea. *Ibid.* **35** : 871–91.

SAXENA, K. N., 1955, The anatomy and histology of the digestive organs and Malpighian tubules of the Jassidae (Homoptera). *J. zool. Soc. India* **7** : 41–52.

SNODGRASS, R. E., 1935, *Principles of Insect Morphology.* New York.

WEBER, H., 1930, *Biologie der Hemipteren.* Berlin.

WIGGLESWORTH, V. B., 1931, The physiology of excretion in a blood-sucking insect, *Rhodnius prolixus* (Hemiptera, Reduviidae). *J. exp. Biol.* **8** : 411–51.

WILLIS, D. M., 1949, The anatomy and histology of the head, gut and associated structures of *Typhlocyba ulmi*. *Proc. zool. Soc. Lond.* **118** : 984–1001.

VI. EXPLANATION OF FIGURE LETTERING

Acc. S.G., accessory salivary gland
Ant. Div., anterior diverticulum of mid-intestine
Aor., aorta
Caec., gastric caeca
Chit., chitinous intima
Cut., cuticular outgrowth
Gr., basophilic apical granules
Ht., heart
Il., ileum
In. Sh., inner layer of sheath
Inc., brown cell inclusion
Int., mid-intestine tube (fig. 1) ; tubular intestine (fig. 4)
Int. Ep., epithelium of mid-intestine
M.G.S., midgut sac
*M.G.*3, third region of midgut
M.T., Malpighian tubule
Mus., muscle strand
Nuc., nucleus
O. Sh., outer layer of sheath
Oen., oenocytic cells
Oes., oesophagus
Oes. V., oesophageal valve
Per., peritoneal membrane
Rec., rectum
Rec. Org., rectal organ
S.G.A., anterior lobe of salivary gland
S.G.P., posterior lobe of salivary gland
Sep., septum
Sh., intestinal sheath
Sh. Cav., sheath cavity
Tr., trachea

APPENDIX 3

STUDIES ON THE FUNCTIONAL ANATOMY OF THE INTESTINES OF HETEROPTERA

BY

A. J. P. GOODCHILD

Department of Agricultural Biology, Makerere University College, Kampala, Uganda

[Accepted 9th October 1962]

(With 18 figures in the text)

Recent studies on the physiology of the malpighian tubules of insects have indicated that they play only a minor role in osmotic regulation. The major part of this function is now thought to be performed by glandular structures in the hind-gut. The Heteroptera are of especial interest from this viewpoint, since they are a group in which a wide range of food choice occurs, so that species can be found which are adapted to either extreme of osmotic stress, from dry seed diets to the water superfluity of sap sucking. A comparative study of intestinal anatomy and histology was undertaken, in the hope that correlations between structure and dietary water content would be revealed, and in particular to elucidate the mechanism of water disposal in the sap-sucking forms (which lack the specialized filter chamber of Homoptera). The first part of this paper describes the results of this study, and a case is made out for the interpretation of the gastric caeca possessed by sap-sucking species as water excretory organs. In the second part, the phenomenon of intestinal discontinuity is shown to be widespread among the most specialized sap-sucking Heteroptera, and its relationship to the water excretion theory of gastric caeca is discussed.

CONTENTS

INTRODUCTION

No comparative account of this aspect of the internal anatomy of Heteroptera has previously been published, although general surveys of gross intestinal anatomy have been made (Glasgow, 1914 ; Elson, 1937 ; Yanai, 1952), and the structure of the salivary glands compared (Bugnion & Popoff, 1908, 1910 ; Baptist, 1941 ; Southwood, 1955 ; Nuorteva, 1956). Other comparative accounts of Heteroptera have referred to the bacterial symbionts and the organs which harbour them (Kuskop, 1923 ; Schneider, 1940), to stridulatory mechanisms (Leston, 1957), and to genitalia (Pruthi, 1925 ; Marks, 1951 ; Scudder, 1959). Yanai (*op. cit.*) gives a brief classification of the types of intestine, and of the structure at the junction of mid- and hind-intestines, but publishes only simplified diagrams.

The present writer was stimulated to undertake the work which is recorded in this paper as a result of studies on the cacao capsid bugs of West Africa (Goodchild, 1952). The structure of the intestine of those insects provided a puzzle by being widely different from any heteropteran previously described, and the feeding behaviour and nature of the excreta drew attention to the problem of water disposal which affects the plant sucking Hemiptera. It is clear that many Heteroptera are no less suckers of plant sap than the Homoptera but so far no structure with a function comparable with the well known filter chamber of the latter, has been recognised in the former group. It has in the past been assumed that the Malpighian tubules performed such a function of water excretion, but the researches of Ramsay (1950, 1952, 1955, 1958) have revealed that the urine produced by these tubules is more or less isotonic with the haemolymph. The Hemiptera appear to be unique among terrestrial forms of life, in that their exploitation of plant sap as the sole food of all stages in the life-cycle causes them to dispose of an excess of water, a problem otherwise found only in the inhabitants of fresh water. That such a problem exists is evident from the complexity of the Homopteran filter chamber, for which no other purpose can be imagined. It is not just a question of ingesting a fluid food of low osmotic pressure, as the figures which are available for the osmotic pressure of plant sap show that it is often high (e.g. Pfeiffer, 1937). It must be borne in mind, however, that where sap is the sole food, the insect utilizes the solutes which are responsible for the osmotic pressure, unlike, for instance, bloodsucking arthropods, which rapidly excrete a fluid isotonic with the ingesta, leaving the true food, blood corpuscles and protein, to be digested later. Since the Heteroptera include not only sap sucking types, but also species which feed on dry seeds, it was thought that a comparative study would reveal significant differences, which might give an indication of the tissues or organs involved in water balance in this group. Obviously, physiological experiments

are desirable to confirm the conclusions wihch have been reached on a mainly histological basis.

Following the studies of Leston, Pendergrast, & Southwood (1954), and also those of Scudder (1959), it is clear that the Heteroptera fall into two quite distinct groups of families, the Pentatomomorpha, which are predominantly phytophagous, and the Cimicomorpha (with which Scudder associates the Amphibicorisae and Hydrocorisae, but not Corixidae), which are almost entirely zoophagous. Marked differences in intestinal and salivary gland structure are found between the two groups, and it seems probable that the original dichotomy in the evolutionary history of the Heteroptera was between phytophagous and zoophagous modes of feeding. Among existing species, however, there are examples of feeding habits opposite to the general trend, to be found in both groups. In the Pentatomomorpha, the Asopinae (Pentatomidae) are all zoophagous, and this would seem to be the case in Geocorinae (Lygaeidae) and several species of Rhopalidae, while in the Cimicomorpha, the Tingidae seem to be entirely, and the Miridae are mostly, phytophagous. It is reasonable to expect, in these atypical families or sub-families, changes in the structure of the digestive organs from that normal to the group. This does occur to some extent, but it is found that the altered structure, though it must possess functional similarities with that of the opposite group, still retains the histological detail characteristic of its own group. Thus, the grouping together of superficially similar types of intestine, as, for instance, the Reduviidae and Asopinae, or the Pyrrhocoridae and Hydrometridae, as done by Yanai (1952), is not supported by their histological structure. For this reason, the descriptions that follow will be arranged according to accepted taxonomic categories.

MATERIAL AND METHODS

This work has been based on dissections, microscopic examinations of fresh tissues (using phase-contrast microscopy where appropriate), and stained serial sections. The latter were prepared from whole intestinal systems dissected out with the minimum of disarrangement, fixed in Bouin's fluid, wax embedded, and sectioned at a thickness of 10 μ. The fixative was chosen for convenience in handling large collections, since immediate dehydration and embedding was not usually possible. It gave satisfactory results with most of the cell types encountered. It was noticeable, however, that osmotic damage regularly occurred in certain tissues, these being such organs as rectal glands, where active water absorption probably took place in the living insect, and certain other cells where it was thought (on other grounds) that water excretion might be taking place. Since it was found that cells which might reasonably be expected to be in osmotic equilibrium with their surroundings fixed in a very life-like manner, this observation seemed to be significant in indicating tissues where osmotic work was carried out. The stains used were Mayer's haemalum and eosin. A list is given below of those species of which stained sections were examined for the purpose of this study. Some of these were made before the writer arrived in East Africa, and are of British species. The list does not include many species examined by dissection and fresh tissue preparation

only, as the structures found in these merely confirmed the other observations. Minor differences in the histological appearance of tissues from different species have had to be ignored, unless they had some obvious significance. Such differences could be multiplied endlessly, as more species are studied. It is felt that the range of species which have been investigated is adequate to justify the general account given. In a number of species, only a few individuals were available, but where any histological features could be simply an isolated phase in a cyclical pattern of activity, this is taken into account in assessing their significance.

LIST OF SPECIES STUDIED

Cimicomorpha

Family Reduviidae	*Reduvius personatus* (Linn.), *Harpactor tibialis* Stål, *Petalochirus rubiginosus* P. & B., *Vadimon nodamus* Sign., *Sphedanolestes* sp.
Family Nabidae	*Nabis ferus* (Linn.).
Family Cimicidae	*Cimex lectularius* Linn.
Family Anthocoridae	*Piezostethus flavipes* Reut.
Family Isometopidae	*Lindbergiola aureopilosa* Carv.
Tamily Tingidae	*Habrochila africana* Drake, *Ammianus wahlbergi* Stål.
Family Miridae	Subfamily Bryocorinae: *Chamopsis tuberculatus* (Dist.), *Boxia khayae* China, *Lycidocoris mimeticus* Reut. & Popp.
	Other subfamilies: *Proboscidiocoris fuliginosus* Reut., *Stenotus elegans* Popp., *Leptoterna dolabrata* (Linn.), *Psallus impictus* Odhiambo.

Amphibicorisae

Family Gerridae	*Gerris dolosa* Bergr.

Hydrocorisae

Family Belostomatidae	*Lethocerus cordofanus* Mayr.

Pentatomomorpha

Family Lygaeidae	*Spilostethus pandurus* (Fab.), *Pachybrachius capicola* Stål, *Paromius gracilis* Stål, *Dieuches armipes* Fab., *Chauliops rutherfordi* Dist., *Geocoris amabilis* Stål.
Famil Rhopalidae	*Serinetha amicta* Germ., *Corizus nigromaculatus* Stål.
Family Pyrrhocoridae	*Dysdercus nigrofasciatus* Stål, *Myrmoplasta potteri* Mast., *Scantius forsteri* Fab.
Family Coreidae	*Acanthomia tomentosicollis* Stål, *Cletus fuscescens* Walk., *Dulichius trispinosus* Stål, *Hydara tenuicornis* Westw., *Stenocephala luteipes* Stål, *Mirperus torridus* Westw., *Acanthocoris obscuricornis* Dall., *Mygdonia tuberculosa* Sign., *Anoplocnemis signata* Dist.
Family Pentatomidae	*Nezara viridula* Linn., *Caura leggei* Dist., *Agonoscelis versicolor* Fab., *Halyomorpha annulicornis* Sign., *Aeliomorpha divisa* Walk., *Sepontia misella* Stål, *Scotinophara fibulata* Germ., *Sphaerocoris testudo-grisea* DeG., *Hotea subfasciata* Westw., *Platynopus septemdecimmaculata* Drury, *Picromerus bidens* (Linn.), *Aspongopus xanthopterus* Fairm., *Piezosternum calidum* Fab.
Family Cydnidae	*Cydnus indicus* Westw.
Family Acanthosomidae	*Acanthosoma haemorrhoidale* (Linn.).
Family Plataspidae	*Libyaspis flavospersus* Mont., *Coptosoma nigriceps* Sign.

THE CIMICOMORPH FAMILIES

The intestines of these are always simple, consisting of an anterior sac-like region, followed by a tubular region. Temporary accumulations of indigestible residues may cause slight distension in the posterior part of the tubular intestine, but a permanent expansion at this point, as found in many Coreidae, Lygaeidae, and Pyrrhocoridae, does not seem to occur. In the large tropical Miridae Bryocorinae, the tubular intestine is divided by a slight constriction into two consecutive tubular regions with distinctly different cell types. Yanai & Iga (1956) recognize a *Lygus type* of intestine, with a similar external appearance, but do not mention any histological differentiation. The cells lining the cimicomorph intestine are of the type common in Hemiptera, with narrow bases and bulbous tips projecting into the lumen. They are usually binucleate, the nuclei typically having a large central chromatic granule and smaller granules peripherally distributed, and the cytoplasm is often heavily charged with basophil granules, or with self coloured brown, yellow, or green granules. There may be a vacuole, or several small vacuoles, towards the tip, especially in the cells of the anterior sac-like region. This vacuolation is most strongly marked in the zoophagous species (e.g. Reduviidae). In the small, immature, cells, a thin striated (honeycomb) border can usually be detected, but in fully expanded cells this is more or less obliterated. As the cells mature, they seem to exert strong lateral pressure on each other, and assume a tall, narrow, shape. The appearance of the epithelium is dependent upon the phase of the secretion cycle and the degree of distension of the intestine, as these cells seem to be very easily stretched and deformed into the semblance of a regular columnar, cuboid, or even pavement epithelium.

A second type of cell is found in the phytophagous forms, Tingidae and Miridae, lining the anterior tubular region, or the whole tubular region where it is not subdivided. In this type, there is an approximately cuboid cell base, firmly attached to neighbouring cell bases, which bears a large lobe projecting into the lumen. The junction of the lobe with the base is distinctly constricted. There is a broad brush-like border, particularly over the lobe. These cells are very constant in shape, though in different species the lobes vary in their characteristic shape from bulbous to long and finger-like. The cytoplasm is always rather dense, finely granular, and not vacuolated.

In the larger cimicomorph species, the Reduviidae, the internal surface of the intestine is increased by infoldings of the basement membrane so that the cells are clustered on transverse ridges. Cell and nucleus size, over the size range of the species studied (from 1·5 mm to 18·0 mm body length), increases by a factor of about two, from $50\,\mu \times 10\,\mu$ (nucleus 6–7 μ) to $90\,\mu \times 25\,\mu$ (nucleus 13–14 μ) for cells of comparable shape and degree of maturity.

In the Cimicomorpha, the structure of the hind-intestine and the Malpighian tubule junction has been found to be remarkably constant. That of the bloodsucking reduviid, *Rhodnius prolixus* Stål, was described and illustrated by Wigglesworth (1931), and may be summarized briefly. The intestine, of the type described above, joins a pear-shaped rectum at the middle of its broad end. The wall of the rectum is thin with many small nuclei but no distinct cell boundaries. There is a network of fine muscle strands outside

this layer, and a chitinous intima within, the whole system being capable of considerable distension. When contracted, the inner lining and syncytial layer are thrown into many small folds. It would appear that the muscle strands are not adherent to the syncytial layer, for they remain as an even layer on the outside of the contracted rectum. At the anterior end of the rectum, surrounding the opening of the mid-intestine, there is a region, the rectal gland, where the cells are of very distinct type, unlike any found elsewhere in the alimentary canal. This region is shaped like a cap over the broad end of the rectum, extending to about three-quarters of the diameter of the normally distended rectum. The cells of the rectal gland are large, approximately cuboid, and fairly constant in shape irrespective of the degree of distension. They present a level, or gently undulating, surface to the lumen, the internal border being a sharp line which seems to have the nature of a chitinous intima. The single large nucleus (about twice the size of mid-intestine nuclei) is situated towards the base, and contains many small chromatic granules. In larger species especially, intracellular tracheoles and accompanying cells can be detected. The distal part (lumenward) of the cell is densely striated perpendicularly to the border, and minute irregularities in the latter correspond to the striations. Perhaps because of these dense striations, cell boundaries are indistinct. The striations are strongly eosinophil, and the striated region seems to maintain a fairly constant width of about 15 μ, although the cells, in species of different sizes, vary from 20 μ to 60 μ in depth. Typical cells of different species are illustrated in Fig. 1 (A, B, & C). It has been noticed that osmotic stress in fixation forces the border and striated zone together, away from the basal part of the cell. Where this extreme damage does not occur, the spaces between the striations often appear distended with fluid. In the larger species, there is an inwardly projecting fold of the gland epithelium around its perimeter, presumably to overcome a less favourable area to volume ratio. The junction of the gland epithelium with normal rectal epithelium is usually somewhat abrupt.

The central opening through the rectal gland is in the form of a hollow cone of very small columnar cells, which projects into the rectal lumen. This, which may be termed the ileo-rectal valve, is the most anterior point to which the proctodaeal chitinous intima extends. Between this and the mid-intestine proper there is a very small chamber, the ileum, into which the Malpighian tubules open. In Cimicomorpha, the ileum extends into four radially spaced flask-shaped ampullae, with a tubule entering each on its anterior side. These ampullae, in most species, can be seen to be bound to the anterior end of the rectum by a delicate peritoneum. The cells within the ampullae are remarkable in being produced into long filaments which may project into the lumen of the rectum, or occasionally into that of the mid-intestine. These cells occupy the base of the ampulla, distal from the intestine, and number from 10 to 12 in small species to 40 or 50 in the largest. The single large nucleus (very similar to rectal gland nuclei) almost fills the base, and the cell tapers evenly into the long filament. The cytoplasm contains many long basophil fibres, but no other intracellular structure. Unlike the rectal gland, there are a number of smaller nuclei between the bases of the fully developed ampulla cells, presumably being those of replacement cells.

The neck of the ampulla is formed of normal cuboid cells, which join the ileo-rectal valve, posteriorly, and a similar valve-like structure, the ileo-intestinal valve, anteriorly. The latter valve is composed of cuboid to columnar cells grading into those of the intestine, and distinctly larger than those of the ileo-rectal valve. The ileo-intestinal valve may be level, like a diaphragm, or it may project slightly into the mid-intestine. In some of the smallest species

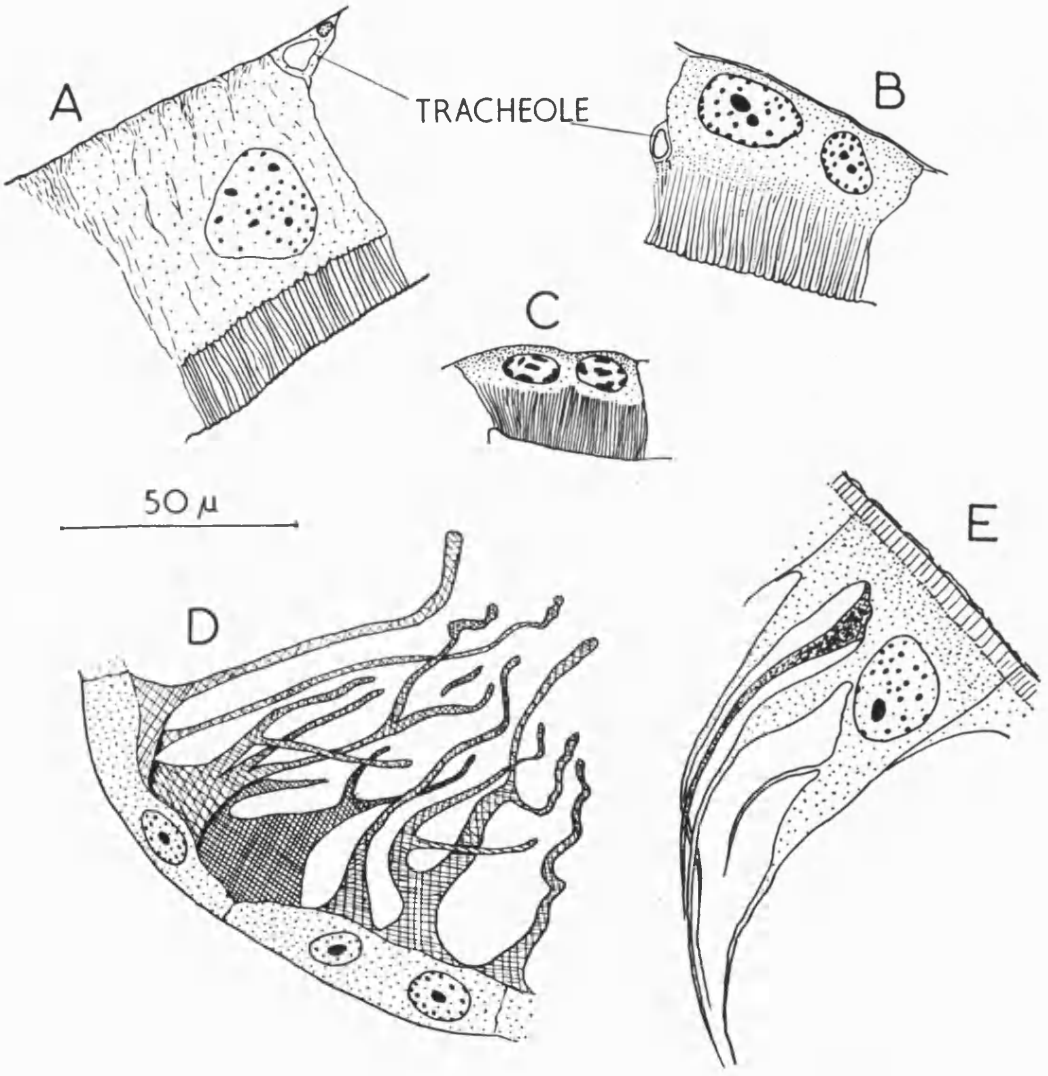


Fig. 1—A, B, C. Rectal gland cells of Cimicomorpha (A—*Petalochirus rubiginosus*. B—*Proboscidiocoris fuliginosus*. C—*Habrochila africana*). D. Filamentous cells of posterior mid-intestine of *Lycidocoris mimeticus*. E. Filamentous cells in proximal region of Malpighian tubule of *Petalochirus rubiginosus*.

it has been found to project posteriorly, into the ileum (e.g. *Lindbergiola aureopilosa*, Isometopidae), or in others (e.g. *Psallus*, *Habrochila*.) the intestine opens into the ileum without restriction. Between the ampullae, the cells of the ileo-rectal valve continue forward to meet those of the ileo-intestinal valve. A typical example of this set of structures is shown in Fig. 2A. Essentially similar features have been found by Painter (1930) in *Psallus seriatus* Reut. Cragg (1915) studied this region in *Cimex lectularius* but failed to observe the

peculiar structure of the ampullae. The present writer has, however, re-examined this species and confirmed that ampullae with filamentous cells are present. As a result of the present study, it can now be said that this pattern is found in Reduviidae, Cimicidae, Anthocoridae, Nabidae, Tingidae, Isometopidae, and Miridae (except Bryocorinae).

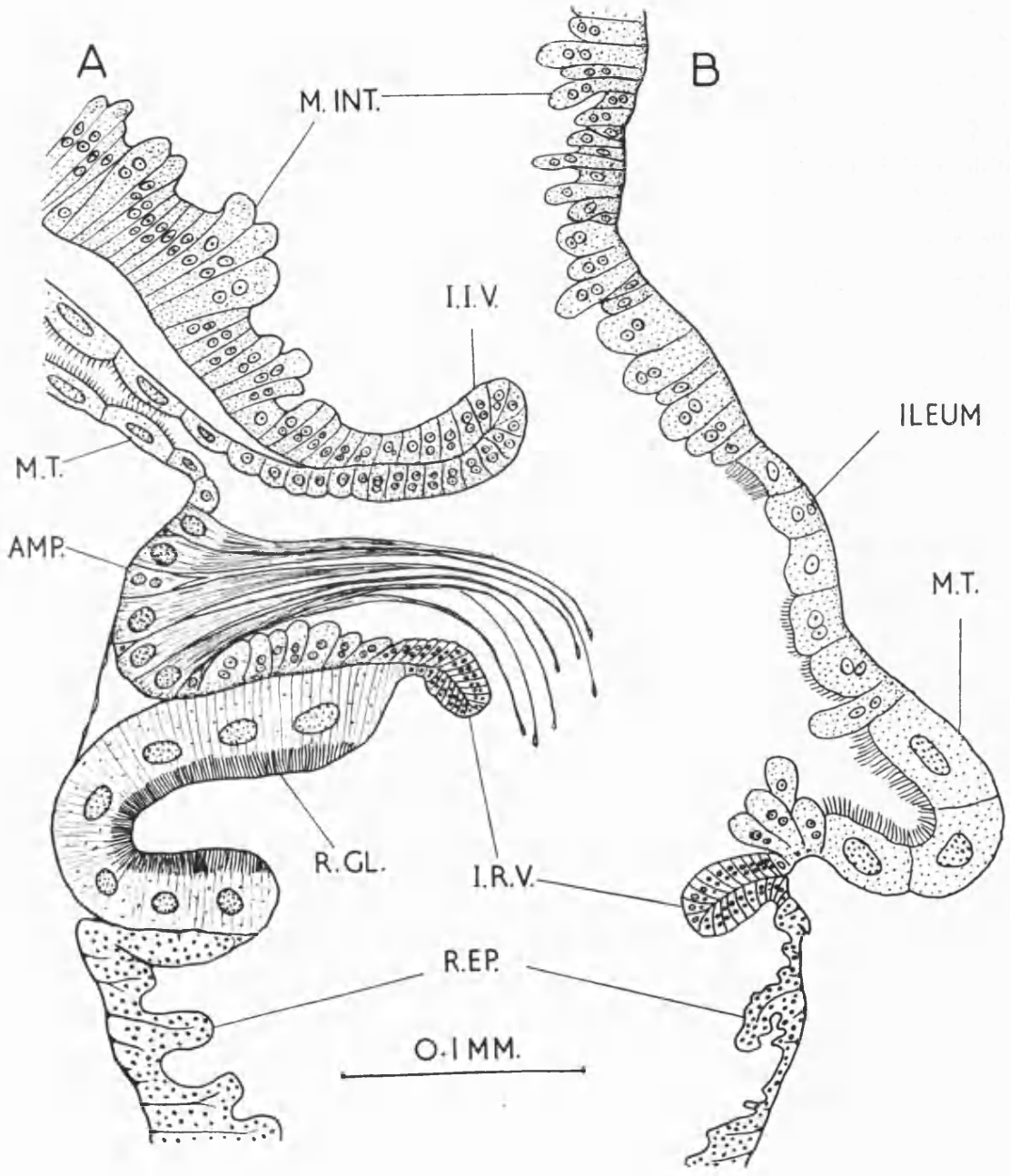


Fig. 2—Half-longitudinal sections from mid-intestine to rectum of A—*Sphedanolestes* sp. (Reduviidae) and B—*Boxia khayae* (Bryocorinae).
Amp.—ampulla, I.I.V.—ileo-intestinal valve, I.R.V.—ileo-rectal valve, M.Int.—mid-intestine, M.T.—Malpighian tubule, R.Ep.—rectal epithelium, R.Gl.—rectal gland.

THE MIRIDAE BRYOCORINAE

The earlier work of the present writer, already referred to, revealed the existence of a structural pattern in the posterior region of the alimentary canal

of certain species of large Bryocorinae, which is markedly dissimilar from all previously described Heteroptera. This has now been found to occur in three more species of the sub-family, thus giving added weight to the previous work. To summarize briefly the structure found in Bryocorinae, the ileum is expanded into a sac partly confluent with the rectum via a rudimentary, widely open, ileo-rectal valve (in the work referred to above, the term " pylorus " was used, following Snodgrass, (1935), but in all other descriptions of Heteroptera the authors use " ileum ", and it seems less confusing to follow them). The lining of the ileum is of large bulbous cells of typical mid-intestine type, which join the intestine anteriorly without any well-defined valve. There is no trace of ampullae or filamentous cells, and the malpighian tubules open directly into the ileum. The rectum has no gland cells, but is entirely composed of thin rectal epithelium. The posterior part of the tubular intestine is lined by cells (Fig. 1D) of an unique type, bearing, from a cuboid or flattened base, long, often branched, filamentous processes, with dense basophil cytoplasm and a surface layer of apparently structureless cuticle. Of the three species recently studied, *Chamopsis tuberculatus* differed slightly in that this region was lined with more normal looking bulbous cells, but these had the same structureless border as the filamentous cells of the other species, and reacted to vital dyes in the same way (i.e. rapid and intense colouration). This species has somewhat unusual Malpighian tubules, in which the distal convoluted portion is reduced to only about one-eighth of the total length, and the proximal portion is wide and more or less banana-shaped, lying alongside the rectum and ileum. In these insects, the Malpighian tubules at the point of junction with the ileum are usually lined with simple rounded cells without a brush border, but in *Boxia khayae* (Fig. 2B) the brush-like border is evident not only at the point of junction, but also on the cells lining the ileum immediately anterior to this point.

Since it would seem that long filamentous processes, either in the ampullae or, in Bryocorinae, in the intestine, must have some important physiological significance, it is of interest to note the existence of such cells in the proximal part of the Malpighian tubules in the largest cimicomorph species studied, *Petalochirus rubiginosus*. This species has a normal ampulla structure, but in addition the cells lining the proximal region (about 2 mm) of the tubules are drawn out into long, dark staining, filaments (Fig. 1E). These are most strongly developed along the edges of the cell base, so that the cell is rather crown shaped. The existence of a supplementary filament bearing region, in addition to the ampullae, in a very large species, is reasonable if they have an absorptive function to perform, since the volume of fluid passing down the tubules will increase with size faster than the available absorptive area in the ampullae.

THE AMPHIBICORISAE

In this group, *Gerris dolosa* has been examined. The rectum was found to contain an extensive rectal gland, with large cuboid cells having typical large, finely granular, nuclei and eosinophil striations at the lumen-ward side. The cells are like those of the rectal gland in the Cimicomorpha, but the eosinophil

region is less sharply defined (Fig. 3A). The gland does not surround the ileo-rectal valve entirely, but covers the whole of the dorsal and lateral walls of the rectum, except for the extreme posterior end (the anal tube). It also extends

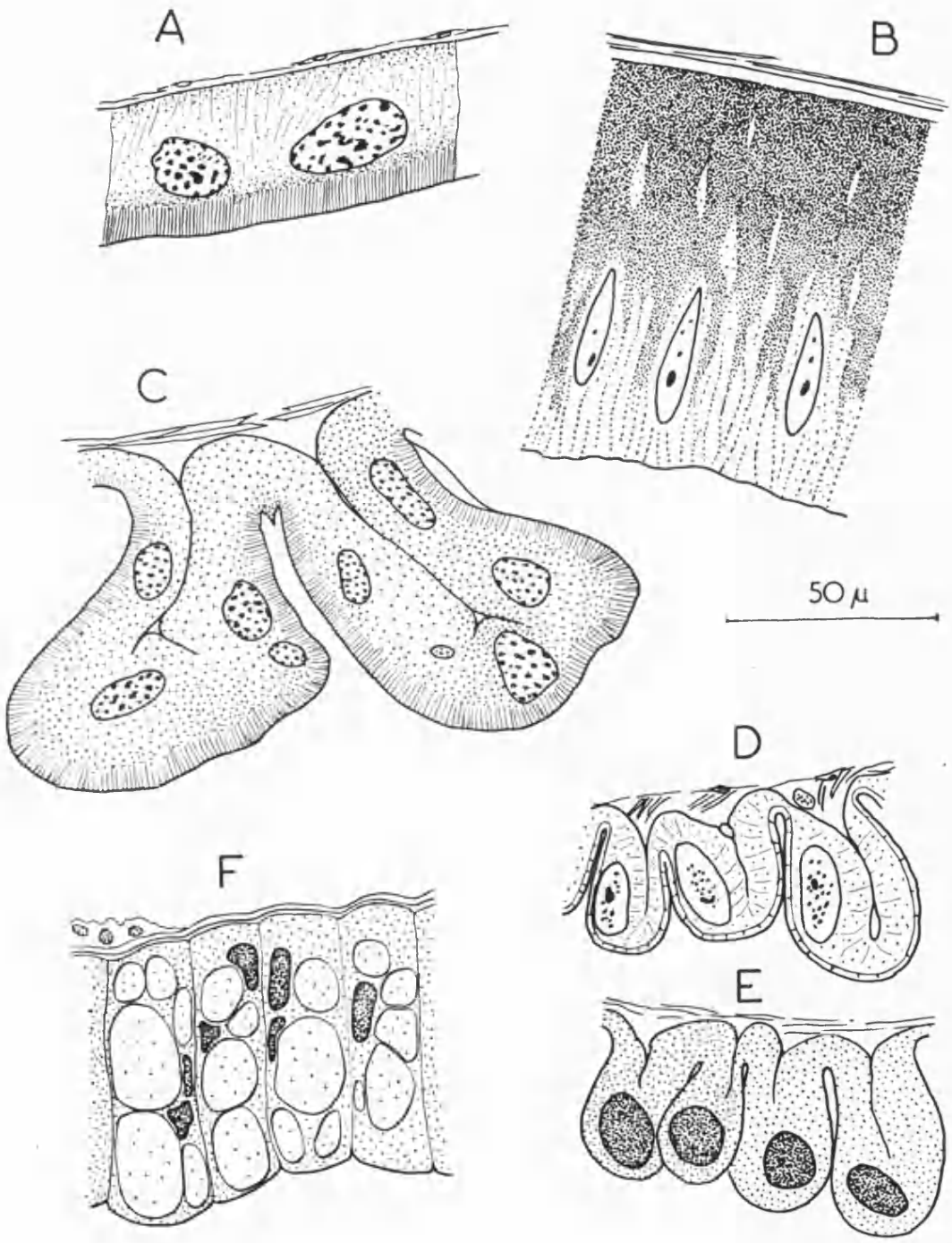


Fig. 3—A to E. Rectal gland cells of A—*Gerris dolosa* (Amphibicorisae). B—*Lethocerus cordofanus* (Hydrocorisae). C—*Dulichius trispinosus* (Coreidae). D—*Scotinophara fibulata* (Pentatomidae) adult. E—*S. fibulata* nymph. F. Vacuolated cells from posterior mid-intestine of *Paromius gracilis* (Lygaeidae). The intestinal lumen is below in each drawing.

into the rectal lumen in the form of two longitudinal folds hanging from the dorsal wall. There is a ventral band of normal rectal epithelium with small

scattered nuclei. The rectal contents are a thick paste of undigested residues. The ileum is even smaller than in Cimicomorpha, with the delicate Malpighian tubules opening at regular intervals around a shallow annular chamber. There are no ampullae. The ileo-rectal valve is the usual cone of small columnar cells, on the outside of which are many strong circular muscle fibres. Anteriorly, the ileum is widely open to the mid-intestine. The ileum cells are small and cuboid, and the simple intestine is lined with bulbous-tipped columnar cells with abundant cytoplasmic granules.

THE HYDROCORISAE (CRYPTOCERATA)

The structure of this group is fairly well known from the work of Kuskop (1923) on *Notonecta glauca* Linn., Hamilton (1931) on *Nepa cinerea* Linn., and Sutton (1951) on Corixidae. All observations are in agreement that the hind-intestine is in two distinct parts, an anterior tube with rectal gland epithelium on its dorsal and lateral walls, and a posterior thin-walled bladder. A similar structure has been found by the present writer in the large water bug *Lethocerus cordofanus* (Belostomatidae), and in a toad bug *Mononyx* sp. (Gelastocoridae). Yanai (1952) recognises this pattern as one of his intestinal types, the *Diplonychus type*. In *Lethocerus*, the rectal tube is not readily distinguishable externally from the tubular mid-intestine, and forms the posterior two-fifths (approximately) of a long convoluted tube connecting the anterior mid-intestine sac with the rectal bladder. The latter is continued forward in the form of a narrow tubular rectal diverticulum, as has been reported in other species of this type. From their point of origin, the long Malpighian tubules are directed backwards and extensively twisted around the rectal tube. The very thick rectal gland (Fig. 3B) is not formed into internal folds, and occupies all but a very narrow ventral strip of the tube. Its cells and nuclei are large, the latter being laterally flattened and situated towards the tip. The cytoplasm has a dense granular eosinophil zone at the cell base, grading into weakly striated or reticular cytoplasm, not notably eosinophil, at the lumenward border. The contents of the rectal tube are mainly fluid with some uric acid granules. No distinct ileum can be found in this species, nor is such a structure reported from other species of this type. The Malpighian tubules open into the terminal part of the mid-intestine. The intestinal wall is thick, and its cells, which are rather small and rounded, are arranged in deep crypts. Among such crypts the Malpighian tubules are inserted, their cells remaining distinct right through to the lumen of the intestine. The only difference in the nature of the intestinal lining at this point is a constriction of the lumen due to the development of a deep stratified layer covering the mouths of the crypts, and formed from cells identical with those in the crypts. Immediately posterior to the Malpighian tubule junction, there is a collar of pale-staining columnar cells with nuclei like those of the mid-intestine, which grades posteriorly into a valve-like arrangement of very tall narrow columnar cells with much smaller nuclei resembling those of rectal epithelium. This valve has a much folded inner border due to varying heights of pale-staining cytoplasm above the basally situated nuclei, and is slightly invaginated into the beginning of the rectal tube.

A chitinous intima is present on the valve cells continuous with that of the hind-intestine.

THE PENTATOMOMORPH FAMILIES

The structure of the ileum and rectum in these families shows characteristic differences from those of the Cimicomorpha, and there is also more variation within this group, in which certain broad evolutionary trends can be distinguished. These seem to be associated with the divergence in feeding habits towards either extreme of water lack, as in seed sucking types, or water excess, as in sap sucking species. It seems likely, moreover, that the latter adaptations have been arrived at independently by at least two different lines. There is a tendency for the opening of the mid-intestine into the ileum to move posteriorly into close proximity to the ileo-rectal valve, so that the ileum becomes excluded from the main line of flow of the intestinal contents, and becomes a diverticulum. In a few species of sap suckers, found in different family groups, a peak of specialisation is reached in which the mid-intestine ends blindly, and has no opening into the ileum. These include Plataspidae (Coptosomatidae) and certain species of Lygaeidae (Schneider, 1940 ; Poisson, 1951), and also certain Coreidae and Pentatomidae which will be described in Part II of this paper.

It is in the Pentatomomorpha that the intestine bears, in most species, the well known " gastric caeca ", which form two (in Pentatomidae, four) rows of thin walled pouches along the last part of the mid-intestine. These are absent or vestigal in zoophagous or seed sucking species. The general characteristics of the cells lining the intestine are not very different from those of Cimicomorpha though they may be slightly smaller for a given size of insect, and often form a more even columnar epithelium. The cells are usually bulbous at the tip, but appear to be more firmly attached to adjacent cells, and are not so greatly distorted by distension of the intestine. No cells of the second type found in Cimicomorpha, with lobes bearing a deep brush-like border, have been seen in the pentatomomorph material examined. In a number of species, the bulbous tip seemed to be slightly pinched off from the cell base, but these had no conspicuous border. A few instances of vacuolation much more extreme than in any Cimicomorpha were found. These were in the middle part of the intestine of *Cydnus indicus* and *Serinetha amicta*, both of which are probably partly zoophagous in habit, and in the posterior end of the mid-intestine of a lygaeid, *Paromius gracilis* (Fig. 3F).

To deal first with the rectum of Pentatomomorpha, this is a large ovoid sac, with thin walls capable of great distension. Although in some text-book accounts, the Hemiptera have been said not to possess rectal glands, there is in these insects always a large area of specialised tissue. This has the same very large granular nuclei found in the rectal glands of the groups previously described, and in most species the cells are similarly divided into a lumen-ward eosinophil striated zone and a basal zone of less deeply staining reticular cytoplasm. The area occupied by this gland epithelium is usually much greater than in Cimicomorpha, covering most of the dorsal wall of the rectum, and extending down the sides to a variable degree in different species. In

most families it does not surround the ileo-rectal valve, but in several species of Pentatomidae and a few Coreidae this does happen. In some Pentatomidae and the closely related Plataspidae, the gland epithelium joins ventrally and encloses the whole rectum. These are all advanced sap sucking types with interrupted intestines, and the gland epithelium is thin and lacks the eosinophil zone. Because of its great extent, the gland epithelium takes part in the expansion and contraction of the rectum, being thrown into deep folds in the contracted state. The cells are much wider and shallower than those of Cimicomorpha (Fig. 3C, D), so that this is easily accomplished. The depth and intensity of staining of the eosinophil striations is greatest in the zoophagous species, and some seed sucking species. In the seed sucking Pyrrhocoridae, however, the striated zone does not seem to be present, although the cells are deep and well developed. In sap sucking types a weakly staining, narrow, striated zone may be found, but the cells are shallow and with little cytoplasm (Fig. 3D). In the zoophagous or partly zoophagous species, the gland cells are much thicker than in phytophagous types, and resemble those of Amphibicorisae. These thicker glands do not seem to undergo folding as readily as the thinner ones, and perhaps for this reason, were more restricted in extent. The extreme case found was that of the lygaeid, *Chauliops rutherfordi*, where the rectal gland is a small group of about a dozen large cuboid cells in the middle of the dorsal wall. In many sap sucking Coreidae and Pentatomidae, and in *Serinetha amicta* (Rhopalidae), there is an anterior ventral diverticulum of the rectum, forming what has been called the rectal pouch. In Coreidae and *Serinetha*, this is clearly a permanent feature, but in Pentatomidae it is more prominent in the distended rectum, as a result of a patch of rectal epithelium in the antero-ventral region stretching more freely than the remaining glandular part of the rectum. In *Serinetha* and in the coreid *Acanthocoris*, the rectal pouch is formed entirely of thin rectal epithelium, but in the largest coreids (Mictinae), represented by *Anoplocnemis* and *Mygdonia*, the rather weakly developed gland epithelium extends forward over the dorsal and lateral walls of this pouch.

The ileo-rectal valve is a narrow opening surrounded by small columnar cells which usually have dark staining nuclei and pale cytoplasm. They do not appear to differ significantly from those of Cimicomorpha. In the Pentatomidae and Pyrrhocoridae the valve tends to be large with many longitudinal folds of epithelium restricting the lumen to a stellate cross-section. In Coreidae, the valve tends to become a narrow tube, while in zoophagous forms the valve tends to be somewhat reduced, leaving the ileum widely open to the rectum. As with the other groups, this valve is the most anterior part of the hind-intestine in which a chitinous intima can be detected.

THE ILEUM IN PENTATOMOMORPHA

In the Pentatomomorpha it is possible to distinguish four types of ileum structure. In general, the fluid capacity of the ileum is always much greater than in Cimicomorpha, and it may form a distinctly swollen bladder. The lining cells are not markedly different from those found in the mid-intestine, and it is often necessary, when examining sections of entire intestines, to seek

the openings of the Malpighian tubules in order to identify the ileum with certainty. The most usual type of cell is rather low columnar, with broad base and rounded tip, usually binucleate with the nuclei situated near the base and surrounded by fairly dense cytoplasm, while the tip may appear almost empty. The rounded tip may be slightly pinched off from the base. The cell border is extremely thin and no striations can be detected.

The simplest type of ileum is found in Lygaeidae and Rhopalidae, and may be termed the *lygaeid type* (Fig. 4). This is a more or less spherical sac, with

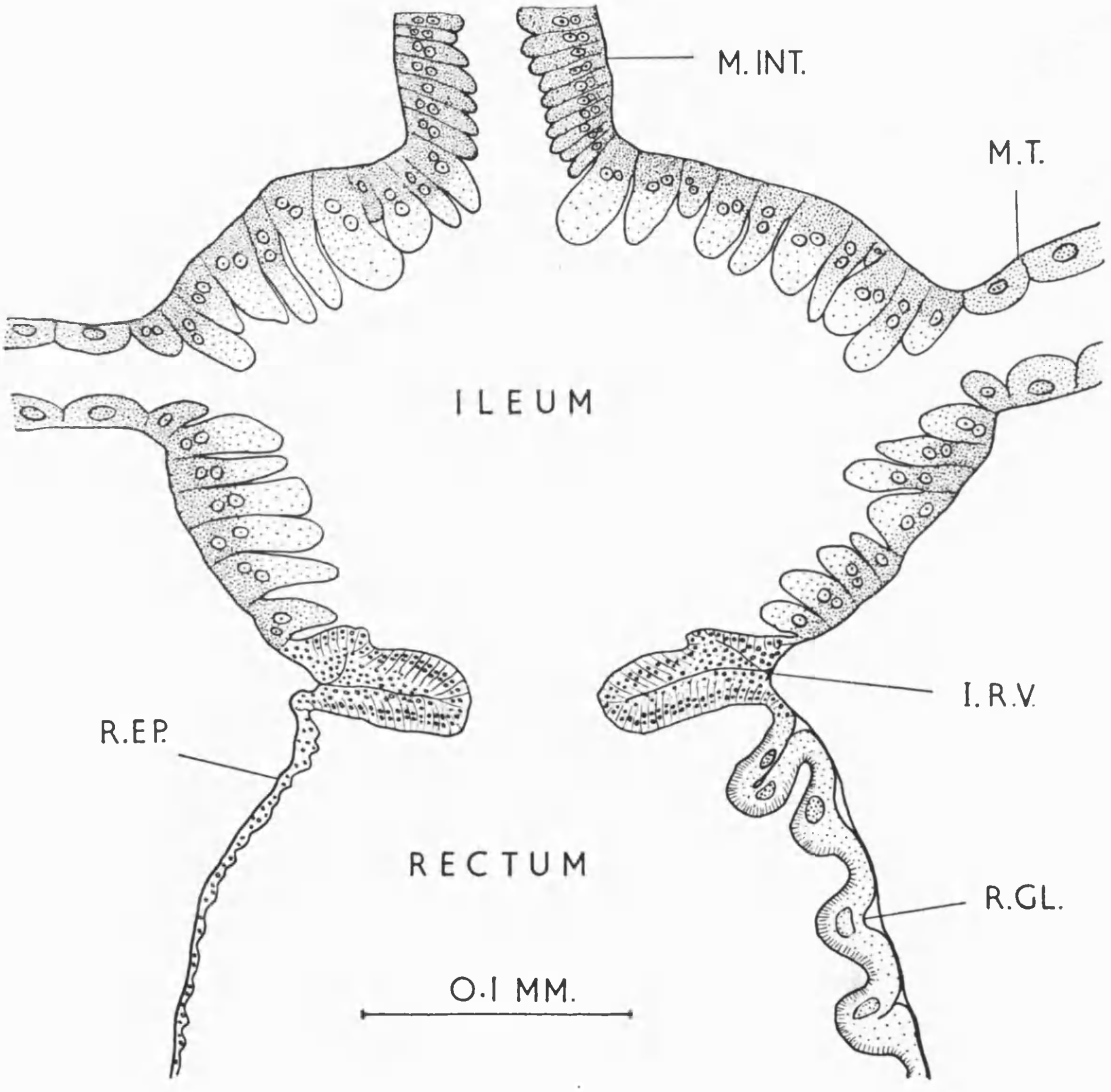


Fig. 4—Oblique longitudinal section through ileum of *Pachybrachius capicola* (Lygaeidae), showing rectal gland (dorso-lateral) and unspecialized rectal epithelium (ventro-lateral). Abbreviations as in Fig. 2, p. 858.

cells as described above, and with the mid-intestine joining it anteriorly, opposite to the ileo-rectal valve. The ileum in these families may sometimes be found to be strongly distended, with the cells stretched to a thin pavement epithelium. The ileum cells grade into smaller columnar cells with dense cytoplasm at the entry point of the mid-intestine, which is, in most species, only slightly constricted. In *Chauliops* and *Corizus*, however, the lumen is not discernible at

this point, and in *Serinetha* there is no connection with the mid-intestine at all. The points of entry of the Malpighian tubules may be radially spaced as in e.g. *Aphanus* sp., but are in many species associated into pairs on each side. Where this is the case, the openings lie in a plane parellel to the axis of the intestine. Apparent fusion of the proximal regions of the tubules occurs in some species (e.g. *Corizus* sp.), but the common region is histologically similar to the ileum, and not composed of tubule cells with their characteristic brush-like border. A slight constriction may be present at the junction of the tubules with the ileum, but the opening is otherwise simple, not involving any special cell types.

In the next type of ileum, characteristic of the Coreidae (and therefore termed *coreid type*), the cells are essentially as in the *lygaeid type*. The Malpighian tubule openings are simple, but their arrangement is now found to be fixed in two lateral pairs. The general shape of the coreid ileum may be compared to a pillow, with its long axis at right angles to that of the insect (in a horizontal plane), and the Malpighian tubules opening at the four corners. The distinguishing feature of this type is the structure of the mid-intestine opening, which is invaginated more or less deeply into the lumen of the ileum in the form of a conical papilla. This consists of an outer layer of small cuboid or flattened cells enclosing an inner layer (continuous with the wall of the mid-intestine) of slightly larger, rounded cuboid, cells (Fig. 5A). The opening of the intestine, at the tip of this papilla, is usually very restricted (15 to 20 μ). In the most advanced sap sucking species, the ileum is strongly constricted in the middle, so that the mid-intestine opens very near to the ileo-rectal valve. In certain Coreidae (Alydinae) which seem to have adopted a dry seed diet, the cells lining the ileum have long, rather irregular, terminal lobes, which stain almost black with haemalum.

The type of ileum found in Pentatomidae (*pentatomid type*, Fig. 6A) is similar in many respects to the above mentioned types, but the lining cells generally have a more uniformly staining cytoplasm throughout the depth of the cell, and the cells are narrower, forming a more compact columnar epithelium. The Malpighian tubules are widely open into the ileum, their proximal part being of comparatively large diameter and lined by cells not very different from those of the ileum. The main difference between this type of ileum and the other types lies in the dorsal and posterior displacement of the mid-intestine opening. The end of the mid-intestine is inserted into the ileum as a tubular invagination, much less restricted at its tip than in Coreidae. At the same time, the point of insertion is backwardly displaced on to the dorsal side of the ileum, so that the actual opening is very close to the ileo-rectal valve. The length of the invagination varies between different species, but where it is fairly long, as in e.g. *Caura leggei* (Fig. 5B), the outer layer of cells is fused to the dorsal wall of the ileum, and the ileum walls wrap closely around the invagination. In many species (e.g. *Aeliomorpha divisa*, Fig. 6A) the invagination is relatively short, but the point of insertion of the mid intestine into the ileum is situated at the posterior edge of the ileum, just above the ileo-rectal valve. This is the position also in zoophagous forms such as Asopinae, but in these, there is no sign of any invagination, and the mid-intestine opening is direct

and unrestricted. In the largest forms which were investigated, the phytophagous species *Nezara viridula*, *Aspongopus xanthopterus*, and *Piezosternum callidum*, the lining of the ileum appears to be folded into ridges or crypts.

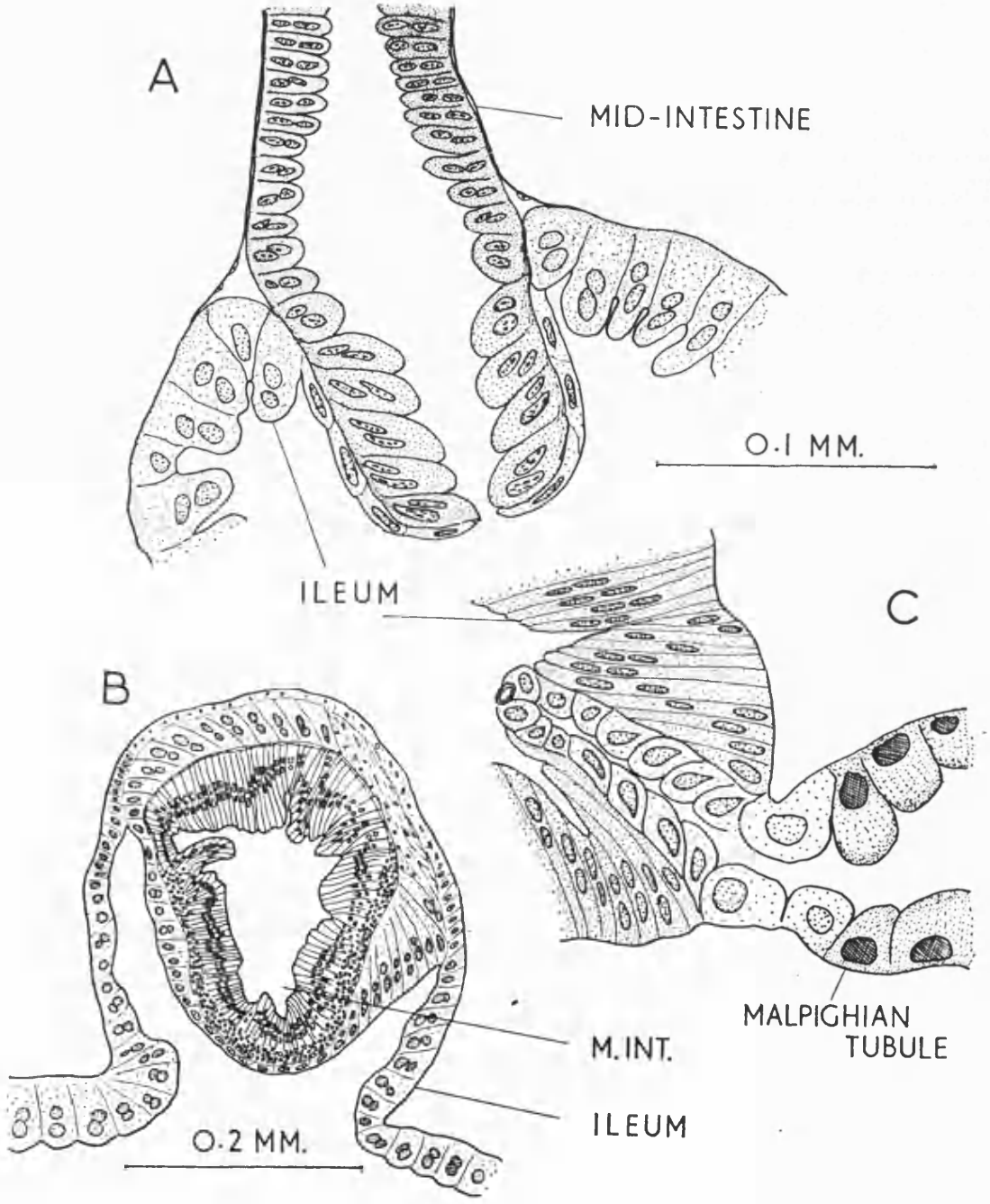


Fig. 5—A. Longitudinal section through mid-intestine–ileum junction of *Dulichius trispinosus* (Coreidae). B. Transverse section through invagination of mid-intestine into ileum in *Caura leggei* (Pentatomidae). C. Section through Malpighian tubule–ileum junction of *Myrmoplasta potteri* (Pyrrhocoridae) along axis of tubule. A and C to same scale.

The fourth type of ileum which can be distinguished is that found in Pyrrhocoridae (*pyrrhocorid type*, Fig. 6B). In this group, a departure from the primitive condition has taken place in an entirely different direction. The

opening of the mid-intestine in these forms is simple, and displaced to the ventral surface of the ileum. The lining cells of the ileum are extremely narrow, rather tall, columnar cells, with cytoplasmic density increasing towards the tip. The cell tips are not bulbous, and stain very deeply, almost

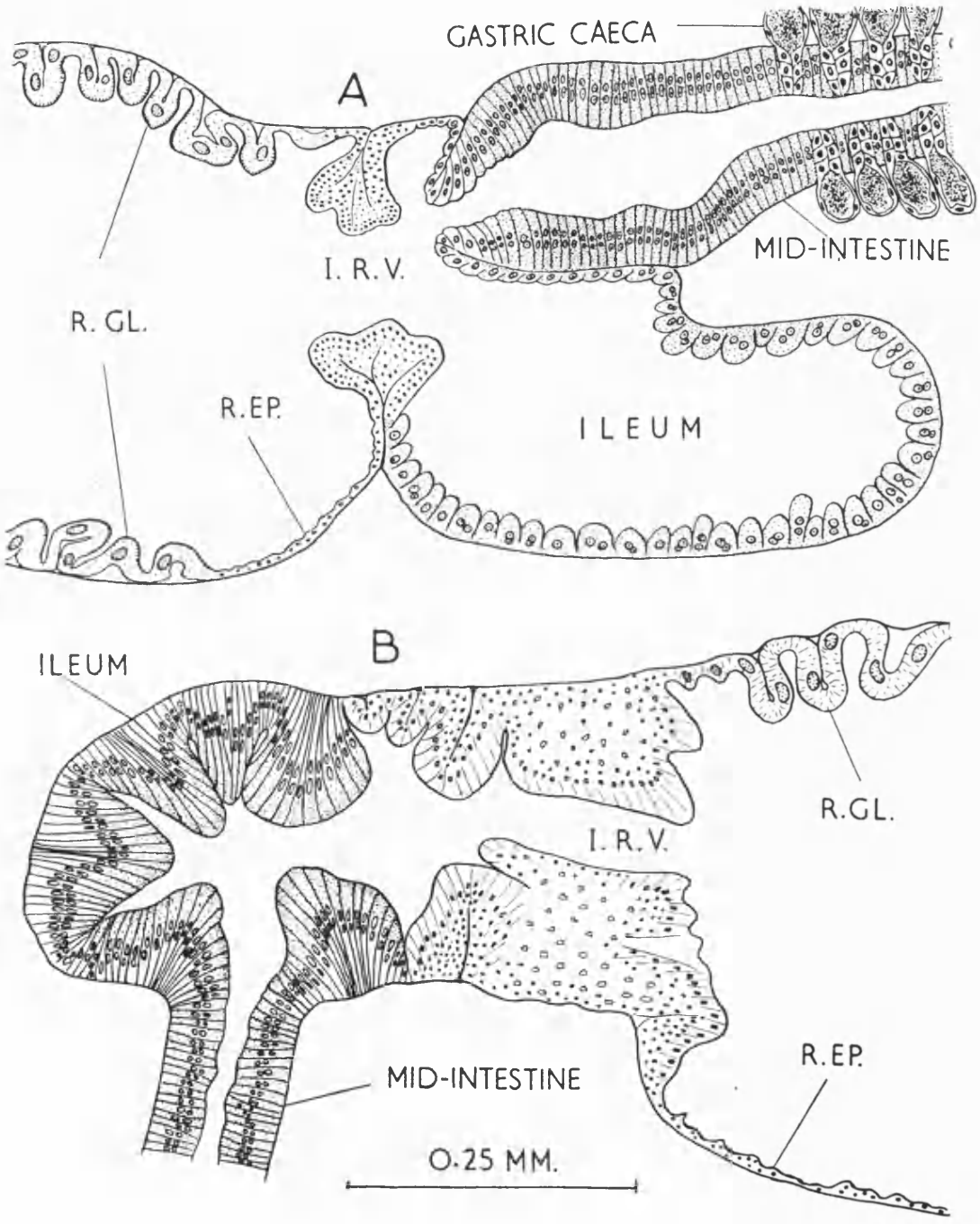


Fig. 6—Sagittal sections from mid-intestine to rectum of A—*Aeliomorpha divisa* (Pentatomidae) and B—*Scantius forsteri* (Pyrrhocoridae). The dorsal surface is towards the top of the page in each. Abbreviations as in Fig. 2, p. 858.

black, with haemalum. This thick epithelial layer is split by deep clefts where the ileum wall changes direction. The Malpighian tubules have a wide proximal region lined with simple rounded cells lacking a brush-like border, but unlike

this region in the *pentatomid type*, there is a marked constriction where they join the ileum. From this point, the lumen of the Malpighian tubule is continued in a narrow tube of pale staining, rounded cuboid cells (Fig. 5C) which passes through the middle of a clump of ileum cells to a restricted opening (about 20 to 30 μ) at the inward side of the clump. The fluid capacity of the *pyrrhocorid type* of ileum appears to be limited in comparison with the other types. It is important to record that the ileum of *Spilostethus pandurus*, representing the larger kind of lygaeid (Lygaeinae) with seed sucking food habits, shows considerable tendencies in the direction of the pyrrhocorid structure.

Among those species (described in Part II) where the mid-intestine has no connection with the ileum, there exists a similarity of structure, due to the absence of the distinguishing marks of position and nature of the mid-intestinal opening. The evidence suggests that these highly specialised types of intestine are developments at the peaks of their respective families, and that the resemblance is the result of evolutionary convergence. Such species are found in Rhopalidae, Coreidae, Pentatomidae, and Plataspidae. In *Aspongopus xanthopterus*, the ileum lining cells, arranged in a crypt-like formation, are strongly vacuolated (Fig. 7B). In *Piezosternum calidum* and in the nymphs of *Nezara viridula*, where a fully developed mid-intestinal connection is present, but apparently imperforate, the cells are similar to those in *Aspongopus*, but without distinct vacuole walls. In the Plataspidae, there is in *Libyaspis* a remarkably close association between the ileum and the posterior end of the first mid-intestine region, the former being almost completely enclosed by the latter (Fig. 7A). In all Pentatomorpha, the ileum is in close contact with the posterior end of the sac-like first region of the mid-intestine, and is usually bound to it by fine tracheal branches, but in *Libyaspis*, sections through the undisturbed alimentary canal show a true invagination to be present. The ileum cells of *Libyaspis* have a primitive appearance, being broad based, rounded cells, with large empty apical swellings while the cells lining the adjacent part of the first mid-gut differ from those of the remainder of that region in their slightly smaller size, and in the absence of the dark coarse granules which are abundant in the other cells.

THE GASTRIC CAECA IN RELATION TO WATER BALANCE

Having described above the structure of the parts which are usually regarded as significant in water regulation, it is necessary to go further, and to present evidence that certain other structures, the gastric caeca, which have not previously been studied in this light, may in fact have a part to play. It must be remembered that it is only among the Hemiptera that one finds species which are adapted to feed on plant juices throughout their whole life cycle. In insects of several other orders, nectar or sap may be imbibed by the adult for the maintenance of life, but does not contribute to growth or production to any great extent. Species with this habit possess a chitin-lined crop developed from the fore-gut. Denisova (1943) has shown that in insects such as mosquitoes, which can feed on vertebrate blood or on plant nectar, the former passes directly into the mid-intestine, while fluids of plant origin are retained in the crop. They are then passed slowly, a little at a time, into

the mid-intestine. When these insects are fed with warm water through a membrane, the warmth causes the crop to be by-passed, and the insect dies rapidly as a result of dilution of the haemolymph. Thus it appears that the impermeable crop is necessary, because the water regulating mechanism can expel water only at a rather slow rate. The sap sucking Hemiptera need to pass sap through their alimentary canal at the highest possible rate, in order to extract sufficient nourishment. That they have successfully adapted

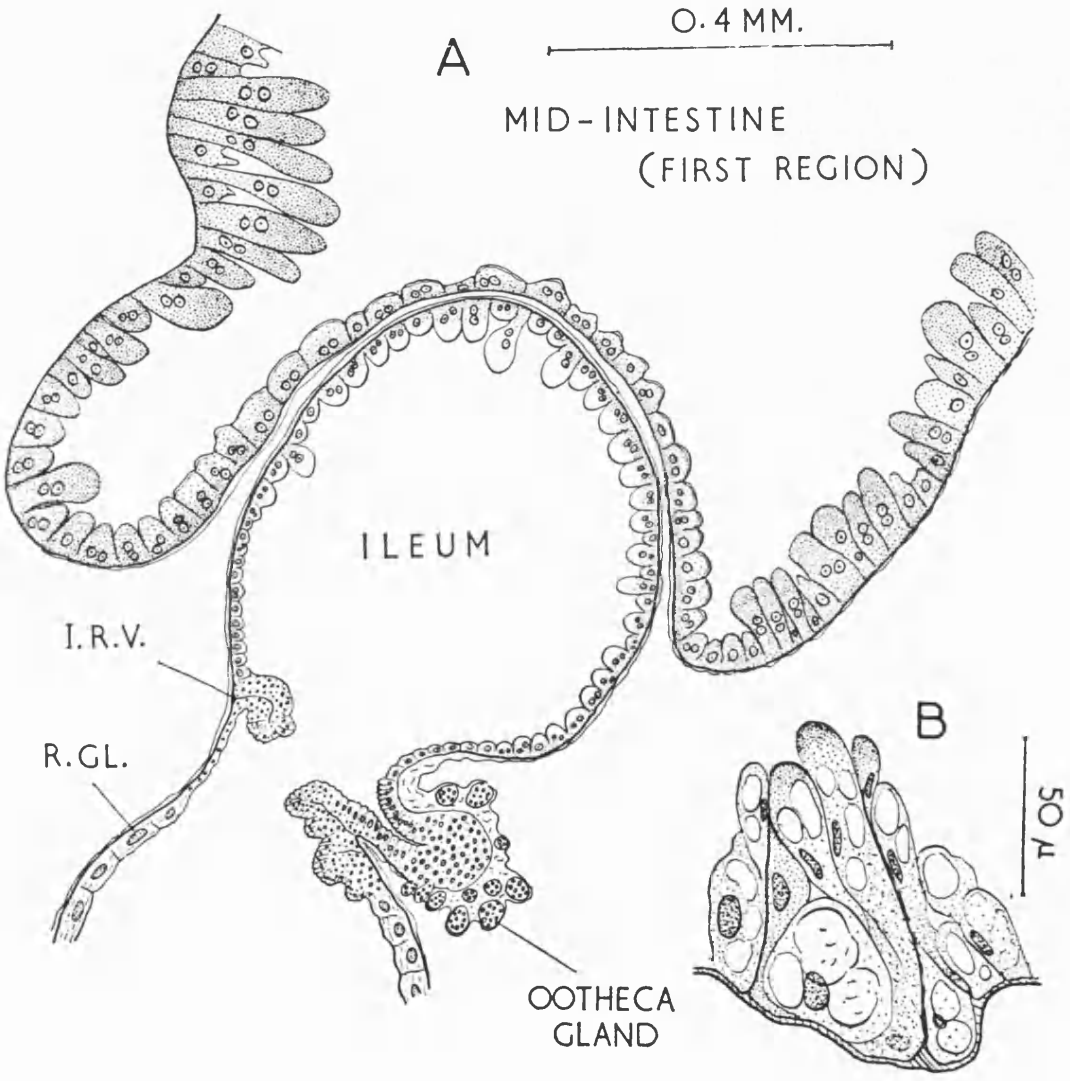


Fig. 7—A. Sagittal section of ileum invaginated into first region of mid-intestine of *Libyaspis flavosparsus* (Plataspidae). Abbreviations as in Fig. 2, p. 858. B. Vacuolated cells from ileum of *Aspongopus xanthopterus* (Pentatomidae, Dinidorinae).

themselves to such a process is evident from the abundance of species, and the large size reached by some of them. Rapid passage of sap is not likely to be achieved by doling it out slowly from an anterior impermeable reservoir, but by accelerating the rate of elimination of excess water. This is probably the reason for the absence of a chitin-lined crop in all Hemiptera. On the other hand, they do possess a large chitin-lined rectum, where excreta of low osmotic pressure may be stored without danger of haemolymph dilution, until it is

convenient to eject it. This organ is often found to be enormously distended with watery fluid. The water repellent character of its lining is readily seen when the organ is opened in dissections, as it will spread out on the surface of the dissecting fluid. The problem is to discover the mechanism by which surplus water is shunted into this safe reservoir. In Homoptera, the complex " filter chamber " evidently functions in this way, but no such structure exists in the Heteroptera. Even if we suppose, in spite of Ramsay's findings (1958) on the isotonicity of the tubule fluid, that the Malpighian tubules might perform this task, the fact remains that the tubules are not conspicuously more developed in sap-sucking than in other Heteroptera. One conspicuous organ system stands out, as being present in sap-sucking Heteroptera, but absent from those of other feeding habits, and from Homoptera, and that is the gastric caecal system. These caeca are nearly always found to contain vast numbers of bacteria in pure culture, and previous conjectures as to their function have been based on the assumption that they existed only to harbour these bacteria. The evidence for the interpretation of the caeca as essentially a water eliminating system can be summarized under several headings, as follows:

1. *Are the bacteria found in the caeca essential to the insect?* While it is probable that caeca, even if evolved for a different purpose, would provide harbourage for bacteria, and that this association would itself be the subject of evolutionary change to the advantage of the insect, it is by no means certain that they are essential to life in every species in which they occur. Furthermore, the evidence suggests that, in so far as these bacteria are valuable to their host, this value may be a consequence of the caecal function which is being proposed here.

With many species, it has proved impossible to rear sterile individuals owing to transovarial infection of the offspring (this transmission does not necessarily imply an essential relationship). Thus, Glasgow (1914) found it impossible to produce sterile *Anasa tristis* (De Geer) or *Murgantia histrionica* (Hahn), in order to study this problem. However, Bonnemaison (1946) with *Eurydema ornatum* L. (Pentatomidae), and Müller (1956) with *Coptosoma scutellatum* Geoffr. (Plataspidae) have managed to rear bacteria-free insects. Although in the latter case there was a high rate of nymphal mortality, both authors recorded successful reproduction by the adults. *Coptosoma* and other Plataspidae are insects in which the bacteria-insect association has evolved to a degree when the bacteria may be essential to the insect, and the specialised means of transmission to the offspring, by globules deposited among the eggs, was made use of, in order to obtain the sterile insects. The present writer has found that in the Bean bug (*Acanthomia tomentosicollis*) the caeca are moderately well developed, but frequently do not contain a normal bacterial population. The Lygaeidae Rhyparochrominae (e.g. *Dieuches armipes*, *Aphanus* sp.) are also variable in this respect.

On the other hand, supposing the caeca to be evolved primarily for the purpose of exposing a larger number of excretory cells to the haemolymph, it is inevitable that bacteria, finding their way into the crypts so formed, would multiply. The water rejecting mechanism is unlikely to be so efficient

that there would not be some sugars and amino acids to provide sustenance for a bacterial population. The origin of the bacteria would be from bacteria contaminating the plant surface. Miles (1958) has described how *Oncopeltus fasciatus* and other phytophagous Heteroptera sample the surface with a drop of saliva which is immediately withdrawn into the food canal for testing by gustatory sensilla. Once the species has acquired an internal flora, then transmission through the nymphs sucking excretory droplets from the adults would occur. It is significant that when identification of the bacteria has been attempted (Steinhaus *et al.*, 1956 ; Huber-Schneider, 1957) the caecal organisms have been found to be very close to common soil inhabiting forms, which one may assume to be present on plant surfaces as a result of rain splash. In the case of the Bean bugs mentioned above, they were collected from ripening pods of Pigeon pea (*Cajanus cajan*) at a level of 4 to 5 feet above the soil. At this height rain splash is not likely to be great, and hence the number of individuals which lacked a normal growth of bacteria. The idea that in many of the caeca-bearing species of Heteroptera, the infection of the caeca is more or less fortuitous, finds support in the number of different strains which can be isolated from different individuals of the same species. Glasgow (1914) found this with *Anasa tristis*, while Steinhaus *et al* (1956), working with the same species, obtained strains of two distinct physiological types. One of these types was found to be virtually identical with organisms isolated from a pyrrhocorid, *Euryophthalmus cinctus californicus* (Van Duzee). The present writer has found in one experiment on culturing caecal bacteria, that a group of four species (*Hydara tenuicornis*, *Cletus fuscescens*, *Mirperus torridus*, *Aspavia armigera*) collected in one spot all yielded a similar organism (paired very short rods, growing in yellow translucent pinhead colonies), whereas in other experiments this was typical of *Aspavia armigera* only, the other species (Coreidae, while *A. armigera* is a pentatomid) producing creamy white opaque colonies. Unspecialised, easily cultured, bacteria are found chiefly in Coreidae, and only a few species of Pentatomidae have been found to contain such organisms. In Pentatomidae, evolution of the symbiotic association has led to special means of transmission to the offspring by smearing the eggs (Rosencranz, 1940), and the bacteria tend to become highly modified involution forms, unresponsive to artificial culture. Failure to grow in culture also afflicts the usually tractable coreid caecal organisms, if maturing adults, particularly females, are used as the source of inoculum. The organisms, in these insects, appear to be in a state of degeneration, irregularly clumped together, and with many abnormal shaped forms. The caeca themselves appear transparent, instead of their usual opaque creamy colour. How far this is the result of action by the insect tissues, and how far it is the normal degeneration of an old bacterial colony, it is impossible to say at present. An interesting point is that in cultures, many of these strains grow slowly and reach only a very small colony size (0·5 to 1 mm), which could be comparable to the volume of a single caecum. While it is also difficult to determine whether the Coreidae in which this degenerative phase occurs can derive benefit from the absorption of breakdown products, there is no doubt that there is a trend, as seen in the histology of the anterior end of the caecal region and its contents

(See Part II), in Coreidae and perhaps even more in Pentatomidae, towards a continuous breakdown and digestion of bacteria, even while they are actively growing in the caeca.

The theory put forward by Glasgow (1914), that the main function of caecal bacteria was to prevent the growth of other, perhaps less benign, parasitic organisms in the intestine, was a reasonable deduction from his observations. It is now well known, however, that many, if not all, soil micro-organisms secrete antibiotic substances. The purity of the caecal cultures would therefore be due to a process of natural selection, the most strongly antagonistic organism finally surviving to colonise the caeca. The organism which inhabits the intestine of the blood-sucking reduviid, *Rhodnius prolixus*, (*Actinomyces rhodnii* Erikson.) has this sort of effect, and when isolated from *Triatoma infestans* (Klug.), gave only pure cultures (Goodchild, 1955). The possibility that caecal bacteria may secrete accessory nutritional factors has also to be considered. They may well do so, but in insects where this is known with more certainty to be the reason for symbiosis with micro-organisms, the bacteria are much fewer in number, and occupy relatively small organs in the body cavity, or live in the ordinary intestine lumen. The most highly developed caecal regions are 70 per cent of the total length of mid-intestine, and 1·5 per cent of total body weight, while a rough count of numbers in an average species, using a blood counting chamber, gave 19×10^6 bacteria per insect.

The activity of caecal bacteria in locking up useful materials, which would otherwise pass out with the excreta, must surely be regarded as sufficiently beneficial to the insect to bring about the specialisations seen, for instance, in the Pentatomidae, while not contradicting the suggested function of water excretion.

2. *Can essential bacteria be harboured other than in caeca ?* Apart from the fact that in most other kinds of insects, symbiotic micro-organisms are harboured in many ways quite unassociated with the alimentary canal, the evidence from other members of the Hemiptera favours an affirmative answer to this question. In *Rhodnius prolixus*, organisms, proved to be necessary for completion of the life history (Brecher & Wigglesworth, 1944), are found in the lumen of the intestine. Fresh blood meals are reinfected from coccoid forms which are retained in the crevices between the bulbous cell tips. In *Dysdercus* sp., long chains of bacteria grow in the lumen of the sac-like third mid-intestine region (though these are unspecialised types which grow vigorously in culture, and have not been proved to be essential). In *Cimex lectularius*, symbionts are harboured in a special organ, the mycetome, separate from the intestine, and in the Homoptera (which are all sap suckers), a wide variety of symbionts are contained in mycetomes in the body cavity. The caecal region in some Heteroptera becomes transformed into a mycetome, by the obliteration of the passages from the caeca into the central intestinal tube, or by interruption of the lumen of the intestine at both ends of the caecal region. In those species where the former condition occurs, the food is usually of a dry kind, as in the pentatomid *Agonoscelis versicolor*, which in Uganda feeds on fully ripe seed heads

of *Bidens pilosa* (Compositae). Rosencranz (1940) claimed that the caeca of *Acanthosoma haemorrhoidale*, which feeds on hawthorn fruits, were cut off in this way, but the present writer finds that a continuous, but very narrow, passage exists between caecum and intestine in this species. Species with the intestine interrupted so as to isolate the caecal region are relatively few in number, and highly specialised. They may have, as in *Aspongopus* and *Libyaspis*, histological evidence suggesting that the ileum may have taken over a water excreting function, or it may be that the highly developed exploitation of the bacteria enables them to reduce the rate of intake of sap. They are all sap suckers, with very well developed caecal regions. Mycetome-like structures are reported from the lygaeids *Nysius*, *Ischnorrhynchus*, and *Ischnodemus* (Schneider, 1940), which do not possess caeca, but these small bugs may not feed entirely upon sap, but take whole cell contents. In spite of the above exceptions, the vast majority of species seem to possess caeca which are in communication with the intestine, at least freely enough to pass water through the connection.

3. *Do the anatomical relationships of the caeca with other parts of the mid-intestine support a water excreting function ?* In the Homoptera, it is the elaborate intertwining of the anterior and posterior ends of the mid-intestine which draws attention to the possibility of water transfer between the two regions. Huber-Schneider (1957) suggested that the anterior ventral diverticulum of the rectum was, in *Mesocerus marginatus* L. (Coreidae) the equivalent of a filter chamber, on the grounds of its close anatomical relationship with the first mid-intestine region. Mere physical proximity, however, is by itself no proof of such a function. After all, the internal organs cannot avoid touching one another to some extent. The possibility of anatomical proximity having a physiological significance is increased if it involves extreme modifications of shape, as in the homopteran filter chamber, or if the adjacent parts of the organs concerned have modifications of the epithelia at the point of contact. The epithelial structure should also at any rate in the receiving organ, show some evidence of ability to perform the necessary osmotic work, unless passive osmosis is to be regarded as the operative mechanism. These criteria seem to be fulfilled in the ileum of *Libyaspis*, but not for the rectal diverticulum of Coreidae. The thin rectal epithelium is not likely to be able to perform osmotic work, in fact it only appears capable of secreting the chitinous intima. There is no deviation from normal in the epithelia over the area of contact, and, *a priori*, passive osmosis cannot be considered to take place. Furthermore, an anterior prolongation of the rectum is not found in all sap sucking Heteroptera. The most probable interpretation of the rectal diverticulum is as a device for obtaining the maximum rectal volume within the limits imposed by the shape of the insect. Pesson (1944) seems to interpret the invagination of the filter complex into the rectum of certain coccids in a similar way.

Regarding the gastric caeca in the light of the above criteria, it may be said that there is always a close contact between the caeca and the posterior dorsal part of the sac-like first mid-intestine region. It is most strikingly exemplified by the arrangement in Rhyparochromine Lygaeidae. In this subfamily, the caeca

are long tubes, relatively few in number, with their openings into the intestinal tube bunched rather closely on a short length of intestine. In their natural position, these pass forwards and wrap closely around the first region, as

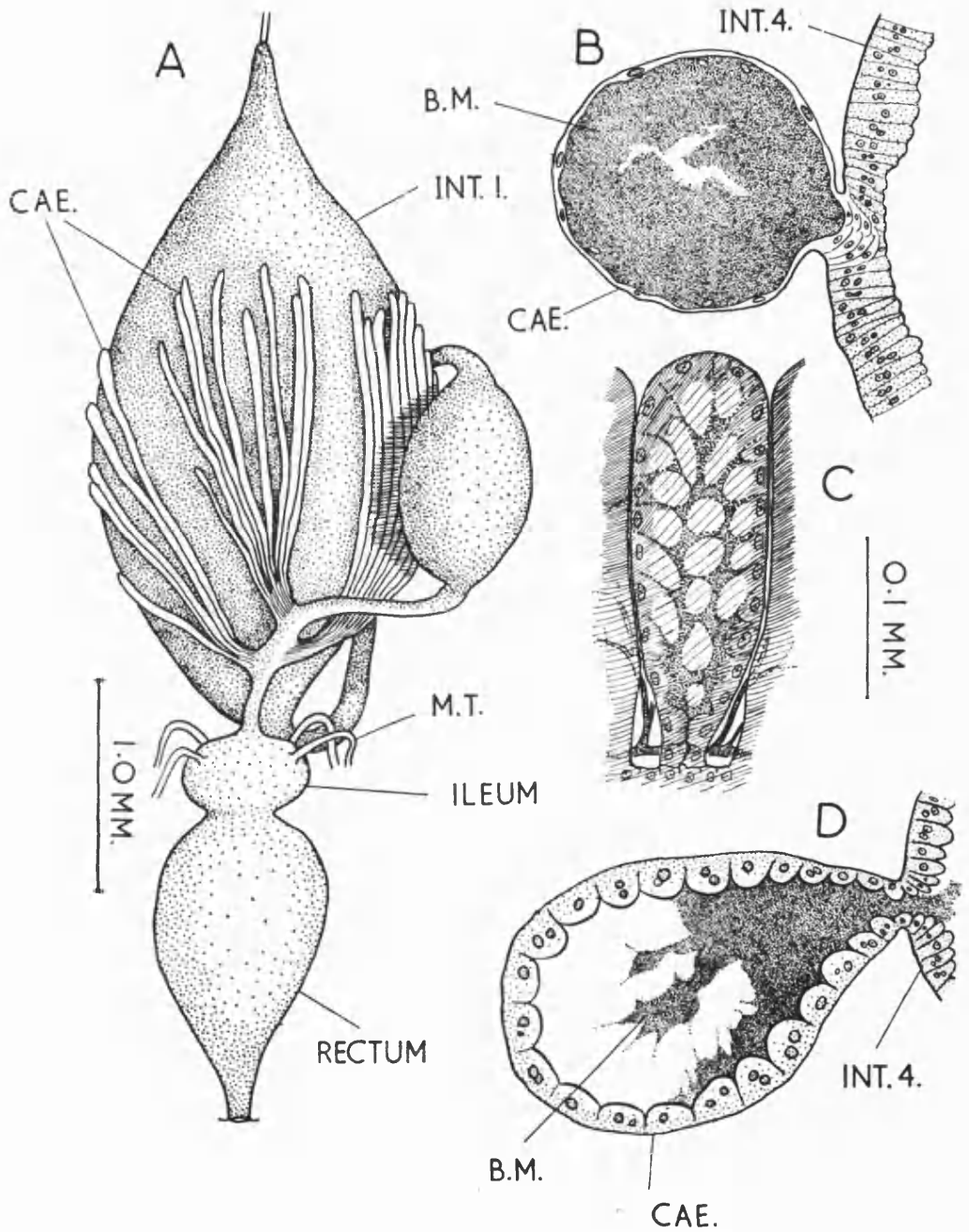


Fig. 8—A. Alimentary system of *Dieuches armipes* (Lygaeidae, Rhyparochrominae) showing natural relationships of parts. B. Section through gastric caecum of *Agonoscelis versicolor* (Pentatomidae). C. Optical section through living gastric caecum of *Cletus fuscescens* (Coreidae). D. Section through gastric caecum of *Acanthomia tomentosicollis* (Coreidae).
B.M.—bacterial mass, Cae.—gastric caecum, Int. 1—first region of mid-intestine, Int. 4—fourth region of mid-intestine, M.T.—Malpighian tubule.

shown in Fig. 8A. In all previous accounts of the heteropteran intestine, this type has been illustrated with the parts in an extended position (e.g. Glasgow, 1914 ; Kuskop, 1923), and the caeca separated from the anterior part of the

intestine. Thus, the obvious parallel with the homopteran filter chamber has not been apparent. In the most advanced types of sap sucking Heteroptera, the caeca are in the form of many short pouches. It may be that these are more efficient in water excretion than long tubes, and since there are many of them, the total caecal volume is probably greater than with fewer long tubes.

It is also a significant feature of the anatomy of the caecal region in relation to the more anterior parts of the mid-intestine, that there is always a more or less marked constriction between the two. This amounts to complete intestinal interruption in a number of species. In all sap sucking Heteroptera, insoluble residues accumulate in the mid-intestine region just anterior to the caeca-bearing region, and are only expelled at long intervals, in many species not until the adult stage is reached. The caecal region is thus isolated from the region of active digestion. This has long been recognized, and no author has ever regarded the bacterial flora as in any way connected with the digestive process.

4. *Is the histology of the caecal region consistent with water excretion?* Although there is nothing in the anatomical relationships which contradicts the hypothesis, such evidence is not in itself compelling, and it is necessary to consider whether the caecal cells have a histological structure appropriate to this function. All the published illustrations of sections through caeca show the wall as a very thin cytoplasmic layer with flattened nuclei. In fairness to the authors concerned, it must be admitted that this is probably the best interpretation of a confusing histological picture. Such a structure does not suggest an active role for the caecal cells. In the living state, however, the cells are often enormously expanded by large apical vacuoles, and the lumen of the caecum contains large transparent spheres which must be derived from such cells. (Fig. 8C). The bacterial mass occupies the irregular spaces between the expanded cell tips and the spheres, and no bacteria can be detected, even with phase-contrast illumination, within the spheres, the vacuoles, or the cell cytoplasm. Attempts were made to fix and stain these fresh preparations, with aceto-carmine or methyl-green-acetic, but in every case the vacuoles and spheres could not be seen after treatment. It seems, therefore, that these structures are extremely delicate. Nothing resembling the appearance of the living cells survives normal histological processes, though in many preparations the bacterial mass is separated from the caecal wall by a wide space, or may adhere to it in a layer broken by numerous gaps. It is likely that these spaces were occupied by vacuolated cell tips. A slight improvement was found when non-aqueous fixative, picro-chloroform-acetic, was used, and some of the cells could be seen to contain small vacuoles. These were probably in an early stage of vacuole development.

The caeca were not invariably vacuolated in this manner, and in all species, specimens were found which, in both fresh and sectioned preparations, showed the commonly accepted picture of thin flattened cells enclosing a continuous mass of bacteria (Fig. 8B). In sections of these specimens, it is always possible to find some nuclei which appear to be embedded in the outer layer of the bacterial mass, rather than in the cytoplasm of the caecal wall. In fresh

preparations, the contents of the caeca include objects which appear to be degenerating nuclei, surrounded by a thick cluster of bacteria. These observations suggest that vacuole production is a cyclical process, resulting in cell destruction, with the bacteria actively breaking down the spent nuclei. The vacuoles may discharge their contents while still attached to the cell, or the cell tip may be pinched off from its base, and become an isolated " transparent sphere ". The latter do not usually pass into the main intestinal tube, but discharge at the narrow neck of the caecum. There exists, therefore, in the caeca, a pattern of cell behaviour which can be conceived as appropriate to the present hypothesis. The very abundant tracheal supply to the caeca also indicates a high level of physiological activity.

In the Bean bugs, *Acanthomia* spp., which, as mentioned above, do not always have a normal bacterial population, it was interesting to find that the cells of the caeca preserved a bulbous shape in sections (Fig. 8D). As these insects feed upon ripening seeds rather than sap, the cells may not be very active in vacuole formation, or it may be that the pressure of the growing bacterial colony causes the collapse of the cells in other species. The caecal cells of *Acanthomia* are very like those found in the ileum of many Lygaeidae, Coreidae, and Plataspidae. Seen under the microscope in the living state, such ileum cells may occasionally release an expanded cell tip to float off in the lumen like a soap bubble. The mechanism involved seems to be the squeezing from adjacent similarly expanded, cells. There do not seem to be any records of comparable " water pumping ", epithelia in other Insecta. The passage of water into the distal end of a Malpighian tubule appears (Ramsay, 1958) to be a passive filtration, while the mechanism of water regulation in fresh water insects seems to involve the active recovery of solutes from such a filtrate (Ramsay, 1950). Perhaps the nearest known mechanism is the contractile vacuole of Protozoa.

As a postscript to these considerations, the writer has been fortunate enough to discover, in a lygaeid, *Paromius gracilis*, which does not possess gastric caeca, a cell pattern in the last part of the mid-intestine which suggests a stage in the evolution of caeca. In this species, the cells lining a short segment of the intestine immediately anterior to the ileum contain several large vacuoles (Fig. 3F). The intestinal epithelium of this region is not vacuolated in any other species. These cells form a continuous layer in *P. gracilis*, but one may imagine that if small groups of such cells developed among the ordinary intestinal cells, the pressure of the expanding vacuoles might force them to bulge outwards into a crypt-like structure.

5. *The evidence from correlation of caeca with sap sucking diet.* While it is well known that zoophagous and seed sucking species lack the gastric caeca, the evidence for a relationship between presence of caeca and phytophagous diet is obscured by the abundant species of Tingidae and Miridae, and many Lygaeidae, in which caeca do not occur. With regard to these, however, there is no clear evidence that sap alone constitutes a major portion of the diet. From observation of a number of species, it appears that such rich sources of food as ovules or developing seeds are frequently, perhaps constantly, selected. Carayon (1944) suggests that the passage of liquid excrement by *Ischnodemus*

sabuleti, a species in which the intestine not only lacks caeca, but is discontinuous, proves that the Malpighian tubules are the source of the liquid excreta in all species. It is likely, however, that the excreta of these insects is more or less isotonic with the haemolymph, and produced in relatively small amounts. The families named above are composed of species of small or very small size. This makes it possible for the insects to feed by sucking out the whole cell contents, thereby gaining a more concentrated and balanced source of nutrition It is significant that many small Homoptera which feed in this manner (e.g. many Jassidae, *vide* Saxena, 1955), lack the filter chamber.

In the phytophagous Cimicomorpha (Tingidae and Miridae) there are present, in the salivary gland structure organs, the accessory salivary gland vesicles, which are lacking in Pentatomomorpha. These vesicles are thin walled, and closely applied to the wall of the first mid-intestine region. The purpose of these vesicles seems to be the recirculation of water, in order to maintain a flow of saliva. The value of this to zoophagous insects would be a more efficient washing out of the contents of the prey, broken down by salivary proteases. In the most highly developed blood-sucker, *Rhodnius prolixus*, the vesicles are absent. They are well developed in Miridae and Tingidae, and in the feeding of these insects, a toxic saliva is used to kill the plant cells around the puncture, and the dissolved contents are ingested. This sort of diet would not contain a great excess of water. There is evidence from the Bryocorinae, which are probably the largest of the phytophagous Cimicomorpha, and which make a large lesion into which sap may exude, that excess water can be excreted through the salivary gland vesicles. Goodchild (1952) records the appearance of droplets of a clear fluid from the mouth parts soon after finishing a feed.

The exceptional cases where caeca are present in species which have adopted a dry diet, and those where the caecal region is not in communication with the rectum, have already been referred to. For the reasons which were mentioned, it is not thought that they constitute an insurmountable obstacle to the acceptance of a water excreting function for the caeca in general, but represent evolutionary divergences from the sap sucking basic stock.

6. *The evidence from the fate of injected dyes.* In studying the cacao capsid bugs (Goodchild, 1952), it was found that the parts of the intestine which became stained with injected methylene blue were those which, on other grounds, it was thought that passage of water from the haemocoel to the intestinal lumen might be taking place. Similar work on Pentatomomorpha, carried out at the same time but unpublished, showed clearly that the caeca took up the dye. This was most striking in the pyrrhocorid *Physopelta* sp., which possesses a normal series of pocket-like caeca. Unfortunately, it has not been possible to collect this genus for further study, and in this present work only seed sucking Pyrrhocordiae were studied.

DISCUSSIONS AND CONCLUSIONS

The conclusions which may be drawn from the facts outlined above seem to be as follows. Taking the Order Hemiptera as a whole, the existence of a separate compartment of the intestine into which the Malpighian tubules open

(the so-called " ileum ") can be demonstrated only in the Heteroptera Gymnocerata. It is not present in Homoptera (*vide* Licent, 1912 ; Kershaw, 1913 ; Pesson, 1944 ; etc.), or in Heteroptera Cryptocerata (*vide* authors quoted earlier). The ileum, in those species in which this organ occurs, arises in the embryo as a fusion of vesicles formed at the base of each malpighian tubule (Srivastava & Bahadur, 1961), and is generally agreed to be endodermal, both on embryological grounds (e.g. Henson, 1932), and on histological grounds (absence of chitinous intima recorded by numerous authors). It must therefore be regarded as a segment of the mid-intestine, and not homologous with the ileum in other insects, which is part of the hind-intestine (Snodgrass, 1935). In the zoophagous lines of Gymnocerata (Cimicomorpha, Amphibicorisae), the ileum remains very small, and it is only in the Pentatomomorpha that a disinct sac develops. Increasing size is probably sufficient reason for the lateral migration of the Malpighian tubule openings, which thereby avoid compression against the dorsal and ventral body walls. The Lygaeidae, in which the ileum retains its primitive position as a continuous part of the intestine, and is histologically least specialised, seem to be a family in which there is the greatest variety of form in the development of gastric caeca and mycetome-like structures (as shown by the list given by Schneider, 1940). One may regard it as a family in which many " experiments " in intestinal structure have been attempted. There is also a great variety of feeding habits. Though many Lygaeidae feed on ripening or dry seeds (Sweet, 1960), others are undoubtedly predatory (e.g. *Geocoris*), sap sucking (e.g. *Blissus*), or of uncertain diet which may include coprophagy or necrophagy (Miller, 1956). In Uganda, *Dieuches armipes* is usually found among rotting fruits beneath plants of various species of Cucurbitaceae. In the other families of Pentatomomorpha, the pattern of the gastric caeca has become stabilised in the form of rows of small pouches, and there is a trend towards the exclusion of the ileum from the main axis of the intestine. While it is in the Rhyparochrominae that the gastric caeca are most strikingly comparable to the filter chamber of Homoptera, these changes in the ileum, in the families (Coreidae and Pentatomidae) in which sap sucking has become most highly developed, support the evidence already given for that interpretation of caecal function. If the chitin-lined rectum can be regarded as a place where excreta hypotonic to the haemolymph can be stored, then the tendency for the mid-intestine opening to be brought into close proximity to the ileo-rectal valve may be a device for facilitating the transfer of hypotonic material from the caecal region to the rectum, with the minimum of contact with the permeable walls of the ileum. The differences in the manner in which this is achieved, and the different number of caecal rows in the two families, suggest that the Coreidae and Pentatomidae have evolved separately towards a sap sucking mode of life. The tendency to by-pass the ileum makes it difficult to understand why this organ should have evolved at all, as it is obviously correlated with a phytophagous habit. On the basis of the evidence from such forms as *Libyaspis* or *Aspongopus*, it seems possible that the ileum was itself the original water excreting region, but became superseded by caeca, which could offer a greater surface to the haemolymph, and be more closely applied to the first region of the intestine. The,

probably fortuitous, invasion of the caeca by bacteria has been the subject of further evolutionary modification and specialisation, but from the beginning, these bacteria would be of value in locking up useful materials which would otherwise be lost. This function of the bacteria, complementary to the conception of the caeca as a water excreting system, is emphasised by the discovery (See Part II) that mechanisms exist, and are developed to a high degree in some species, by which the bacteria can be digested, and the locked-up nutrients released and absorbed.

In the species which have become specialised for a dry diet, such as ripe seeds, the gastric caeca are not developed, or are rudimentary, or are closed off from the lumen of the intestine. The ileum is reduced in volume, and its cells have a dense, deeply staining, cytoplasm. In the Pyrrhocoridae the cells are tall and narrow with strongly basophil tips, and in Coreidae (Alydinae) they have long basophil terminal lobes. The staining reaction of such cells is similar to that of the ampulla cells of Cimicomorpha. Wigglesworth (1931) has shown that the latter cells actively absorb water, and it is therefore reasonable to assume that this is the case in the Pentatomomorpha which have adopted a dry diet. The significance of the extreme development seen in the ampullae of the Cimicomorpha may be in the essentially zoophagous diet of this group. This must involve a considerable amount of protein metabolism, and consequently a high rate of uric acid production. As Wigglesworth showed, the absorption of water in the ampullae is important in precipitating out the uric acid.

Turning now to the hind-intestine, we find that in spite of negative statements in textbooks, there exist in this region cells which are distinct from the thin rectal syncytium, and which justify the term rectal glands. Such specialised areas are found in species with every type of feeding habit, and although the appearance differs considerably between the extreme cases, yet they are connected by a full range of intermediate forms. The function usually ascribed to rectal glands is water absorption, though in Cryptocerata a respiratory function has been suggested (Poisson, 1924) on account of the numerous intracellular tracheoles. Hamilton (1931) refuted the latter explanation on the grounds of lack of a demonstrable water flow, and the fact that such tracheoles occur also in most terrestrial Heteroptera would seem to confirm this opinion. The conclusions of Ramsay (1958), on the passage of amino acids and other useful materials through the Malpighian tubules, show that rectal glands must absorb these materials to a large extent. This concept is of value in understanding the position in Heteroptera. While, in the terrestrial species, the connection between degree of gland cell development (particularly the eosinophil border) and dryness of diet, suggests the commonly accepted function of water absorption, this is most unlikely to be necessary in an aquatic species. Yet it is these species which have the most extensive and highly developed rectal glands of any. The Cryptocerata, being underwater feeders, must suffer the dilution of their ingesta with water, which would pass freely through the wall of the intestine. This may, incidentally, be the reason for the retention (or re-development) of the peritrophic membrane in Corixidae. The turgor pressure set up in the haemolymph would drive a large volume of

filtrate through the Malpighian tubules. The rectal gland in this group must, therefore, be regarded as an organ for the recovery of solutes from this flow, finally yielding a copious hypotonic urine. In the structure of the cells, it is noticeable that the striated eosinophil border is absent, and the eosinophil material is concentrated in the basal part of the cell. These differences may be the histologically visible indications of the functional bias towards solute absorption rather than water absorption. With regard to *Gerris*, with its surface skating habits, there would not necessarily be any danger of unwanted water intake. Nevertheless, water is plentifully available for drinking, and the absence of the ileum ampulla cells which occur in fully terrestrial predators may be the result of this. The presence of much solid matter in the rectum suggests that some water absorption does take place in *Gerris*, so that the presence of a striated zone is not anomalous.

When we come to the terrestrial predators and seed suckers, it is obvious that there cannot be any excess water in the diet, and conservation will be necessary if the insect is not to be restricted in its movements by the need for sources of water. In the extremely dry diet of seed suckers, some drinking is inevitable, and such forms as *Dysdercus* are known to pierce soft plant tissues or resort to cannibalism or necrophagy, in search of water. As has been described, the glands are well developed in these species. The differences in area and cell thickness between Cimicomorpha and Pentatomomorpha do not seem to indicate any great difference in function, but are probably the result of chance divergence in the course of evolution. More difficult to understand is the great extent of the gland in the most highly developed sap suckers. The cells, it is true, have little cytoplasm and lack the conspicuous striated zone, so that we are not faced with the anomalous presence of water absorbing structures in insects with a water surplus. An explanation on the lines of Cryptocerata faces certain difficulties. The gland cells in Cryptocerata are thick, with dense cytoplasm, whereas those in the most advanced sap sucking Heteroptera are apparently rather degenerate. The shape of the glandular region in Cryptocerata, and also in cicadoid Homoptera, where a similar process probably occurs, is that of a narrow tube. This shape gives a high area to volume ratio, as would be expected, whereas the rectum of Pentatomomorpha is an expanded sac having a low area to volume ratio. The possibility that the gland epithelium in the latter insects is simply non-functional is difficult to reconcile with the large area involved (more than any other terrestrial species). It would have been expected that loss of function would have resulted in replacement of gland area by the more elastic rectal syncytium. A possible explanation is that the fluid stored in the rectum, although it consists of water which is in excess when the insect is actively feeding, may be retained and slowly absorbed to replace evaporative losses, while the insect is migrating in search of a new host plant, or for reproductive purposes. It is noticeable that insects of this type survive much longer in the laboratory without food than Homoptera such as Cercopidae, which have no rectal storage capacity. There is also some evidence from differences in the gland epithelium between nymph and adult. In sections, the chitinous intima seems to be separated from the cytoplasm by a narrow non-staining zone in adults, but is in direct contact

with the rather dense granular cytoplasm in nymphs (Fig. 3 D,E). In *Libyaspis*, it has been observed that the nymphal rectum is more transparent, in the living state than that of the adult.

The curious case of the Miridae Bryocorinae which have neither rectal gland nor ampullae, may now be considered. Superficially, their structure is very like that of Lygaeidae, but for the absence of the gland. It does not seem reasonable, however, to regard this sub-family as a link between the two major divisions of terrestrial Heteroptera, owing to the specialised nature of the terminal segment of the mid-intestine. This, with its long filaments, is quite unlike any other Hemipteran intestine. Experiments have shown that it is clearly a region of intense water absorption. The resemblance of the filamentous processes to those of ampulla cells, together with their similar function, suggests that the last part of the mid-intestine of Bryocorinae represents the fused and extended ampullae. Clearly, embryological studies would be of interest here. No provision seems to be made for water recovery posterior to the malpighian tubules, and the widely open ileo-rectal valve suggests that the excreta are isotonic with the haemolymph. On the other hand, the water available is economically used, because nearly all that present in the mid-intestine must be absorbed by the filamentous cells, and utilized in the excretory process by the Malpighian tubules. The possibility that excess water, if any, may be excreted by the accessory salivary gland vesicle, has already been mentioned. The overall impression is that these insects have a well balanced diet, with no urgent need either to conserve or to excrete water, which may be the result of the Cimicomorph cell destruction type of feeding applied to soft, succulent, tissues with high water content. They tend towards the sap sucking Pentatomomorpha in their feeding habits, but are limited in structural adaptation by their ancestry. This paper does not set out to make taxonomic decisions, but there does seem to be a case for raising Bryocorinae to the rank of a family.

Certain other phylogenetic considerations are suggested as a result of this work. While the divisions set out by Leston *et al.* (1954) are endorsed, the evidence from intestinal structure indicates that Lygaeidae are the most primitive group, and that the simplified structure, with loss of caeca, found in Asopinae (Pentatomidae) and certain Pyrrhocoridae, has been secondarily acquired. While no trace of caeca remains in Asopinae, rudiments are to be found in the females of Pyrrhocoridae. In *Dysdercus*, they are closed vesicles containing a clear viscous secretion which stains strongly with eosin. They are connected to the intestine by a solid thread of cells inserted among the cells of the intestinal epithelium. The total appearence is very like that of the thyroid follicles of vertebrates, and it would be interesting to investigate possible endocrine activity in these organs. In *Myrmoplasta* the caeca are represented by small bundles of cuboid, pale staining, cells, and no lumen can be detected. The connection of these to the intestine is by thin strands of the peritoneal membrane. In these species, and also in *Scantius*, where no caeca could be found, the terminal part of the mid-intestine is of the kind found where normal caeca are present, a narrow, rather thick walled, tube composed of tall columnar cells. Caeca may regress and eventually disappear in species which adopt a dry diet, if the cells which would excrete water lack the stimulus

to do so. It may be that the expansion of the caeca depends upon the pressure of the secreted fluid in a manner comparable to the expansion of the vertebrate allantois by the secretions of the embryonic kidney.

II. Discontinuity Of The Intestine In Heteroptera

INTRODUCTION

In the current text-book accounts (e.g. Imms, 1957) of the structure of the alimentary canal in the Insecta, the existence of an interruption of the continuity of the lumen, between mid- and hind-intestine, is described as occurring in the larval stages of Hymenoptera Apocrita, Neuroptera Planipennia and viviparous Diptera. It is also mentioned, as a remarkable state of affairs in certain Heteroptera (the lygaeid *Ischnodemus sabuleti* Fall. and Plataspidae) and in the homopteran family Diaspididae. In the general account of Heteroptera by Poisson (1951), in addition to the above mentioned cases, discontinuity of the intestine is recorded in *Dimorphopterus spinolae* (Sign.) (Lygaeidae, Blissinae). More recently, interrupted types of intestine have been described by Carayon (1955) in the six species of Aradidae which he studied, and by Schorr (1957) in *Brachypelta aterrima* Forst. (Cydnidae). The purpose of the present communication is to describe some further instances of this structural feature in the intestines of adult Hemiptera, and also in the nymphal stages of Pentatomidae. In view of the widespread occurrence of discontinuity of the intestine in Heteroptera, which the present work has revealed, it is remarkable that no reference to it can be found in general accounts of intestinal morphology of this group, such as those of Glasgow (1914) or Yanai (1952). Indeed, the latter author gives as his type genus for the normal continuous intestine of Coreidae, the genus *Acanthocoris*, the tropical African species of which have a discontinuous intestine with strongly marked atypical features. Since these features could hardly have escaped notice, this shows that evolution of the intestine can take place without change in outward appearance exceeding generic limits, in widely separated populations. In the endopterygote larval stages with interrupted intestines, and in some Hemiptera, the non-functional segment of the intestine is readily visible as a narrow thread in which a lumen is lacking. In many other Hemiptera, however, the discontinuity is between two bulbous regions closely pressed against one another, and the lack of connection can only be revealed with certainty by sectioning the organs. This would explain the failure of authors to recognize discontinuity.

THE INTESTINAL ANATOMY OF PENTATOMOMORPHA

All the records of intestinal discontinuity in the Heteroptera, including those to be described here, are found in that sub-division termed by Leston *et al.* (1954) the Pentatomomorpha. These are predominantly phytophagous, and in fact discontinuity has not been found in any purely zoophagous species of the group. It is well known that in most Pentatomorpha, the mid-intestine consists of four distinct regions, which may be briefly described as follows. A very short, chitin-lined, tubular fore-gut joins, in the prothorax, an inflated sac-like first region of the mid-intestine (which is conveniently designated *M*1).

A valve-like oesophageal invagination may be developed at the junction, but the peritrophic membrane is rudimentary or absent. The region *M*1 extends backwards to about the middle of the abdomen, and there joins a tubular region (*M*2), the length of which varies in different species from a half to as much as twice that of *M*1. The *M*2 forms a loop in the ventral part of the abdomen, and is followed by a third region (*M*3), which is another expanded sac, lying in the right-hand side of the abdominal cavity. Whereas *M*1 is usually almost empty, in the adults of sap sucking species (though it may contain abundant whitish pasty material in seed suckers), *M*3 is always distended by a pasty mass of insoluble residues. In many small Lygaeidae, *M*2 is wide, and the intestine appears as a string of three sac-like regions. On the other hand, in many Pentatomidae, *M*3 does not seem to be a permanent organ, but only a temporary swelling of the posterior end of *M*2, which may vanish for a while after defaecation. In the pentatomid sub-family Scutellerinae *M*2 is very long, and appears to lack even a temporary terminal swelling, while in many specimens of large Pentatominae, *M*2 has been found to be in the form of a string of several bead-like temporary swellings. Except in the Aradidae, Piesmidae, and Rhopalidae, in many Lygaeidae and Pyrrhocoridae, and in the pentatomid subfamily Asopinae, *M*3 is followed by a narrow tubular region, which may be as long as the preceding regions together, and in some species reaches as much as two and a half times their combined length. This fourth region (*M*4) bears rows of thin-walled pouches, the well known "gastric cacea ". It occupies the posterior dorsal part of the abdomen, running transversely over the posterior part of *M*1, and backwards to join the expanded ileum, into which the Malpighian tubules open. In many species there is a slight swelling at the anterior end of *M*4, before the beginning of the caecal rows. This swelling is usually well developed in species where a discontinuity occurs between it and *M*3 (though in *Brachypelta aterrima* (Schorr, 1957), the swelling appears to be absent). The junction of *M*3 and*M*4 is the point where discontinuity of the intestine occurs in all Heteroptera *. In species in which *M*4 is not developed (e.g. *Ischnodemus sabuleti* and Aradidae), discontinuity may be found between *M*3 and the ileum. In these, the discontinuity may seem to be between mid-and hind-intestine, as in the larval Endopterygota, while in species having a caecal region, it seems to be in the middle of the mid-intestine. In fact all cases have the constant feature that discontinuity is posterior to *M*3. In some of the species with discontinuity between *M*3 and *M*4, there is also discontinuity between *M*4 and the ileum, and this second, more posterior, discontinuity may be more extreme than the first. No instances have yet been found, however, where a discontinuity between *M*4 and the ileum is the only interruption present in the intestine. In the Plataspidae, the *M*4 — ileum discontinuity is found only in the male, the posterior end of *M*4 in the female being transformed into the highly specialised " oothecal gland ". Since all other cases of intestinal discontinuity are found equally in both sexes, it is probable that from the viewpoint of intestinal function, the posterior mid-intestine of female Plataspidae is effectively discontinuous.

* I am indebted to Dr J. Carayon for confirming this for *Dimorphopterus spinolae*, of which no illustration or full description has been published.

Even when a complete, anatomical, interruption of the intestine cannot be demonstrated, there exist between the successive regions of the pentatomomorph intestine marked constrictions of the lumen, which could act as valves in regulating the flow of the intestinal contents. It has been observed that in dissections of species with apparently continuous intestines, it is often difficult to displace the contents of *M*3 into *M*4 by exerting pressure on *M*3. Miles (1958 a) showed, by feeding nymphs of *Oncopeltus fasciatus* (Dall.) with chinese black in their water supply that excreta of mid-intestinal origin were not passed until after the final moult. Total discontinuity of the intestine is therefore only the ultimate development of a strong tendency to restrict movement of the intestinal contents, which is found throughout the Pentatomomorpha.

ACANTHOCORIS SPECIES (COREIDAE, PHYSOMERINAE)

The intestine of species of this genus has been found to possess a total discontinuity between *M*3 and *M*4, and between *M*4 and the ileum, in both sexes and in later nymphal stages. The early nymphal stages have not been available for study. The most common species in the neighbourhood of Kampala is *A. obscuricornis* Dall., but all other locally occurring species were found to have intestines of this type, as also was the closely related *Choerommatus indutus* Stål. These insects are about 13 mm long (excluding antennae), of typical coreid proportions, and dull grey-brown colour, with a mealy surface. *Acanthocoris* has the hind femora inflated, and tibiae flattened, and the legs and pronotum with many small tubercles or spines,while *Choerommatus* has rather thin, unspecialised legs, and a smoother cuticle. The habitat of these species seems to be at ground level, among mixed grasses and herbs. Individual adults are often found in humid forest regions, resting on low herbage, and occasionally larger groups of (presumably) newly moulted adults occur in this situation. No particular species of plant is chosen, and feeding has not been observed. The nymphs are of usual coreid form, grey in colour with a conspicuous white mealiness, and may be found in the surface layers of the soil. In the writer's own garden, colonies of nymphs have been found associated with plants of a leguminous herb, *Indigofera spicata*, and with Sweet potato (*Ipomoea batatas*).

The anatomy (Fig. 9) and histology of the intestine is as follows. A narrow fore-gut joins the mid-gut by means of a moderately well developed oesophageal valve. The *M*1 region is of the normal sap-sucking heteropteran type, a rather thin walled sac, usually flattened and containing only a little flocculent cell debris, but sometimes distended by air bubbles, in which case vigorous muscular contractions take place. The epithelium consists of the bulbous tipped, irregularly columnar, mostly binucleate cells usually found in Hemiptera The anterior half of *M*1, where the salivary glands are closely applied to it, is a translucent orange tint, and its cells are small to moderate size, with dense, finely granular basophil cytoplasm. The posterior half is opaque creamy colour, and has larger cells which vary in appearance according to the phase of their secretory cycle (Fig. 10A). This seems to resemble that described (Goodchild, 1952) in cacao capsid bugs. The cells have a basal zone with little

stainable material, a middle zone with the two nuclei in a tandem arrangement along the long axis of the cell, and a bulbous tip which is either moderate in size and densely packed with coarse brown granules, or else expanded and empty. In the latter case, the nuclei stain poorly and the cell is evidently degenerating. There is a narrow striated border in the immature cells, but

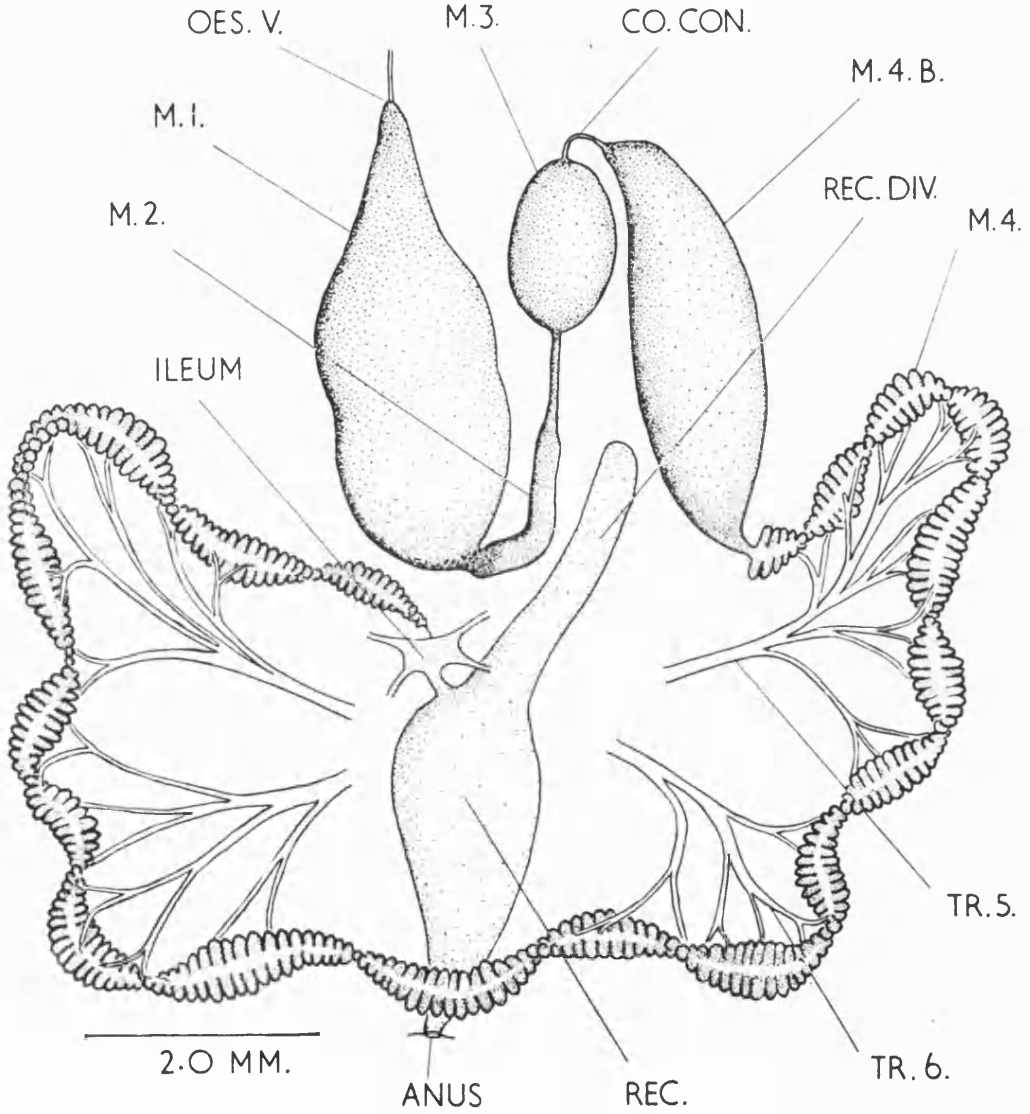


Fig. 9—Whole intestine of *Acanthocoris obscuricornis* Dall.
Co. Con.—cord connection, M.1—first region of mid-intestine, M.2—second region of same, M.3—third region of same, M.4 B.—anterior bulb of fourth region, M.4—fourth region (bearing gastric caeca), Oes. V.—oesophageal valve, Rec.—rectum, Rec. Div.—rectal diverticulum, Tr. 5—tracheae from fifth abdominal spiracle, Tr. 6—tracheae from sixth abdominal spiracle.

this is obliterated by the stretching of the cell wall as maturation proceeds. Cells in the epithelium may be at various stages in development, but as in the cacao capsids, starvation brings about a uniform condition, with cells all immature. A short constricted region of the gut, with slightly smaller, columnar, densely staining, cells, connects *M*1 to a short *M*2, which loops under *M*1 and passes anteriorly. The first part about three-fifths) of *M*2 is rather inflated,

while the last part is a narrow tube, lined with small cuboid to columnar cells, which opens into the sac-like *M3*. The epithelium of the first part of *M2* consists of moderate sized columnar cells, with finely granular cytoplasm and nuclei situated towards the base (Fig. 10B). The cell border appears to have a basophil striated zone within the cell boundary, and a slightly wider, non-

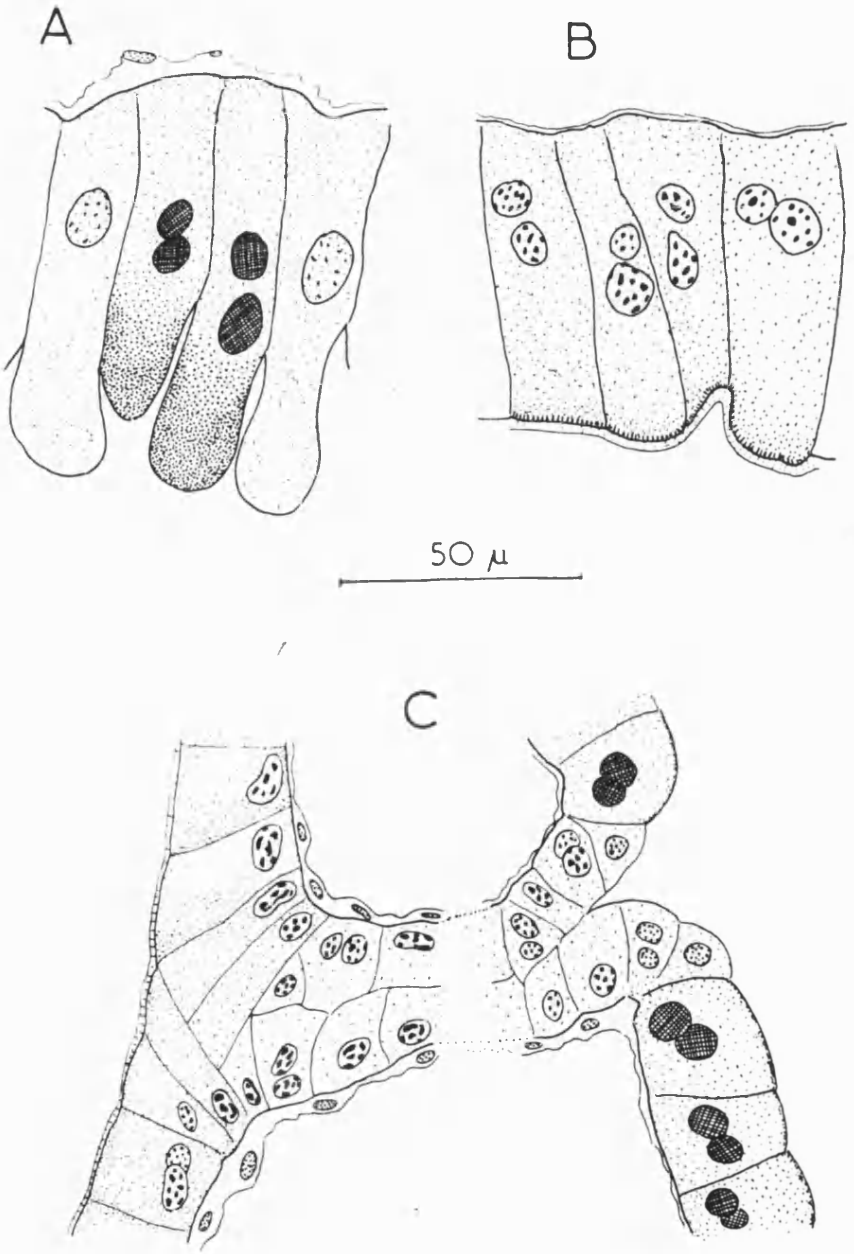


Fig. 10—Cells from mid-intestine of *Acanthocoris obscuricornis* Dall. A. Cells from posterior part of first region. B. Cells from second region. C. Section along cord connection between third (on left) and fourth (on right) regions.

staining, brush-like border extending into the gut lumen, but it would seem that a phase occurs in which the cells are narrow and crowded, with expanded lobe-like tips lacking striations.

In *M3* the cells are always found in a state of tension due to the accumulated residues in the lumen, and they form a pavement-like epithelium under these

conditions. Their general appearance, and particularly the striated cell border, show that they are essentially similar to the cells of *M*2. There is no opening in the epithelial layer at the point where the intestine should make its exit from *M*3, and instead a thin solid cord of small, rather pale staining cells joins the epithelia of *M*3 and the anterior bulb of *M*4. This cord, about 0·5mm long, is enclosed by the peritoneal membrane and muscle layer covering the rest of the intestine, and has a vestigal lumen where it joins *M*4 (Fig. 10 C). The cord-like junction lies far forward in the abdomen, and the regions which it connects lie side by side. The *M*4 bulb is of very large size, comparable with *M*1, and is always distended by a mass of light brown pasty material (the contents of *M*3 are a much darker brown colour). There is a marked difference in the appearance of the contents of *M*3 and the *M*4 bulb in histological sections, those of *M*3 being granular and distinctly eosinophil, while the contents of the *M*4 bulb are non-staining clumps of hyaline appearance, with scattered granules and plate-like or rod-like crystals. The cells forming the lining of this bulb are, as in *M*3, for the most part stretched to a thin pavement-like epithelium, but where the bulb narrows to the caeca-bearing region, they are able to expand to a more normal shape (Fig. 11). At this point it is clear that the cells very much resemble those of the posterior part of *M*1, with coarse brown granules in the cytoplasm. The granules seem to be distributed into the basal part of the cell to a greater degree than in the *M*1 cells, and while the terminal lobe may contain small vacuoles, it does not expand to the extent seen in *M*1. It is not easy to see to what extent the contents of the *M*4 bulb include debris of spent cells, such as occurs in *M*1. A point of the greatest significance is that, where the bulb narrows to the succeeding tubular region, a mass of strongly basophil material, identical in appearance with the bacterial contents of the gastric caeca, is to be seen intruding into the bulb, and grading, through an eosinophil zone, into the typical non-staining contents.

A short tubular region of columnar cells connects the bulb to the caecea-bearing region (Fig. 11), the cells of which are small domed pavement type, with brownish granular cytoplasm. The cells of the connecting region have lobed tips which contain a mass of coarse basophil granules, and between lobes of adjacent cells there is a deep eosinophil brush border. Circular muscle is well developed in this region, more so than in the more anterior constrictions of the intestine. The caecal pockets are exceedingly numerous finger shaped outgrowths, each one about 150 μ by 40 μ, arranged in two opposing rows which twist around the intestine in a helical pattern. The length of this region is about 70 per cent of the total mid-intestine. Inside each caecum is a dense mass of bacteria of a paired short ovoid rod type, probably close to that studied by Huber-Schneider (1957) in another coreid species, *Mescoerus marginatus* L. The caeca open widely to the central tube, and in the living state are a creamy colour, the central tube being almost transparent. The cells of the caeca are flattened and slightly domed, with large flattened nuclei against the base, brown granular cytoplasm, and one or a few clear spherical vacuoles. The caeca-bearing region as a whole is arranged in a sinuous manner around the abundant tracheal branches from the spiracles of the fifth and sixth abdominal segments on each side, like the small intestine of a vertebrate

around the mesenteric blood vessels (Fig. 9). In dissections, *M*4 seems to be connected to the ileum, but closer examination, or the study of serial sections, shows that in fact it ends blindly, and the connection is by means of fine tracheal branches, with not even the peritoneal nor the muscle layer crossing the junction. The ileum, which receives the openings of the Malpighian tubules, is of simple structure, lined with moderate sized bulbous cells which

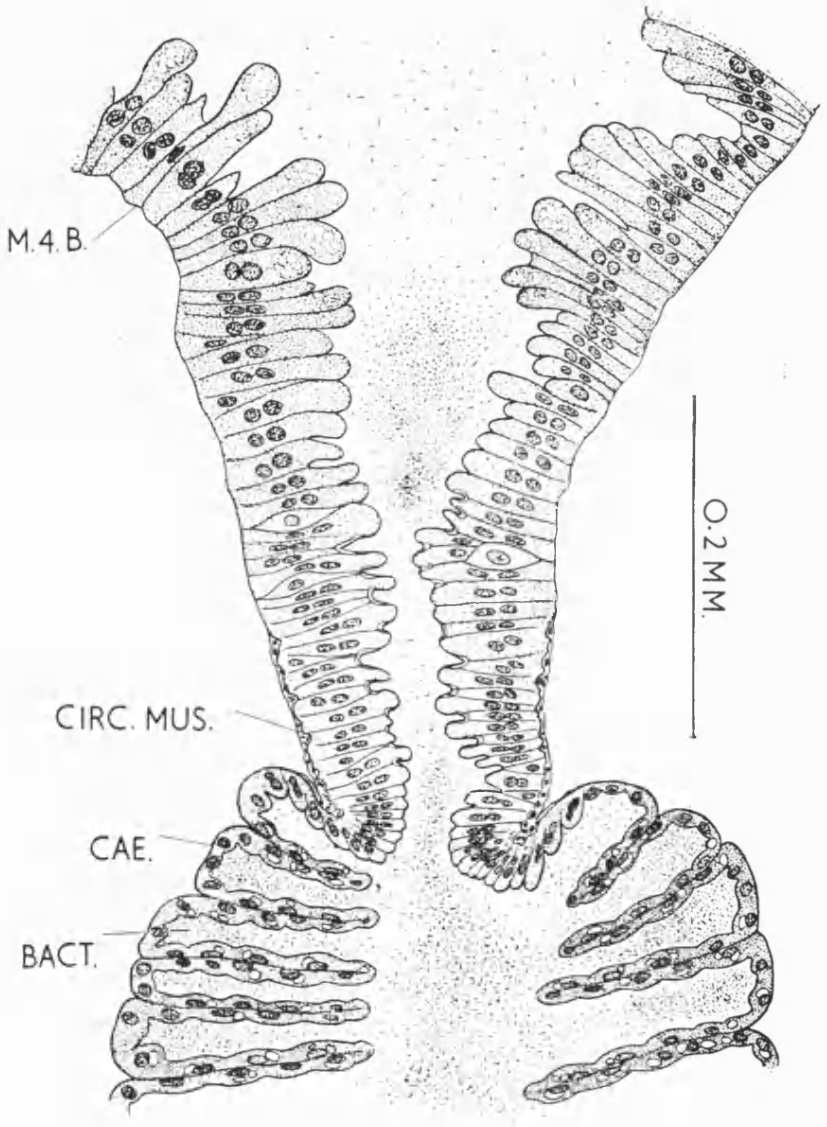


Fig. 11—Section along junction of anterior bulb and caeca-bearing region of fourth mid-intestinal region of *A. obscuricornis*.
Cae.—gastric caecum, Circ. Mus.—circular muscle fibres, M.4 B.—anterior bulb epithelium, Bact.—symbiotic bacteria.

have dense cytoplasm at the base, in which the nuclei are situated, and expanded tips in which there is little stainable material. The ileo-rectal valve is of the usual coreid type and the rectum has a dorsal glandular area. The gland cells lack an eosinophil striated border, and the nuclei appear to be curiously lobed.

ASPONGOPUS XANTHOPTERUS Fairm. (PENTATOMIDAE, DINIDORINAE)

This is the only species belonging to this subfamily which has been available for examination, and so it is not clear to what extent its peculiarities of structure are shared by related forms. Rastogi (personal communication) finds that the internal structure of a related Indian species, *Coridius janus* (F). differs in many ways, but has not yet published his description. *A. xanthopterus* is a dull black insect of typical pentatomid aspect, with yellow veins on the corium, and yellow wing membranes. It is about 15 mm in length, excluding antennae. It is essentially a tropical rain forest inhabitant, being usually collected from ground level shrubs or herbs in that habitat. Feeding has been observed by the writer, on stems of a creeper, *Adenia cissampeloides* (Passifloraceae). The early nymphal stages have not been found, but the later nymphs are not in any way remarkable in appearance. The structure of the intestine is the same in both sexes.

The general plan of the intestine is so similar to that of *Acanthocoris* that it need not be illustrated. The oesophageal valve is rather widely open (Fig. 12A), with a thin tubular invagination. From this, a double sheath of chitinous material extends some way beyond the tip of the invagination (about 0·2 mm), and the mid-intestine epithelium around this sheath is distinctly different from that elsewhere. The cells are cuboid to shallow columnar, extending into the lumen in a finger- or cone-shaped lobe. This cell shape is found in absorptive regions of the intestines of many Hemiptera (e.g. Miridae, Tingidae, Fulgoroidea — unpublished observations of present author). It must be presumed that, owing to the restricted space between the epithelium and the oesophageal invagination, normal secretory processes of the *M*1 epithelium are of no value, and these cells may be modified to absorb simple molecules which diffuse into their neighbourhood. In view of the fact that these insects are sap suckers, with an ingesta of high water content, it is interesting to compare the oesophageal valve with that of Corixidae (Sutton, 1951). Since the Hemiptera have no chitin-lined crop, water passes rapidly from the intestine to the haemolymph, and the extended oesophageal valve may serve to reduce this effect in the region occupied by the important locomotor musculature, thus protecting this system from the paralysing action of haemolymph dilution.

The remainder of *M*1 has the same orange anterior part and creamy posterior as in *Acanthocoris*, and the cells are similar, but taller and narrower (Fig. 12B). The scanty contents appear in sections as a flocculent precipitate At the posterior end, *M*1 is prolonged into a short tube which joins, by way of a constriction, a very short *M*2, which in turn connects to the expanded *M*3. There is only a slight constriction between these last two regions, and they are both lined with cells similar to those in the equivalent regions of *Acanthocoris*. The intestinal interruption between *M*3 and *M*4 is more complete than in *Acanthocoris*, and no cellular cord is present. Instead, the unperforated epithelia come into close contact, and the organs are bound together by a delicate peritoneal layer and by muscle strands. This appears to be the common pentatomid pattern where intestinal discontinuity is concerned,

and in the course of dissection the parts *M*3 and *M*4 can be stretched apart slightly to reveal the " ligamentous connection " as found by Schneider (1940) in *Coptosoma scutellatum* Geoff. The bulbous region at the anterior end of *M*4

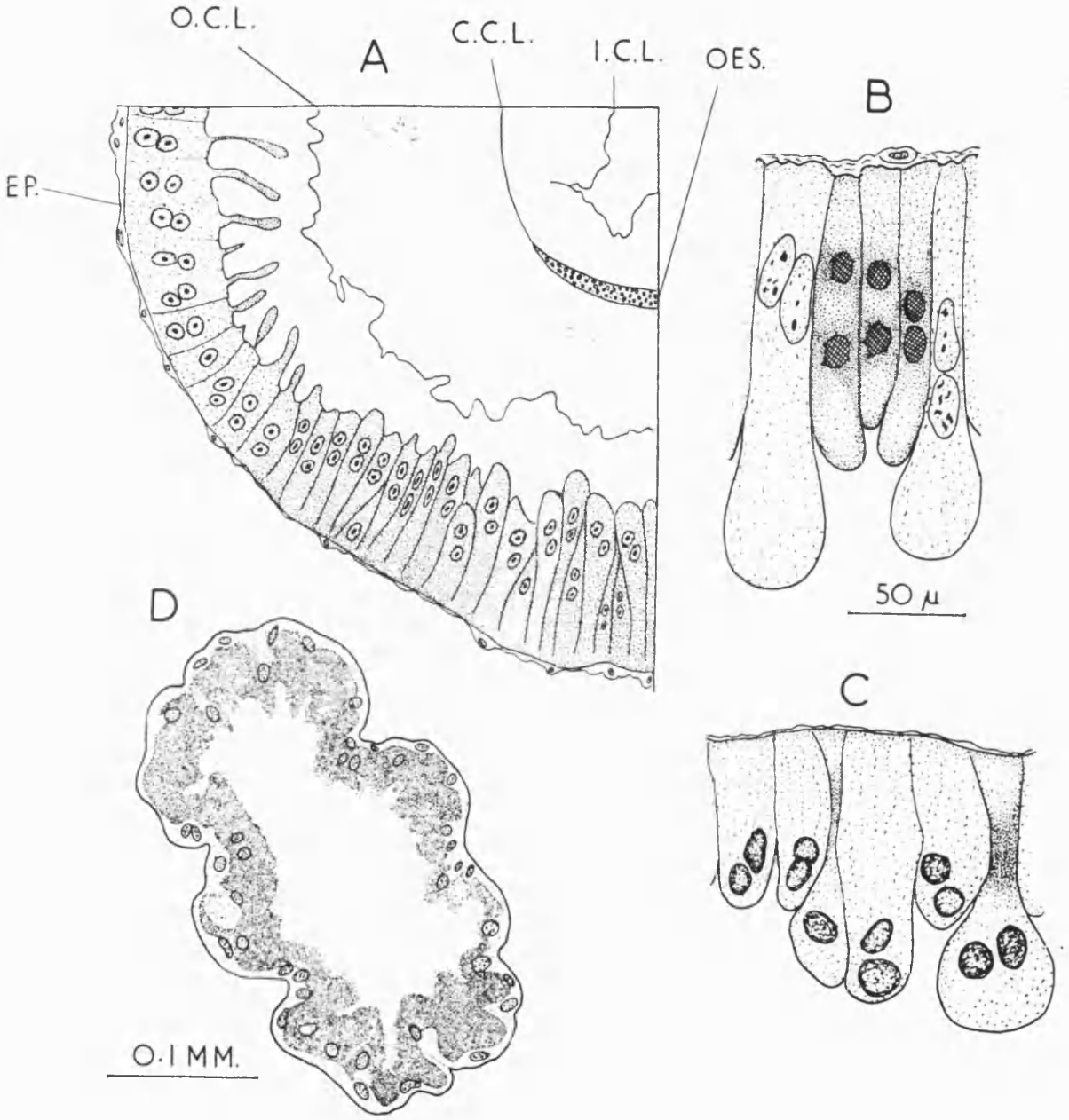


Fig. 12—Cells of mid-intestine of *Aspongopus xanthopterus* Fairm. A. Transverse section through oesophageal valve (slightly oblique, with top left more posterior than bottom right). Oes.—oesophageal epithelium, C.C.L.—central chitinous lamella, I.C.L.—inner chitinous lamella, O.C.L.—outer chitinous lamella, Ep.—epithelium of mid-intestine. B. From first region of mid-intestine. C. From anterior bulb of fourth region of same. D. Transverse section through caeca-bearing region.

is not so large as that of *Acanthocoris*, being only about half the length of *M*1, and about equal to *M*3. There is the same histological distinction between the contents of the two adjacent bulbs, and a slight difference of colour in the living state, *M*3 being orange-brown, and *M*4 a lighter orange shade. The

cells lining the *M*4 bulb, as in *Acanthocoris*, resemble those of *M*1 more than those of *M*2–3 (Fig. 12C), but they are broader based than the *M*1 cells of their own species, and approach the shape of cell found in the *M*1 of *Acanthocoris*. In the specimens sectioned, the *M4* bulb cells had evidently just passed the phase of secretion, since the cells themselves were expanded and empty, while the typical hyaline contents were surrounded by a sheath of granular eosinophil material. The tubular region of *M4* in *Aspongopus* is rather different from that of *Acanthocoris*, and very similar to that of the Plataspidae, in that the caecal pockets have become wide and shallow so as to almost merge into the central tube (Fig. 12D). In fact the caecal system is perhaps better described as a succession of shallow, irregular, annular chambers. It is relatively very long, and may amount to more than 70 per cent of the total mid-intestine, but also very narrow (about 0·33 mm). It appears to be longer in the females, as is commonly found in this part of the intestine in other species. In a male which was measured, *M*4 was 20 mm, long, *M*1, *M*2, and *M*3 being respectively 5 mm, 2 mm and 2 mm but in a female *M*4 was recorded at 58 mm, the other regions not being measured but approximating to the dimensions quoted for the male. The cells of the caecal tube appear to be of the usual type for this region, but are less easy to distinguish from the bacterial mass than those in *Acanthocoris*. They are thickly coated with a deep staining mass of rod-like bacteria, and appear to have little cytoplasm and large nuclei. A few vacuoles can be detected.

The *M*4 bulb is joined to the tube by a collar of small, pale staining, columnar cells, but there is no marked constriction. Connection to the ileum of the posterior end of M4 is by tracheal branches only. The ileum is unusual in having many crypt-like foldings of the lining, the cells of which are highly vacuolated. This has been illustrated in Fig. 7B. The rectum is composed entirely (except for the extreme posterior part) of large pavement-like cells with large nuclei, as found in rectal glands.

PIEZOSTERNUM CALIDUM Fab. (PENTATOMIDAE, TESSARATOMINAE)

This is another moderate to large (17–18 mm) forest dwelling species, which has been found feeding on creepers (*Momordica cissoides* and *M. foetida*, Cucurbitaceae). It is of normal pentatomid appearance, and dark green in colour. No nymphs have been found. The intestine of this species is interrupted anterior to the *M*4 bulb, but there are certain differences from those described above. The general anatomy is illustrated (Fig. 13). In gross appearance, the *M*1 region is deeply indented by the contraction of the circular muscles, so as to form a series of pouches. In the living state, this pattern is constantly changing, as the contents are churned about by muscular activity.

In sections, the following details are revealed. The oesophageal valve is weak, but secretes a deep fold of chitinous material into the anterior end of *M*1. There is a thick collar of columnar mid-intestine cells around the valve, but the *M*1 epithelium does not seem to enclose the introvert as closely as in *Aspongopus*, and no distinct cell types occur at this level. The cells of *M*1 are relatively small for this region, and are mainly concentrated upon a series of transverse ridges formed by inpushing of the basement membrane. There

are about thirty-five of these ridges, spaced about 0·1 mm apart, and extending the full length of the *M*1 region, so that in longitudinal sections the wall of *M*1 is scalloped into a series of annular troughs. These troughs are of very regular size, and are clearly of a permanent nature, unlike the grooves formed by muscle action. The cells, particularly those in the troughs, have unusually well developed brush-like borders, and dense apical concentrations of brownish granules. In most cases no stainable matter was present in the gut lumen of

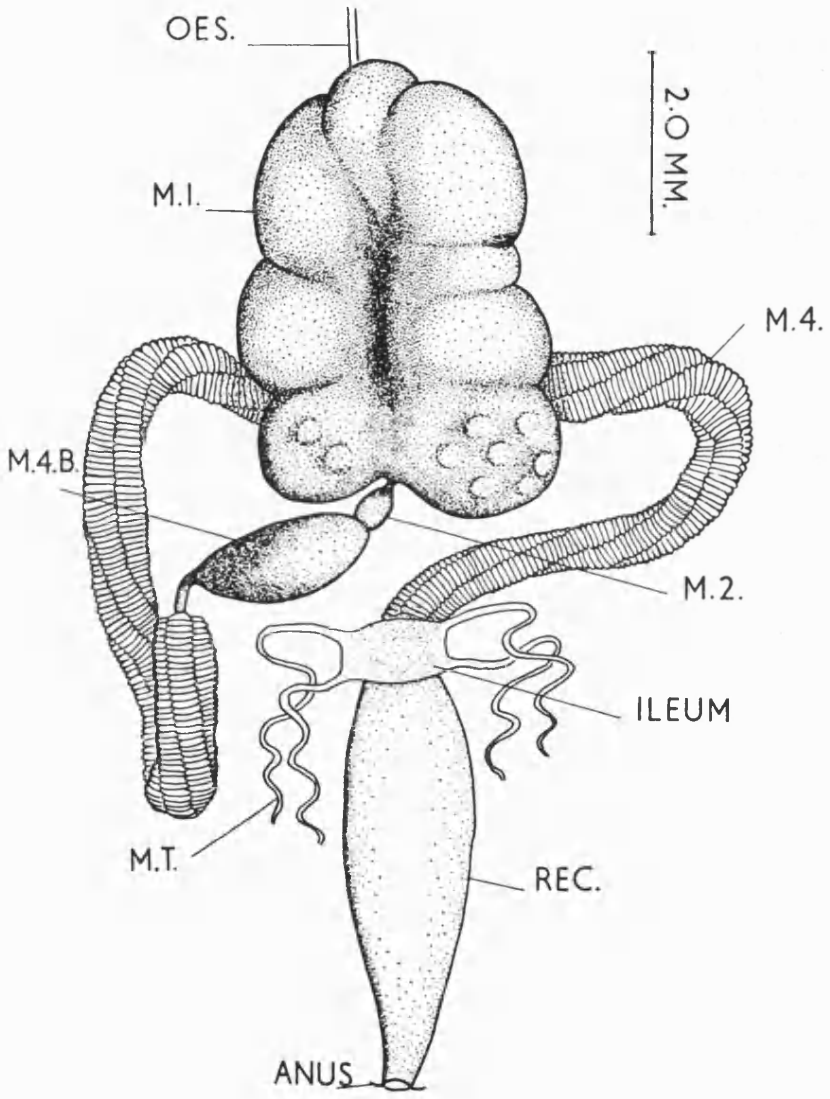


Fig. 13—Whole intestine of *Piezosternum calidum* Fab. (from fixed specimen).
Oes.—oesophagus, M.T.—Malpighian tubules (curtailed), remainder as in Fig. 1, p. 857.

this region, but one individual was found in which the contents were a fairly dense mass of eosinophil granules.

The connection to *M*2 is an exceedingly narrow tube about 0·1 mm long and with a lumen only 20 μ wide, while *M*2 itself is very small (0·35 mm. diameter), and is lined with small columnar cells of even height with a brush like border. The lumen at its maximum width is only 0·15 mm wide, and the

contents are a scanty reticular precipitate. The region of the mid-intestine which follows this *M*2 is connected to it by a delicate solid cord connection 0·1 mm long, and is clearly shown, by its hyaline contents, and its relation to the caecal region posteriorly, to be the *M*4 bulb. There is thus no distinct *M*3 region.

The *M*4 bulb is ovoid, 0·8 mm in diameter, and with lining cells of a wide bulbous type, with thin striated border. Over most of the organ, the cells were found to be stretched into a pavement epithelium, but those near the junction to the caecal region appeared to be normal cells of *M*1 type, in an immature state. The cells contained no granular material, and the lumen contents were entirely of the hyaline variety. A moderately constricted tube of small columnar cells joins the bulb to the caecal region, which is of the usual Pentatomid type. There are four rows of ceaca, twisting in a slight helix around the central tube. Each caecum is laterally expanded, so that the rows almost touch, and the central tube is hidden, but longitudinally compressed by adjacent caeca into a disc-like shape. The opening of each caecum to the central tube is wide and unrestricted. The caeca are filled with a rich culture of bacteria of a twisted rod-like type, and have very thin walls with extremely flattened nuclei. The *M*4 region occupies about three-quarters of the total mid-intestine, the high proportion being due more to the abbreviation of *M*2 and the absence of *M*3, than to excessive size of *M*4, which is not much larger than that in other sap-sucking Pentatomidae of similar size.

The connection of *M*4 to the ileum is in the dorsal and backward position usual in Pentatomidae, and is in the form of a thick plug of cells (Fig. 17C), in which a continuous lumen could not be detected. The possibility cannot be excluded that a passage for fluid, if not for solid matter, may exist, or may be formed periodically by relaxation of the muscles. The ileum is lined with bulbous cells arranged in crypts as in *Aspongopus*, but these are less deep than in that species, and the cells do not show well marked vacuoles. The cytoplasm of the ileum cells shows only a sparse reticulate precipitate of stainable material. The ileo-rectal valve is well developed, with several folds of very small columnar cells, and the rectum, like that of *Aspongopus*, is composed almost entirely of large flattened gland-type cells.

It is to be hoped that specimens of other species of this subfamily will become available for study, as the structure of the epithelium of the apparent *M*1 gives grounds for wondering whether it is not in reality a modified *M*2, the missing segment being not *M*3 but *M*1 itself.

LEPTOCORIS (*SERINETHA*) *AMICTA* Germ. (RHOPALIDAE)

This moderate sized (11–13 mm) insect is a common inhabitant of floor litter in forest of various kinds. Its food probably consists mainly of plant seeds, though it will readily feed carnivorously on other insects when confined in laboratory cages. The structure to be described here is common to both sexes, and also occurs in the related species *L. griseiventris* Westw. and *L. haematica* Germ. The general anatomy of the intestine (Fig. 14) is not complicated and may be regarded as three regions of approximately equal length of 4–5 mm, *M*4 being absent in these species. The oesophagus joins *M*1 by way of a

greatly reduced valve, though in the most anterior part of *M*1 the tall columnar lining cells almost occlude the lumen, and could have a valve-like function. The cells of *M*1 are broad based, moderately bulbous tipped, and with nuclei situated basally. Usually one or a few small vacuoles can be seen in the tips, and there is a sparsely reticulate cytoplasm. This region contains a light coloured paste with many small oil droplets, the contents being eosinophil

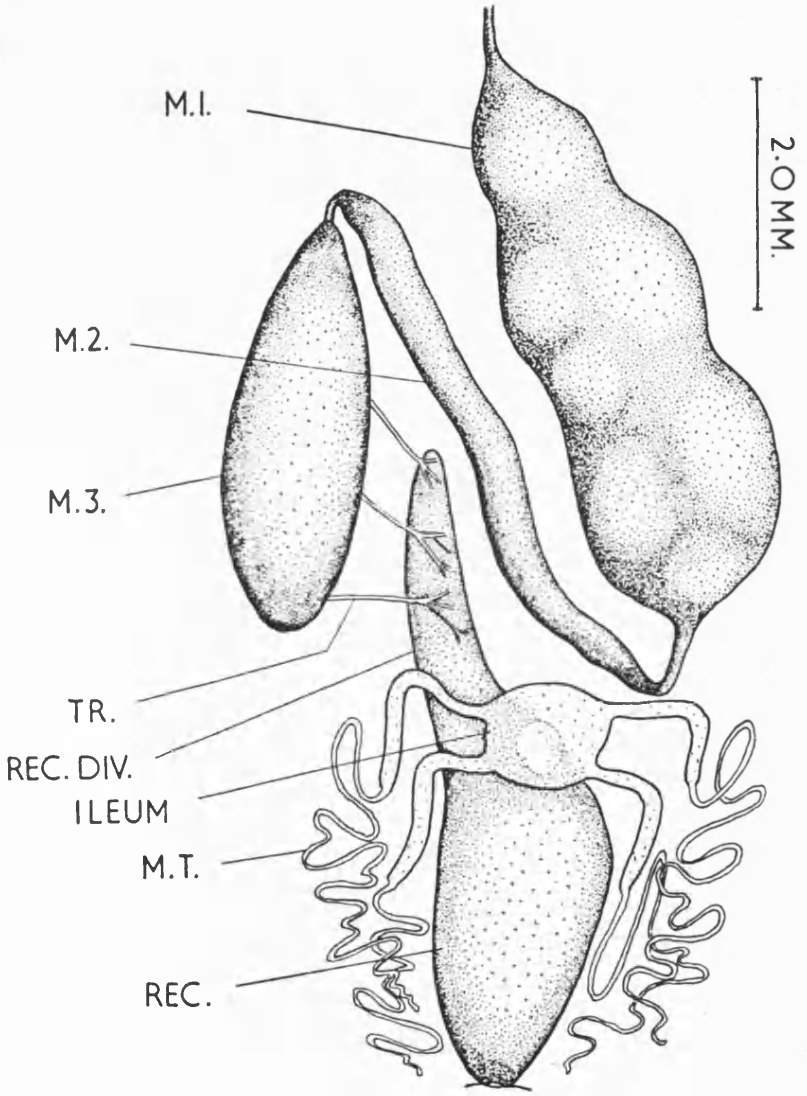


Fig. 14—Whole intestine of *Leptocoris* (*Serinetha*) *amicta* Germ.
M.T.—Malpighian tubules, Tr.—trachea binding mid-intestine to rectum, remainder as in Fig. 9, p. 885.

in stained sections. At the most posterior end of *M*1, there is a distinct valvular constriction, with three or four inwardly projecting ridges of small columnar cells, with central nuclei and many small vacuoles at their tips. From this valve, *M*2 runs forwards on the right-hand ventral side of the abdomen, as a tube about 0·5 mm in diameter, with columnar cells much as in *M*1, but strongly vacuolated at their tips (Fig. 15A). The intestinal contents in *M*2 appear to become more coarsely granular, and brown in colour (remaining so

in stained sections). A slightly constricted region joins *M*2 to *M*3 at the level of the anterior end of the abdomen, and from there, the moderately inflated *M*3 runs posteriorly, on the left side, to end blindly at the anterior end of the rectum. The cells lining *M*3 are low columnar, with an even, brush-like, border. The cytoplasm in the cells of the posterior half of this region (Fig. 15B) is finely granular, with some reticulation and occasional vacuoles, and in this part the contents are a dark brown pasty mass. In the anterior half, however, the contents are a single large oil drop, orange-brown in colour, with a few needle-like crystals suspended in it. Here the cells are strongly vacuolated throughout their depth, but with less distinct vacuole outlines than in *M*2. This appearance may be a fixation artefact.

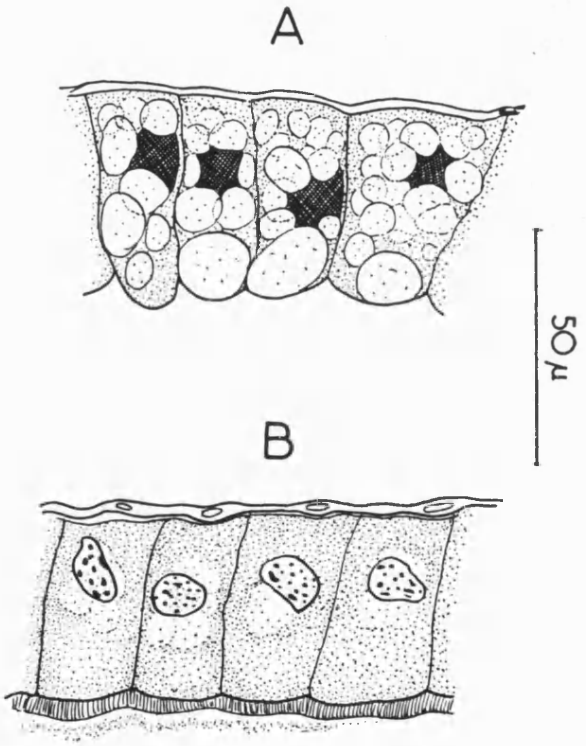


Fig. 15—Cells from mid-intestine of *Leptocoris amicta*.
A. From second region. B. From posterior end of third region.

There is no attachement of *M*3 to the ileum in the original axis of the intestine, but three or four fine tracheae on each side couple *M*3 at points along its length, to a ventral anterior diverticulum of the rectum which lies between *M*2 and *M*3. The ileum is a thin-walled bladder receiving the openings of the Malpighian tubules, with a rather rudimentary, widely open, ileo-rectal valve connecting it to the large thin-walled rectum. The contents of ileum and rectum are a brown transparent fluid, with some powdery uric acid deposit. The rectum had a dorsal area of rectal gland cells, which are thin, and lack the eosinophil border found in most Heteroptera which do not possess gastric caeca.

THE NYMPHAL STAGES OF PENTATOMINAE

In the course of numerous dissections, undertaken in connection with other studies, it has become apparent that complete intestinal discontinuity occurs in the nymphal stages of many species of Pentatominae. In the nymphs of a wide range of species examined, some degree of constriction is found between *M*3 and *M*4, with another, less extreme, constriction just anterior to the gastric caeca, forming a distinct *M*4 bulb. This bulb is almost spherical, and usually very small, being only slightly wider than the central tube of the *M*4 region. In the species in which intestinal discontinuity has been detected it may be a little larger, up to about twice the central tube diameter, and is tightly pressed against *M*3. The presence of a ligamentous connection may be confirmed from sections (Fig. 16A), which show that the epithelia of the two regions are separated by a thin layer of peritoneal cells. The contents of the *M*4 bulb are histologically identical with the hyaline material found in this region in the species described above, and in the living state there is a very marked colour difference between *M*3 and this bulb. This difference is, in fact, much more conspicuous than has been found in those other species, *M*3 being deep green or greenish-brown, and the *M*4 bulb a pale creamy white. In species where a complete discontinuity is not present, the bulb usually contains fluid and brownish flocculent matter.

It has not been possible to investigate in detail the number of species in which discontinuity occurs, but histological evidence has been obtained for it in *Scotinophara fibulata* Germ., *Platacantha lutea* Westw. and in *Caura singeri* Dist., while the gross appearance of the parts strongly suggests it in *Euryaspis signoreti* Stål, *Acoloba lanceolata* Fab., *Antestiopsis lineaticollis* Stål, and *Nezara viridula* L. One of the species investigated, *Halyomorpha annulicornis* Sign., was found to have the *M*3 to *M*4 connection in the form of a tube of exceedingly narrow bore, the lumen being less than 5 μ wide. This type of connection is commonly found in certain Coreidae (*vide infra*), and must be distinguished from a mere constriction of the intestine. It is a characteristic of this type (which might be termed a " capillary connection ") that its cells are sharply differentiated from the epithelia of the regions which it joins, being pale staining, somewhat lens-shaped and partly overlapping, and being inserted among the cells of those epithelia in a shallow, nipple-like, projection (Fig. 18). Unlike the simple intestinal constriction, which is lined with cuboid or columnar cells, the change in diameter of the intestinal lumen is abrupt, and the capillary diameter is constant throughout its length. No normal intestinal contents can be seen in a capillary connection, but the lumen is sometimes filled with a structureless eosinophil material. A connection of this kind must be regarded as virtually discontinuous. To judge from the appearance of the intestines in dissections, many other species of pentatomine nymphs may have intestines of this sort. These include many unidentified nymphs picked up by chance on collecting trips. On the other hand, in those species (of the genera *Aspavia*, *Carbula*, *Agonoscelis*, etc.) which feed on seeds or ripening inflorescences normal continuous intestines are found. In general, it would seem that the tendency towards discontinuity is greatest in sap sucking species, but as yet the exact sources of food of many species is obscure.

The discontinuity breaks down, and a continuous flow through the intestine is established, at some time during the maturation of the adult. Nearly all the adults which have been examined, have been found to lack the distinct *M*4 bulb, and the greenish-brown contents of *M*3 can be seen passing down the central tube of *M*4. At the same time, the expanded *M*3 shrinks considerably. It has been possible to observe this process taking place, in a dissection of a newly moulted adult *Acoloba lanceolata*. In this species, nymphs are rarely found, and are probably to some extent subterranean, while the adult is a long narrow insect not uncommon on the inflorescences of the grass *Hyparrhenia rufa*. On one occasion, a full grown nymph was found in this situation, and moulted in the laboratory. The displacement of the white contents of the *M*4 bulb by green *M*3 material was actually taking place when the intestine was examined. The histological changes which occur when continuity is established are approximately as follows. In the completely discontinuous intestine, as in the adult forms described earlier, the epithelia of the closely opposed parts of *M*3 and *M*4 do not differ from that found in the region as a whole. This may be the case in the earlier instars of pentatomine nymphs, but in the final instar cellular activity, with many mitoses, causes the cells nearest to the ligamentous connection to take on a distinct appearance. This occurs first in the *M*4 bulb, a shallow hump of cells being formed, with low columnar cells around the edge, and a cluster of lens-shaped cells in the centre. At this stage, also, the peritoneal cells start to withdraw from between the adjacent epithelia, (Fig. 16A). Next, mitotic activity may be observed in the epithelium of *M*3, and the cells at the point of contact lose the appearance typical of that region, becoming finely granular, uniformly staining, columnar type, with a cluster of stratified cells like that of the *M*4 bulb. By this time, the peritoneal cells have withdrawn further, and form a thick collar around the junction (Fig. 16 B). The inner cell clusters of the two regions are now in contact, and the beginning of a lumen is shown by the separation of the columnar cells at the centre of the cell hump on either side of the junction. It must be presumed that the inner cell mass is fairly rapidly reorganised into the columnar epithelium of a normal constricted connection, since no intermediate condition has been observed. The structure of the junction just before a lumen appears is strikingly similar to the cell plug connecting the *M*4 region to the ileum in *Piezosternum calidum*. There is probably some variation between species in the exact relation of this process to the life history, as it has been found that in final instar nymphs of *Nezara viridula*, it is already nearly complete, while in an adult *Scotinophara fibulata*, the histological changes were still in progress. Further study might also reveal differences, in this respect, between the sexes, as it is only in the female that competition from developing ova, for space in the body cavity, makes it desirable to evacuate the gut contents.

It is of great significance that in the discontinuous intestines of pentatomine nymphs, two other structural features occur which have been found in the intestines of the adult insects described earlier, and which are evidently an integral part of the physiological adaptation involved. They are, firstly, the lack of a detectable lumen in the junction between *M*4 and the ileum, which is of the nature of a cell plug, and secondly, the widely open gastric caeca.

Taking the Pentatomomorpha as a whole, the most common appearance of the *M*4 region is that of a moderately thick-walled tube, lined with columnar or cuboid cells, with each thin-walled caecum (the cells of which are flattened, pavement epithelium) connecting with this central tube by way of a narrow neck of small cuboid cells (Fig. 17 B). The neck is about six cells long, and has a very restricted lumen, in the region of 5 μ diameter. The cells of which it is composed can be seen to be inserted between those of the main intestinal tube, being carried right through to the lumen of that tube. Narrow though this

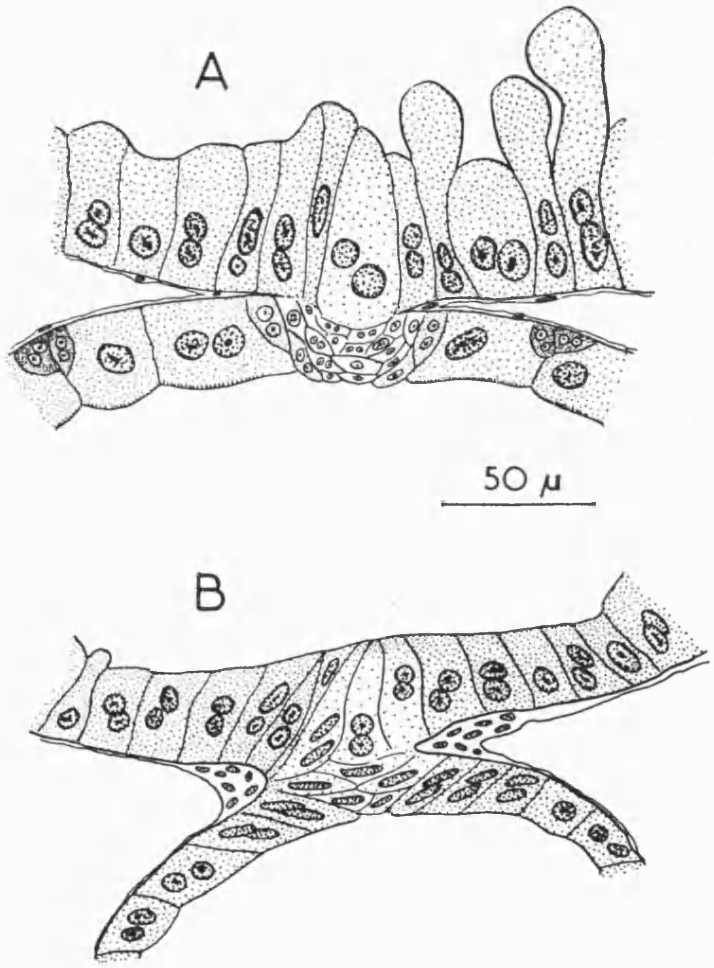


Fig. 16—Sections through intestinal discontinuity of *Scotinophara fibulata* Germ.
A. In fifth instar nymph. B. In young adult.

connection is, a flow of fluid and suspended bacteria may be seen to pass through it, when living tissues are observed microscopically. This description covers the majority of Coreidae, adult Pentatomidae, the nymphs of many species of Pentatominae, and also the Lygaeidae and Pyrrhocoridae. Where there is discontinuity between *M*3 and *M*4, however, the cells forming the central tube of *M*4 are thin and flattened, and the openings of the caeca are not constricted into a tube, but are as wide as the caecum itself (Fig. 17) in the longitudinal axis of the intestine. The opening is equally wide in the transverse direction, but owing to the disc-like shape of caeca in Pentatomidae, it is much

less than the maximum extent of the caecum in this plane. In those species where the adult retains the intestinal discontinuity, there is little difference between the cells of the caeca, and those of the central tube. In *Piezosternum* and *Acanthocoris*, the external distinction between tube and caeca remains, but, as has been described, in *Aspongopus* and Plataspidae this tends to disappear. In the Pentatomine nymphs, the central tube cells are not as flattened as those of the caeca, and small clusters of rounded cells can be seen between adjacent caeca. The change from this condition to that typical of the adult occurs rapidly, before continuity of the intestine is established. Since mitotic activity has not been observed, it must be assumed that this takes place by means of a change in the character of existing cells. A lumen appears in the *M*4 to ileum junction at the same time.

The change in the nature of the caecal opening is remarkable for its rapidity and completeness. As an illustration of this, it may be noted that the relevant figures (Figs. 17A, 17B) are from the same individuals as those of the *M*3–*M*4 junction (Figs. 16A, 16B). This change is clearly connected with the discharge of the contents of *M*3, as a means of protecting the caeca from invasion by this material. It is interesting to note that in *Halyomorpha annulicornis* nymphs, where there is a capillary lumen between *M*3 and *M*4, the caeca are widely open, and *M*4 joins the ileum by way of another capillary connection.

RESTRICTION OF THE INTESTINE IN COREIDAE

Since it is evident that in Pentatomidae, the cases of intestinal discontinuity in the adult are merely the result of prolongation into the adult stage of a structural pattern not uncommon in the nymphs of other species, it is of interest to discover whether *Acanthocoris* is the representative of a similar tendency in Coreidae. There are a number of genera of large Coreidae of purely sap sucking habit (*Anoplocnemis*, *Mygdonia*, etc. of the subfamily Mictinae) to be found in the tropics, of which the intestines are essentially the same as in *Acanthocoris*, with a large *M*4 bulb (equal to, or slightly larger than *M*3). In the adults of these genera, the *M*3 to *M*4 connection, and that between *M*4 and the ileum, are of the capillary type, with lumen diameter of 15 to 20 μ, containing structureless eosinophil material. The caecal openings, however, are of typical restricted type. The contents of the *M*4 bulb present the hyaline appearance, with stainable bacteria passing into the posterior end from the caecal region and grading into non-staining material, as seen in *Acanthocoris* and the pentatomid intestines. In the nymphs of these Coreidae the structure has been found to be exactly the same as that of the adult (Fig. 18). In most of the common Coreidae of East Africa, which inhabit various species of small herbs, and suck inflorescences and developing fruits as well as sap, no *M*4 bulb is developed, and the intestine is continuous. These are rather smaller insects, of the genera *Cletus*, *Hydara*, *Dulichius*, *Stenocephala*, etc. In the caecum-free tubular segment of intestine which joins the caeca-bearing region to the usually well developed *M*3 sac, it is frequently possible to detect masses of hyaline material of the sort associated with the *M*4 bulb in discontinuous intestines.

DISCUSSION

When attention was first drawn to discontinuity in the intestine of Heteroptera (Schneider, 1940), a comparison was made with the known cases in larval stages of certain Endopterygota. The common factor was thought to

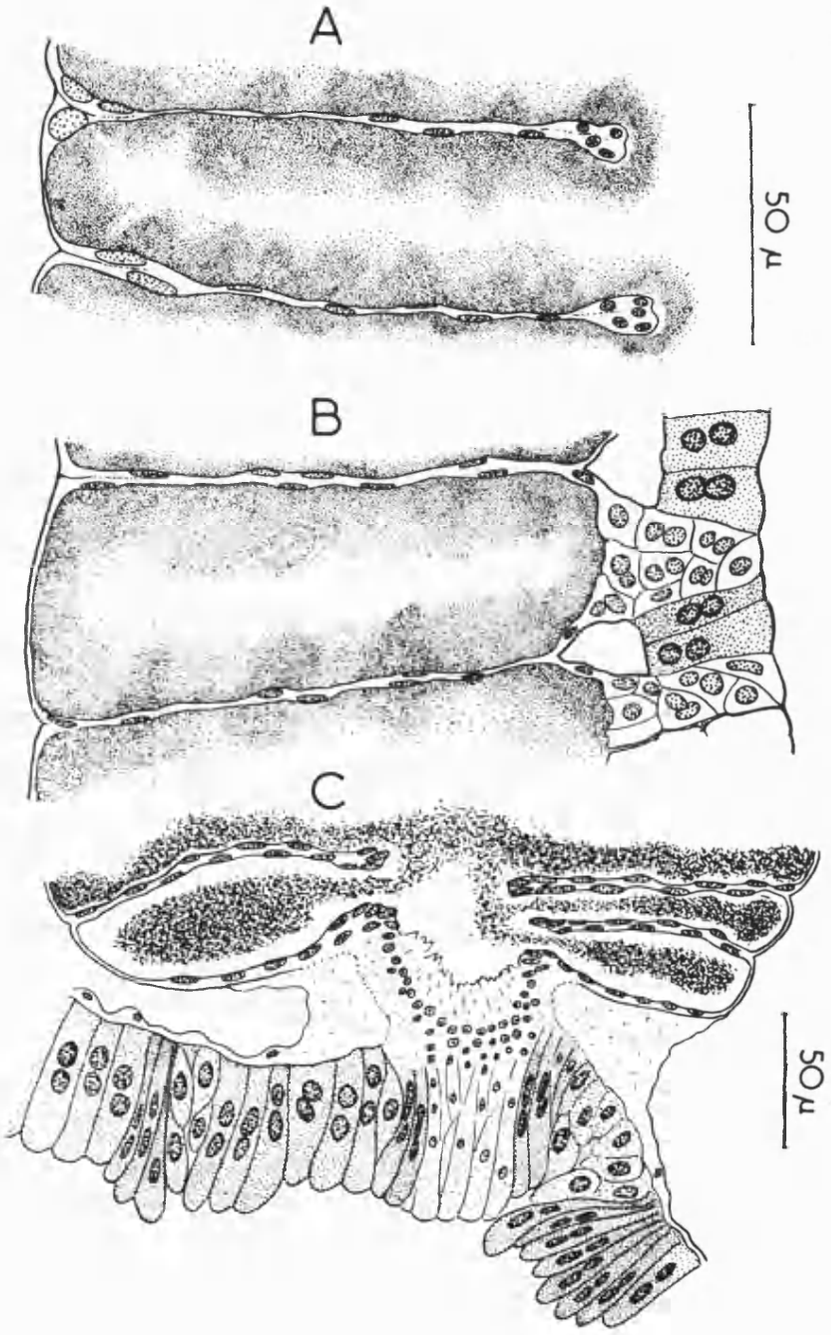


Fig. 17—A. Longitudinal section of gastric caecum of *Scotonophara fibulata* (fifth instar nymph). B. Ditto of *S. fibulata* adult, showing wall of central tube at right. C. Longitudinal section of mid-intestine to ileum connection of *Piezosternum calidum*, showing gastric caeca above and ileum cells below.

be the ingestion of a nutritious fluid diet, with little solid residue. There are two possible criticisms of this view. One is the obvious value to these endopterygote larvae of retention of faeces, to avoid soiling the larval habitat, whether

it be the confined cell of the honey bee larva, the uterus of viviparous Diptera, or the delicately balanced sand pit of the ant lion. This aspect is completely lacking in any of the Hemiptera under consideration. The other problem is that it is not absolutely proved that nutritious fluid diets do not leave any residue. In this respect it is significant that the Reduviidae, which feed in a manner comparable to the ant lion, do not have closed intestines, and pass faeces in a normal manner. It is proposed to examine, first of all, this question of faecal accumulation, in the plant sucking Hemiptera in which discontinuity occurs.

The food which is ingested by Hemiptera is not entirely fluid, but will contain solid particles of microscopic size, such as starch grains, protein granules, and so on, which can pass along the stylet channels. These may

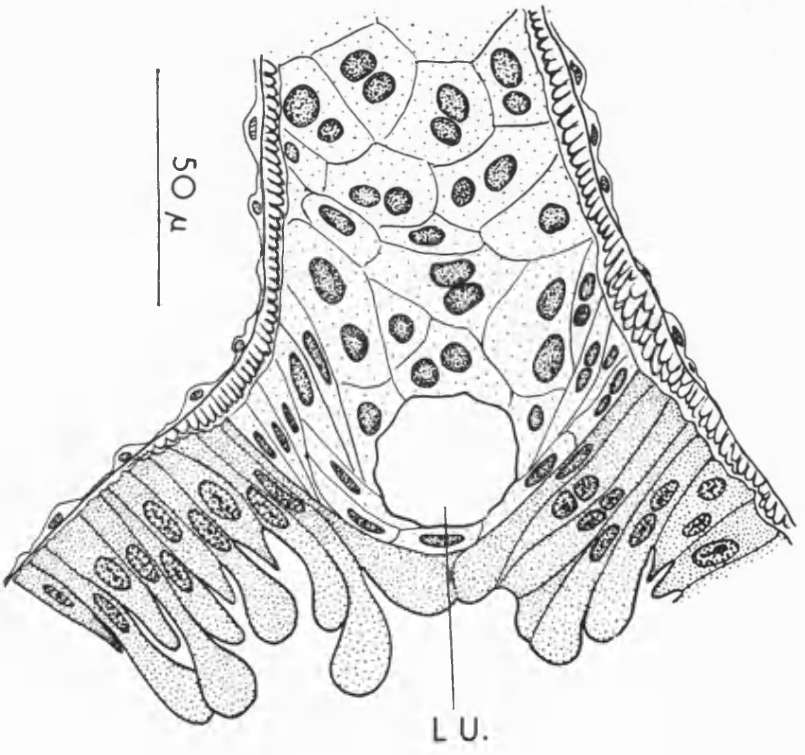


Fig. 18—Section (oblique longitudinal) of junction of capillary connection to fourth mid-intestinal region (anterior bulb) of *Mygdonia tuberculosa* Sign. (fourth instar nymph).
Lu.—lumen of capillary.

remain undigested if there is adequate, or more suitable, nutritive material in solution. In fact, if the solid particles represent an excessive amount of one dietary factor, such as starch, to digest them might upset the balance of the diet. For example, Saxena (1958) showed that *Dysdercus koenigii* Fab. does not digest starch. Another confirmation of this view is found in the very complete utilisation of the ingesta in bryocorine Miridae (Goodchild, 1952), which feed on plant material rich in carbohydrates, but by destroying whole cells with their toxic saliva supply a balanced amount of protein. As a further source of solid residues, it is likely that sparingly soluble materials of high molecular weight will be precipitated out in the first region of the intestine, as water is lost into the haemolymph. Some of the soluble constituents of the

diet may be converted into substances of higher molecular weight by the action of intestinal enzymes. Saxena & Bhatnagar (1961) have found that in the gut of *Oxycarenus hyalinipennis* (Costa), invertase action not only breaks down disaccharides, but also assembles some of the glucose and sucrose into a trisaccharide, when glucose is in excess. Some such balance of breakdown and synthesis must take place in the intestines of most Hemiptera, not only to regulate dietary balance, but also to avoid the disruptive effect of a sudden increase in the osmotic pressure of the gut contents. As there is no peritrophic membrane in Hemiptera, the wall of the intestine has little resistance to the passage of water under the influence of osmotic pressure differences.

There are, therefore, sound reasons for expecting solid residues to occur in the intestine of Hemiptera. The presence of such residues is very evident in the contents of the bulbous *M*3 region of the Pentatomomorpha. The present writer is not aware of any chemical analysis of the pasty contents of this region having been published (the observation of Pick (1956), does not refer to this). The material is undoubtedly heterogeneous, containing granules of different characteristic shapes and staining reactions. It is difficult to consider it as other than a faecal accumulation, as it does not diminish with starvation. There is no region in the continuous intestines of zoophagous Heteroptera equivalent to this *M*3 sac, and the abundant solid residues found in the intestines of such insects as Reduviidae, Gerridae, Nepidae, and related families, pass through without accumulating for long periods. Turning to the other groups of phytophagous Hemiptera, the various families of Homoptera, it is again found that no special region is devoted to retention of faecal matter, although the diet of some smaller forms such as Jassidae may include whole cell contents, and semi-solid masses may be found in the stomach-like anterior expansion of the mid-intestine (Willis, 1949) or among the excreta (Storey & Nichols, 1937). None of the larger Homoptera with truly sap sucking diet have discontinuous intestines, but it is in larger Heteroptera with this kind of diet i.e. showing similar feeding behaviour in their attachment to one spot for very long periods with no serious damage to the plant, that this feature occurs. It is conceivable that a sap sucking insect might tap a source of nourishment giving a low proportion of solid residue. In *Piezosternum*, for instance, the absence of *M*3 suggests that this is the case. The other species described, however, have abundant pasty deposits in their *M*3 regions, and it is certain in the mictine Coreidae, which are relatively common and easily observed, that no other food but plant sap is taken. At present, there is insufficient knowledge of two important aspects of the feeding of Heteroptera of this kind, namely the exact chemical nature of the pasty material, and the part of the plant vascular system from which food is obtained.

It is clear that in the larger Hemiptera, discontinuity of the intestine is not correlated with phytophagous (sap sucking) habit, but is restricted to Heteroptera of that habit. It may be suggested that this is a consequence of the gastric caecal system found only in the latter. It has been suggested in Part I of this paper that the gastric caeca may have developed originally as a water excreting system, acting as the heteropteran equivalent of the homopteran filter chamber. On this hypothesis, the contents of *M*4 would be

hypotonic to the haemolymph, and also to the contents of the more anterior parts of the intestine, so that a restriction between *M*3 and *M*4 would be necessary to prevent a wasteful osmotic flow from the latter to the former, and to direct the flow backwards into the rectum. This is in accord with the finding in all phytophagous Heteroptera of such restrictions, though in most they are not permanent discontinuities. The conflict between the need to isolate the gastric caecal system, and the need to discharge faecal residues, is resolved by limiting the latter function to long intervals, and in many species to the adult stage only, when space in the body cavity is needed for maturation of the gonads.

In order to understand why this state of affairs has been carried by a few species to the extent of permanent discontinuity, it is necessary to consider another function of gastric caeca, namely the harbourage of bacteria. In the water excreting hypothesis, the presence of bacteria is regarded as originally due to accidental colonisation by organisms of common soil inhabiting types, but the ability of such a flora to absorb useful substances passing out with the water flow would be of sufficient advantage to the insect to bring about evolutionary specialisation of both the bacteria, and the mechanism of transmission of these to the offspring. These specialisations are greatest in Pentatomidae. In order that the absorbed materials shall be returned to the use of the insect, some harvesting process must occur. It has already been mentioned that the curious hyaline material which almost certainly represents digested bacteria can be detected in many relatively simple coreid intestines, and in sap sucking Pentatomidae, the *M*4 bulb of the nymphs contains such material. In Coreidae, the bacteria in the caeca of adults are usually in a state of degeneration, and will not grow in culture, whereas those from nymphs grow fairly freely. Possibly the evolutionary beginnings of the harvesting process are to be found in a natural process of disintegration of old, overcrowded, bacterial cultures, from which the insect may be able to reabsorb useful substances. In the large sap sucking Heteroptera with discontinuous intestines, the mechanism of harvesting bacteria by digestion in the *M*4 bulb, with its secretory type of epithelium, is very highly developed, and the gastric caecal system is significantly more extensive than in simple, continuous intestines. The case of *Brachypelta aterrima*, in which the *M*4 bulb is completely lacking, is of interest in exhibiting a different approach to the harvesting of the bacteria. The discontinuity, according to Schorr (1957), develops only from the fifth instar onwards, and although the caeca shrink during oogenesis, they become swollen after the hatching of the eggs, and the young are infected with the symbiotic bacteria during a brooding period. It is highly likely that the young are not merely being infected, but are receiving a rich source of nourishment for their early life.

From the point of view of interpreting the gastric caeca as water excreting organs, the presence of discontinuity between *M*4 and the ileum, in these sap sucking species, poses a problem. Since there is evidence, from histology and from anatomical relationships, that the ileum cells can perform a water excreting function, it is clear that the large extent of *M*4 in these insects is not developed to deal with a high rate of water transfer. It must, therefore, be concerned

with a more intense exploitation of the bacterial flora. This in its turn seems to imply that the bacteria are utilising some component in the ingesta which the insect alone could not use. If this is so, then the insects might be able to manage with a lower rate of sap intake than less specialised forms (including Homoptera of similar habit). The Malpighian tubules and ileum cells could then suffice to maintain water balance, and furthermore, the rate of accumulation of solid residues in *M*3 would be lower. As a result, the complete isolation of the caecal system would become physiologically possible.

As to the identity of the component utilised by the bacteria, breakdown of material such as cellulose (the substrate of symbiotic organisms in many other types of insects) can be ruled out, since it must diffuse through the caecal wall from the haemolymph. The remaining possibilities are inorganic nitrogen (e.g. nitrate) or gaseous nitrogen. Nitrogenous substances absorbed by plant roots are generally thought to pass upwards in the xylem vessels, and Hemiptera feed in the phloem, but it has been shown (Stout & Hoagland, 1939) that upward moving salts diffuse through the plant tissues and can be detected in the phloem. Since the direction of flow in the phloem is opposite to that in the xylem, the nitrogen content of the former tissue will be highest at the base of the stem. It may be significant that the nymphal stages of many sap sucking Heteroptera have a cryptic, soil surface habitat. Indeed, it may be possible to connect the host plant preferences of these insects with the nature of the nitrogen circulating in the plant. Nightingale (1937) showed that in some plant species (e.g. apple, asparagus, narcissus) this is wholly amino acids, while in others (tobacco, tomato, cucurbits) it is in the form of nitrate. In the case of large Heteroptera, little is known about the exact site in the plant upon which feeding takes place. A comparison of dimensions of the stylet bundle of *Mygdonia tuberculosa* Sign. (4th. instar nymph) with the vessels of the petiole of *Markhamia platycalyx* Sprague, upon which it feeds, shows that the disruption of tissues must be such as to permit access to both xylem and phloem. The sizes are as follows—stylet bundle 30 μ, largest xylem vessels 50 μ, phloem bundle 100 μ × 120 μ, sieve tubes 10 μ.

The possibility of utilization of gaseous nitrogen by Hemiptera (through their symbiotic micro-organisms) has been most thoroughly discussed with regard to the Aphidae (Peklo, 1912 ; Smith, 1948 ; Mittler, 1958). The first named author suggested the possibility of nitrogen fixation on the grounds of morphological resemblence of the symbionts to the bacteria found in root nodules of Leguminosae, but Smith was unable to detect uptake of atmospheric nitrogen by *Myzus persicae* (Sulzer) or *Aphis fabae* Scop., either ground up and cultured in nutrient solution, or (using N–15 as an indicator) in the living state. Mittler showed that the rate of nitrogen accumulation in *Tuberolachnus salignus* (Gmelin) can be fully accounted for by that present in the sap ingested. There is also the theoretical difficulty that the bacteria known to fix gaseous nitrogen, do so best in an environment lacking that element in combined form, whereas the aphid symbionts are enclosed within the highly nitrogenous cell protoplasm. As far as the Heteroptera are concerned, while the caecal organisms can in some cases be grown in culture, few tests for nitrogen fixation have been carried out. Huber-Schneider (1957) cultured the organisms from a rather

unspecialised coreid, *Mesocerus marginatus* L., and found them unable to fix nitrogen *in vitro*. No studies of this sort have been performed on the organisms from a discontinuous intestine, although it is perhaps significant that Müller (1956) showed that bacteria-free nymphs of *Coptosoma scutellatum* Geoffr. (Plataspidae) would survive only if fed on nitrogen rich young plant buds. A considerable amount of work has been done on soil inhabiting and plant symbiotic nitrogen fixing organisms, in recent years. Some of this research supplies favourable, though only circumstantial, evidence for the occurence of this process in the caeca of Heteroptera. The only caecal organisms which have been identified and named were placed (Steinhaus *et al.*, 1956) in the genus *Pseudomonas*, and it is in free living members of this genus that Proctor & Wilson (1958) have recorded nitrogen fixation. It is commonly found that the caecal region of the more advanced sap suckers is coloured pink, orange, or in some species (e.g. *Libyaspis* (Plataspidae), *Halyomorpha*, and terminal part of *M*4, near ileum, in many genera) bright red. This is paralleled by the work of Neweth (1959) and Neweth *et al.* (1961) on production of red pigments of porphyrin or carotinoid type in organisms symbiotic with certain Leguminosae. These pigments were produced in proportion to the amount of nitrogen fixed, and fixation only occurred in the presence of a source of nitrogen in the culture medium. In *Anasa tristis* (De Geer) (Coreidae), Metcalf (1945) found that the red colour of epidermis, salivary glands, and testis sheath was a phaeophorbide derived from chlorophyll, but the green of the fat body was a bilin, presumed to be from the same source. In the large *M*4 bulb of Mictine Coreidae, the contents are distinctly green (those of *M*3 being brownish-green), and since the caeca are pale pink, it is tempting to suppose that the latter is a haemoglobin-like pigment, and the green is a bilin formed in the digestive process of the *M*4 bulb. The pigment granules found in the caeca have been known for a long time (*vide* e.g. Kuskop, 1923) but have previously been regarded as connected with the nourishment of the symbionts. These considerations suggest that further work on the function of gastric caeca bacteria would be worth while.

The arguments detailed above are obviously not valid where those species lacking gastric caeca are concerned. Discontinuity of the intestine is, as far as is known at present, less common in such insects, especially if the numerous cases of virtual discontinuity in Coreidae and pentatomine nymphs are taken into account. Those at present known are the lygaeid, *Ischnodemus sabuleti*, the Aradidae studied by Carayon (1955), and the *Leptocoris* species mentioned in this paper. Of these, a possible explanation of the situation in *Leptocoris* may be put forward. It was found that the *M*3 contents of these insects were largely oily. Since the lining of the mid-intestine is wettable by water, it must presumably resist wetting by oils. The oil therefore would not affect it. The lining of the rectum, however, is water repellent (though the glandular areas in many Heteroptera must absorb water through it), and might permit passage of oily materials, with possibly detrimental results. Miles (1958a) found oil droplets in the *M*3 of *Oncopeltus fasciatus* nymphs, which defaecated only after the final moult. There is no information about the nature of the intestinal contents of *I. sabuleti*, and in the Aradidae the

presence of oil droplets is not mentioned. The seed sucking species of Heteroptera often attack seeds which are rich in oils, and must accumulate large quantities of this material, which may be indigestible, or may not be utilized for the reasons discussed above.

It has been suggested (Miles, *op. cit.*) that the retention of the contents of *M*3 for long periods might enable bacteria in that region to act upon the contents more effectively. Against this, it may be said that the nature of hemipteran feeding precludes the ingestion of such indigestible substances as celluloses, lignins, etc., and what is likely to be present in *M*3 could be dealt with by the enzymes usually found in the insect intestine. If the function of bacteria in *M*3 is to synthesize accessory nutritional factors, the evidence from *Rhodnius prolixus* (Brecher & Wigglesworth, 1944) shows that such organisms can maintain themselves satisfactorily in a continuous intestine.

No mention has been made in this paper of the discontinuous intestine of Diaspididae (" scale insects "). It appears that small Homoptera such as aphids, coccids, etc., may not suck sap actively, but may have it forced through their alimentary canal by the turgor pressure in the plant (Mittler, 1958), and it has been suggested that discontinuity of the intestine has been developed to resist this flow. It is obviously advantageous to these gregarious insects to restrict the flow, and so reduce the contamination of the colony with honeydew. There is no evidence that the larger Heteroptera are faced with this problem. Even supposing that they passively accept a flow of sap, it does not seem that they are unable to utilise all they receive, and they are active insects which eject their excreta well clear of their immediate surroundings.

SUMMARY

(Parts I and II)

1. A study of the intestinal structure of fifty-seven species of Heteroptera is reported in the first part of this paper. The object of this study was to discover if any correlations between structure and food habit existed, with particular reference to the problem of water balance.

2. It was found that the degree of development of the rectal gland was greatest in those species in which a need to conserve water could be inferred, but that a glandular region of the hind-gut was also very strongly developed in the water bugs (Hydrocorisae). In the latter, a solute absorbing function has been suggested.

3. In the terrestrial species with a water surplus problem (the plant sap suckers), a case has been made out for the interpretation of the gastric caeca as water excreting organs, performing a function equivalent to that of the filter chamber of Homoptera.

4. The development of a distinct ileum region at the point of junction of the Malphighian tubules with the intestine has been found to be associated with a phytophagous line of evolution (the Pentatomomorpha), and to undergo progressive modification in the different families of that group.

5. Histological details of this part of the alimentary canal support the accepted taxonomic divisions of Heteroptera, and show that superficial similarities are inadequate as a basis for classifying types of intestine.

6. In the second part of this paper, an account is given of four hitherto undescribed instances of discontinuity of the intestine in adult Heteroptera. The examples are in separate subfamilies of three families. Intestinal discontinuity is also shown to occur, in the nymphal stages only, in a number of genera of Pentatominae, and extreme constriction, amounting to virtual discontinuity, in nymphs of certain other species of Pentatominae and nymphs and adults of several genera of Coreidae.

7. Together with previously described cases in the families Plataspidae, Cydnidae, Aradidae, and Lygaeidae, these records show that the phenomenon is widespread in pentatomorph Heteroptera, and not a peculiarity of isolated species. It is not found in the essentially zoophagous Heteroptera (Cimicomorpha, Amphibicorisae, and Hydrocorisae), nor in the Homoptera except for a few species of Sternorrhyncha.

8. Intestinal discontinuity is, in the majority of cases, associated with a sap sucking diet, but this is not because such a diet is lacking in insoluble residues. The disadvantage of retaining such residues is balanced against the advantage of isolating the highly developed gastric caecal system, with its abundant symbiotic micro-organisms.

9. In species with interrupted intestines, the region posterior to the discontinuity consists of the gastric caecal region together with a bulbous expansion at the anterior end of this region. In this bulb, there is histological evidence for the breakdown of the caecal bacteria by a digestive process.

10. In addition to the interruption of the intestine anterior to the gastric caecal region, the most advanced species have a second discontinuity between the posterior end of the caecal region and the ileum. The significance of this in relation to the possible primitive function of the caeca of water excretion is discussed. It is suggested that the harvesting of the bacteria as a food source increases the efficiency of sap utilization, so that the rate of intake can be decreased to a level where the ileum cells can cope with osmotic regulation.

11. The evidence for utilization by the bacteria of materials not directly available to the insect, such as inorganic or gaseous nitrogen, is discussed.

12. The interpretation of the cases where discontinuity occurs in species not having gastric caeca is discussed. This might be due to retention of this characteristic from caeca-bearing ancestors, but in certain cases it is suggested that it is a means of immobilising food residues of an oily nature.

REFERENCES

BAPTIST, B. A. (1941). The morphology and physiology of the salivary glands of Hemiptera-Heteroptera. *Quart. J. micr. Sci.* **83** : 91–139.

BONNEMAISON, L. (1946). Remarques sur la symbiose chez les Pentatomidae (Hem.). *Bull. Soc. ent. Fr.* **51** : 40–42.

BRECHER, G. & WIGGLESWORTH, V. B. (1944). The transmission of *Actinomyces rhodnii* Erikson in *Rhodnius prolixus* Stål (Hemiptera) and its influence on the growth of the host. *Parasitology* **35** : 220–224.

BUGNION, E. & POPOFF, N. (1908). L'appareil salivaire des Hémiptères. *Arch. Anat. micr.* **10** : 227–268.

BUGNION, E. & POPOFF, N. (1910). L'appareil salivaire des Hémiptères II. *Arch. Anat. micr.* **11** : 435–456.

CARAYON, J. (1944). Remarques sur la circulation de l'eau dans l'organisme des Hémiptères. Le dessèchement des organes abdominaux chex certains Hémiptères hibernant. *Bull. Soc. ent. Fr.* **49** : 86–88.

CARAYON, J. (1955). Quelques caracteres anatomiques des Hémiptères Aradides. *Rev. franç. Ent.* **22** : 169–180.

CRAGG, F. W. (1915). The alimentary tract of *Cimex*. *Indian J. med. Res.* **2** : 706–720.

DENISOVA, Z. M. (1943). On the comparative ecology of the blood-sucking Diptera. (In Russian.) *Zool. Zh.* **22** : 214–221.

ELSON, J. A. (1937). A comparative study of Hemiptera. *Ann. ent. Soc. Amer.* **30** : 579–597.

GOODCHILD, A. J. P. (1952). A study of the digestive system of the West African cacao capsid bugs (Hemiptera, Miridae). *Proc. zool. Soc. Lond.* **122** : 543–572.

GOODCHILD, A. J. P. (1955). The bacteria associated with *Triatoma infestans* and some other species of Reduviidae. *Parasitology* **45** : 441–448.

GLASGOW, H. (1914). The gastric caeca and caecal bacteria of Heteroptera. *Biol. Bull. Woods Hole* **26** : 101–170.

HAMILTON, M. A. (1931). The morphology of the water scorpion, *Nepa cinerea* L. (Rhynchota, Heteroptera). *Proc. zool. Soc. Lond.* **101** : 1067–1136.

HENSON, H. (1932). The development of the alimentary canal in *Pieris brassicae* and the endodermal origin of the malpighian tubules of insects. *Quart. J. micr. Sci.* **75** : 283–305.

HUBER-SCHNEIDER, L. (1957). Morphologische und physiologische Untersuchungen an der Wanze *Mesocerus marginatus* L. und ihren Symbionten. *Z. Morph. Ökol. Tiere* **46** : 433–480.

IMMS, A. D. (1957). *A general textbook of entomology*, 9th edition. London : Methuen.

KERSHAW, J. C. W. (1913). Anatomical notes on a membracid. *Ann. Soc. ent. Belg.* **57** : 191–201.

KUSKOP, M. (1923). Bakteriensymbiosen bei Wanzen. *Arch. Protistenk.* **47** : 350–385.

LESTON, D. (1957). The stridulatory mechanisms in terrestrial species of Hempitera Heteroptera. *Proc. zool. Soc. Lond.* **128** : 369–386.

LESTON, D., PENDERGRAST, J. G. & SOUTHWOOD, T. R. E. (1954). Classification of the terrestrial Heteroptera (Geocorisae). *Nature, Lond.* **174** : 91–92.

LICENT, P. E. (1912). Recherches à l'anatomie et de physiologie sur la tube digestif des Homoptères supérieurs. *Cellule* **28** : 1–161.

MARKS, E. P. (1951). Comparative studies of the male genitalia of the Hemiptera (Homoptera–Heteroptera). *J. Kansas ent. Soc.* **24** : 134–141.

METCALF, R. L. (1945). A study of the metabolism of chlorophyll in the squash bug, *Anasa tristis*. *Ann. ent. Soc. Amer.* **38** : 397–402.

MILES, P. W. (1958). Contact chemoreception in some Heteroptera, including chemoreception internal to the stylet food canal. *J. Insect Physiol.* **2** : 338–347.

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

MILLER, N. C. E. (1956). *The biology of the Heteroptera*. London : Leonard Hill.

MITTLER, T. E. (1958). Studies on the feeding and nutrition of *Tuberolachnus salignus* (Gmelin) (Homoptera, Aphidae). III. The nitrogen economy. *J. exp. Biol.* **35** : 626–638.

MÜLLER, H. J. (1956). Experimentelle Studien an der Symbiose von *Coptosoma scutellatum* Geoffr. (Hem., Heteropt.). *Z. Morph. Ökol. Tiere* **44** : 459–482.

NEWETH, GY (1959). A new nitrogen-fixing micro-organism producing a red pigment. *Nature, Lond.* **183** : 1460.

NEWETH, GY, URESCH, F., FODOR, G. & LANG, L. (1961). Chemical character of the pigments in a new nitrogen-fixing micro-organism. *Nature, Lond.* **191** : 1413–1414.

NIGHTINGALE, G. T. (1937). The nitrogen nutrition of green plants. *Bot. Rev.* **3** : 85–174.

NUORTEVA, P. (1956). Studies on the comparative anatomy of the salivary glands in four families of Heteroptera. *Ann. ent. fenn.* **22** : 45–54.

PAINTER, R. H. (1930). A study of the cotton flea hopper, *Psallus seriatus* Reut., with especial reference to its effect on cotton plant tissues. *J. agric. Res.* **40** : 485–515.

PEKLO, J. (1912). Über symbiontischen Bakterien der Aphiden. *Ber. dtsch. bot. Ges.* **30** : 416–419.

PESSON, P. (1944). Contribution à l'étude morphologique et fonctionelle de la tête, de l'appareil buccale et du tube digestif des femelles de Coccides. *Monogr. Sta. Lab. Rech. agron., Paris* **1944** : 1–266.

PFEIFFER, M. (1937). Die Verteilung der osmotischen Werte im Baum im Hinblick auf die Muench-sche Druckstromtheorie. *Flora, Jena* N.F. **32** : 1–47.

PICK, F. (1956). La cristallisation des proteins vegetale dans le tube digestif de *Corecoris fuscus* (Thun. 1783). *C.R. Acad. Sci., Paris* **244** : 1673–1675.

POISSON, R. (1924). Contributions à l'étude des Hémiptères aquatiques. *Bull. Biol. Fr. Belg.* **58** : 49–305.

POISSON, R. (1951). Ordre des Hétéroptères. *In* Grasse's *Traité de Zoologie* **10** (2) : 1657–1803.

PROCTOR, M. H. & WILSON, P. W. (1958). Nitrogen fixing by gram-negative bacteria. *Nature, Lond.* **182** : 891.

PRUTHI, H. SINGH- (1925). The morphology of the male genitalia in Rhynchota. *Trans. ent. Soc. Lond.* **73** : 127–267.

RAMSAY, J. A. (1950). Osmotic regulation in mosquito larvae. *J. exp. Biol.* **27** : 145–157.

RAMSAY, J. A. (1952). The excretion of sodium and potassium by the malphigian tubules of *Rhodnius*. *J. exp. Biol.* **29** : 110–126.

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

RAMSAY, J. A. (1958). Excretion by the malpighian tubules of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae) : amino acids, sugars. and urea. *J. exp. Biol.* **35** : 871–891.

ROSENCRANZ, W. (1940). Die Symbiose der Pentatomiden (Hemiptera Heteroptera). *Z. Morph. Ökol. Tiere* **36** : 279–309.

SAXENA, K. N. (1955). The anatomy and histology of the digestive organs and malpighian tubules of the Jassidae (Homoptera). *J. zool. Soc. India* **7** : 41–52.

SAXENA, K. N. (1958). Digestion and absorption of carbohydrates in the alimentary canal of the red cotton bug, *Dysdercus koenigii* Fabr. (Heteroptera ; Pyrrhocoridae). *Physiol. Zool.* **31** : 129–138.

SAXENA, K. N. & BHATNAGAR, P. (1961). Nature and characteristics of invertase in relation to utilization of sucrose in the gut of *Oxycarenus hyalinipennis* (Costa) (Heteroptera : Lygaeidae). *J. Insect Physiol.* **7** : 109–126.

SCHNEIDER, G. (1940). Beitrage zur Kenntnis der symbiontischen Einrichtungen der Heteropteren. *Z. Morph. Ökol. Tiere* **36** : 595–644.

SCHORR, H. (1957). Zur Verhaltensbiologie und Symbiose von *Brachypelta aterrima* Forst. (Cydnidae, Heteroptera). *Z. Morph. Ökol. Tiere* **45** : 561–602.

SCUDDER, G. G. E. (1959). The female genitalia of the Heteroptera : morphology and bearing on classification. *Trans. R. ent. Soc. Lond.* **111** : 405–467.

SMITH, J. D. (1948). Symbiotic micro-organisms of aphids and fixation of atmospheric nitrogen. *Nature, Lond.* **162** : 930–931.

SNODGRASS, R. E. (1935). *Principles of insect morphology*. New York : McGraw-Hill.

SOUTHWOOD, T. R. E. (1955). The morphology of the salivary glands of terrestrial Heteroptera (Geocorisae) and its bearing on classification. *Tijdschr. Ent.* **98** : 77–84.

SRIVASTAVA, U. S. & BAHADUR, I. (1961). The development of the malpighian tubules in *Dysdercus koenigii* (Hemiptera, Pyrrhocoridae). *Quart. J. micr. Sci.* **102** : 347–360.

STEINHAUS, E. A., BATEY, M. M. & BOERKE, C. L. (1956). Bacterial symbiotes from the caeca of certain Heteroptera. *Hilgardia* **24** : 495–518.

STOREY, H. H. & NICHOLS, R. F. W. (1937). Defaecation by a jassid species. *Proc. R. ent. Soc. Lond.* (A) **12** : 149–150.

STOUT, P. R. & HOAGLAND, D. R. (1939). Upward and lateral movement of salt in certain plants as indicated by radioactive isotopes of potassium, sodium, and phosphorus absorbed by the roots. *Amer. J. Bot.* **26** : 320–324.

SUTTON, M. F. (1951). On the food, feeding mechanism and alimentary canal of Corixidae (Hemiptera, Heteroptera). *Proc. zool. Soc. Lond.* **121** : 465–499.

SWEET, M. H. (1960). The seed bugs, a contribution to the feeding habits of the Lygaeidae (Hemiptera–Heteroptera). *Ann. ent. Soc. Amer.* **53** : 317–321.

WIGGLESWORTH, V. B. (1931). The physiology of excretion in a blood-sucking insect, *Rhodnius prolixus* (Hemiptera, Reduviidae). *J. exp. Biol.* **8** : 411–451.

WILLIS, D. M. (1949). The anatomy and histology of the head, gut, and associated structures of *Typhlocyba ulmi*. *Proc. zool. Soc. Lond.* **118** : 984–1001.

YANAI, T. (1952). Morphological and histological studies of the mid-intestine of heteropterans with special reference to binucleated epithelial cells. *Annot. zool. jap.* **25** : 427–433.

YANAI, T. & IGA, T. (1956). Further study on the binucleated epithelial cells in the mid-intestine of heteropterous insects. *Cytologia, Tokyo* **21** : 183–187.

APPENDIX 4

Biol. Rev. (1966), **41**, *pp.* 97–140

EVOLUTION OF THE ALIMENTARY CANAL IN THE HEMIPTERA

By A. J. P. GOODCHILD

School of Biological Sciences, University of Bath

(*Received* 5 *April* 1965)

CONTENTS

I. INTRODUCTION

The order Hemiptera, including both Homoptera and Heteroptera, must be regarded as one of the most successful groups in the class Insecta, as judged by such criteria as number of species, abundance of individuals, diversity of ecological adaptation and geographical range. In these respects there is a marked contrast between the Hemiptera and the closely related orders Psocoptera, Thysanoptera, Mallophaga, and Siphunculata, each of which occupies a relatively restricted ecological niche and contains many fewer species. As a consequence of their ecological limitations, these latter orders are very uniform in size, structure and habits, and in the Thysanoptera and Siphunculata particularly, the feeding apparatus is highly specialized in adaptation to their mode of life. On the other hand, Hemiptera are found in almost every ecological niche which supports insect life, failing to nourish themselves only on dry dead organic matter (though many species feed on dry seeds, and others on moist animal or plant remains or on excreta). Thus they do not utilize dry wood or stored products, but they do include the only insects pelagic upon the ocean surface. Their range of size, from minute to among the largest of insects, is a further indication of their biological success.

This successful adaptive radiation suggests that some highly advantageous feature of structure or physiology has been evolved by the Hemiptera. The structural feature which is common to all members of the order is the suctorial feeding apparatus. In this the mandibles and maxillae have become converted into medially grooved interlocking stylets, forming between them both food and salivary canals, the labium has become a long, dorsally grooved, proboscis which encloses and supports the delicate stylets, and the maxillary and labial sensory palps have been lost, being functionally replaced by sensillae at the tip of the proboscis and gustatory organs

within the head. It is probably the most refined piercing and sucking apparatus to be found in the whole of the phylum Arthropoda.

There is no progressive modification of the mouthparts within the order, such as occurs, for instance, in the Diptera, and the variations between different groups in length of stylets, or in length and point of articulation of the proboscis, have no bearing on the problem of the evolution of the hemipteran feeding apparatus from primitive biting mouthparts. The mouthparts of the aquatic heteropteran family Corixidae, which Sutton (1951) regards as primitive in their short and simple stylets, are more probably degenerate in consequence of a detritus-feeding habit, since all other evidence points to the Heteroptera having arisen from ancestral Homoptera, and not vice versa. The structure of the thysanopteran feeding apparatus gives useful clues as to the nature of an intermediate stage in evolution, but the uniformity of the mouthpart structure in living members of the Hemiptera shows that it was fully evolved before the radiation into the existing suborders began, some time during the Permian period.

The specialized form of the mouthparts has not prevented a wide range of feeding habits being adopted by the various families of the order. Not only are the obvious fluids diets of plant sap or animal body fluid taken, but the species which feed upon dry seeds or upon the tissues of other insects compete successfully in these habits with the insects having masticatory mouthparts, and the basic structure of the mouthparts is very little modified by such dietary adaptations. A number of considerations suggest, however, that the ancestral hemipteran was a small insect feeding upon surface cells of plants in a manner similar to that of the present-day Thysanoptera. The Homoptera, which appear earlier in the fossil record (Imms, 1937), and have more primitive wing structure than Heteroptera, are all phytophagous, and for the exploitation of such a food source by a small insect, piercing mouthparts would have an obvious advantage in avoiding the necessity for dealing with tough, indigestible cell wall material. The development of longer stylets, and their formation into a hollow sucking tube, would enable the insect to exploit deeper cell layers, thus avoiding frequent changes of feeding site, and finally the ability to tap the plant vascular system would enable the insect to feed at one point for long periods with the minimum of active movement. It is this ability to survive and grow on a diet of plant sap alone, through all stages of the life-cycle, which is unique to the Hemiptera. Although suctorial mouthparts are found in other orders of insects, and in the Arachnida, they are used to imbibe fluid food of a more concentrated kind than sap, such as blood, body fluids of other insects, or the protoplasmic contents of plant cells. In some cases sap may be ingested, but only as a supplement to reserves accumulated during the larval stages, or to the main diet of a more nutritious material. The failure of insects other than Hemiptera to exploit sap as a source of food does not lie in the mechanics of the mouthparts, since a number of adult Diptera and Lepidoptera are known to pierce plant tissues. Abdel-Malek & Baldwin (1961) were able to prove, by the use of radioactive tracers, that mosquitoes of the genus *Aedes* imbibed material from leaves in preference to taking flower nectar. In the order Lepidoptera the adult takes little nourishment, so it is not surprising to find the fruit-piercing

moths taking no clear advantage from their ability to penetrate plant tissues, but most Diptera require to feed in the adult stage and this is especially evident in the blood-sucking forms. It would not be unreasonable to expect to find sap-sucking developed in this group, but this is not the case. The success of so many species of Hemiptera, in different branches of the order, which have adopted this mode of nutrition must therefore lie in the modifications of the alimentary canal that enable them to deal with a dilute fluid food rapidly and in large quantities.

It is intended in this article to review the knowledge of alimentary canal structure in the Hemiptera and to show that the variety of patterns which are found in the major subdivisions can all be interpreted as adaptations to sap-sucking, in that they facilitate a rapid passage of sap, while preventing dilution of the blood with osmotically imbibed water. The problem is essentially one of osmoregulation, not only because the ingesta are generally of lower osmotic pressure than the blood, but because the nutrients are in the form of small molecules which are assimilated directly, leaving a net excess of water to be excreted. Where large molecules of protein and starch are broken down in the gut, as is the case in Hemiptera which feed upon plant cells (mesophyll feeders), it is possible for the insect to absorb what it needs and still pass excreta isotonic with the blood. Nuorteva (1958) has shown that sap-sucking species have no proteases or amylases in their saliva, whereas these are present in the saliva of mesophyll feeders. In insects of other orders which imbibe plant juices, dilution of the blood is prevented by storing the ingesta in the crop, the lining of which is impervious to water. Honey-bees evaporate water from nectar by exposing globules of it on the mouth parts, until it is sufficiently concentrated to be stored as honey. In the Diptera and Lepidoptera it must be assumed that the transfer of material from the crop to the mid-gut is effected slowly, so that the osmoregulatory mechanism can dispose of the surplus water. In the Hemiptera there is no chitin-lined stomodaeal crop, and the means by which blood dilution is avoided is not restriction of entry into the body but rapid and efficient transfer of surplus water to the impervious hind-gut. It has been known from an early date (Dufour, 1833) that unusual structures existed in the alimentary canals of Hemiptera. Of these, the so-called 'filter chamber' of the Homoptera Cicadoidea, with its intimate association of the anterior and posterior extremities of the mid-gut, has from the time of its discovery been interpreted as a means of transferring surplus water directly from the anterior end of the mid-gut into the hind-gut, since no other function could be conceived for this complex structure. Similarly, filter systems of apparently self-evident function have been found in many Sternorrhyncha (Berlese, 1893). In the sap-sucking Heteroptera it is only recently that an anatomical arrangement evidently having this function has been described in the pentatomid subfamily Phyllocephalinae (Miyamoto, 1961; Goodchild, 1963*a*). In those sap-sucking Hemiptera, in which a conspicuous filter mechanism is lacking, it is reasonable to assume that other structures must exist which perform an analogous task. Hitherto it has been assumed that the Malpighian tubules were the main site of osmoregulatory activities. The work of Ramsay (1950, 1958) has shown, however, that the tubules pass a fluid more or less isotonic with the blood, and that it is the glandular areas

in the hind-gut which are responsible for the control of water balance, either by absorption of water in terrestrial and salt-water insects, or by absorption of solutes in freshwater forms. Sap-sucking Hemiptera resemble freshwater insects in their need to excrete a hypotonic urine, and this transfer of attention to the hind-gut has been most fruitful in understanding their structure. It has made possible a satisfactory explanation of the workings of the Cicadoid filter-chamber, on the basis of solute absorption in the long tubular hind-gut. The development of a similar hind-gut in the aquatic Heteroptera may be explained in the same way. On the other hand, examination of the Fulgoroidea and terrestrial Heteroptera has revealed a hind-gut structure much less competent to effect osmotic control, and thus directed attention to other structures which had not previously been considered in this light.

II. LITERATURE

Apart from numerous studies of individual species, which will be referred to at the appropriate places in the text, certain papers of a comparative or review nature may be mentioned. The early work of Dufour (1833) dealt with the alimentary anatomy of both Heteroptera (aquatic and terrestrial) and the larger Homoptera (Auchenorrhyncha). Since that date, studies have been confined to more restricted groups, Licent (1912) dealing with the Homoptera Auchenorrhyncha, Glasgow (1914) and Kuskop (1923) with the Heteroptera from the particular aspect of symbiotic bacteria, Poisson (1924) with the aquatic Heteroptera and Schneider (1940) with certain families of terrestrial Heteroptera. Parsons (1959) gives an account of the mid-gut of the aquatic Heteroptera with emphasis on histological aspects and Miyamoto (1961) has examined and illustrated the gross anatomy of the digestive systems of most families of Heteroptera. Although Licent (1912) supplied a useful amount of histological detail, this aspect has been relatively neglected in earlier comparative work on Heteroptera. The studies of Yanai (1952), Yanai & Iga (1956), Bahadur (1963), and myself (Goodchild, 1963*b*) have revealed much of interest. The Homoptera Sternorrhyncha have been rather inadequately studied, probably on account of their small size. Weber (1930) summarized the knowledge up to that date, but the greatest contribution has been the series of papers on the Coccoidea by Pesson (1933, 1935, 1936, 1941). The literature on the digestive system of Aphidoidea has been reviewed by Auclair (1963).

III. COMMON FEATURES OF THE INTESTINE

The extreme anterior end of the alimentary canal, the cibarial pump, is essentially the same in all Hemiptera and need not be discussed further. Full accounts of this region in both suborders are given in Grassé (1951). The fore-gut posterior to the head forms the oesophagus, usually a narrow, rather thin-walled, tube, with a lining of small cuboid cells which secrete a chitinous intima. In some species, the oesophageal wall is thickened by longitudinal ridges of columnar cells, giving the lumen a stellate cross-section, and in many of the large phytophagous forms (Licent, 1912;

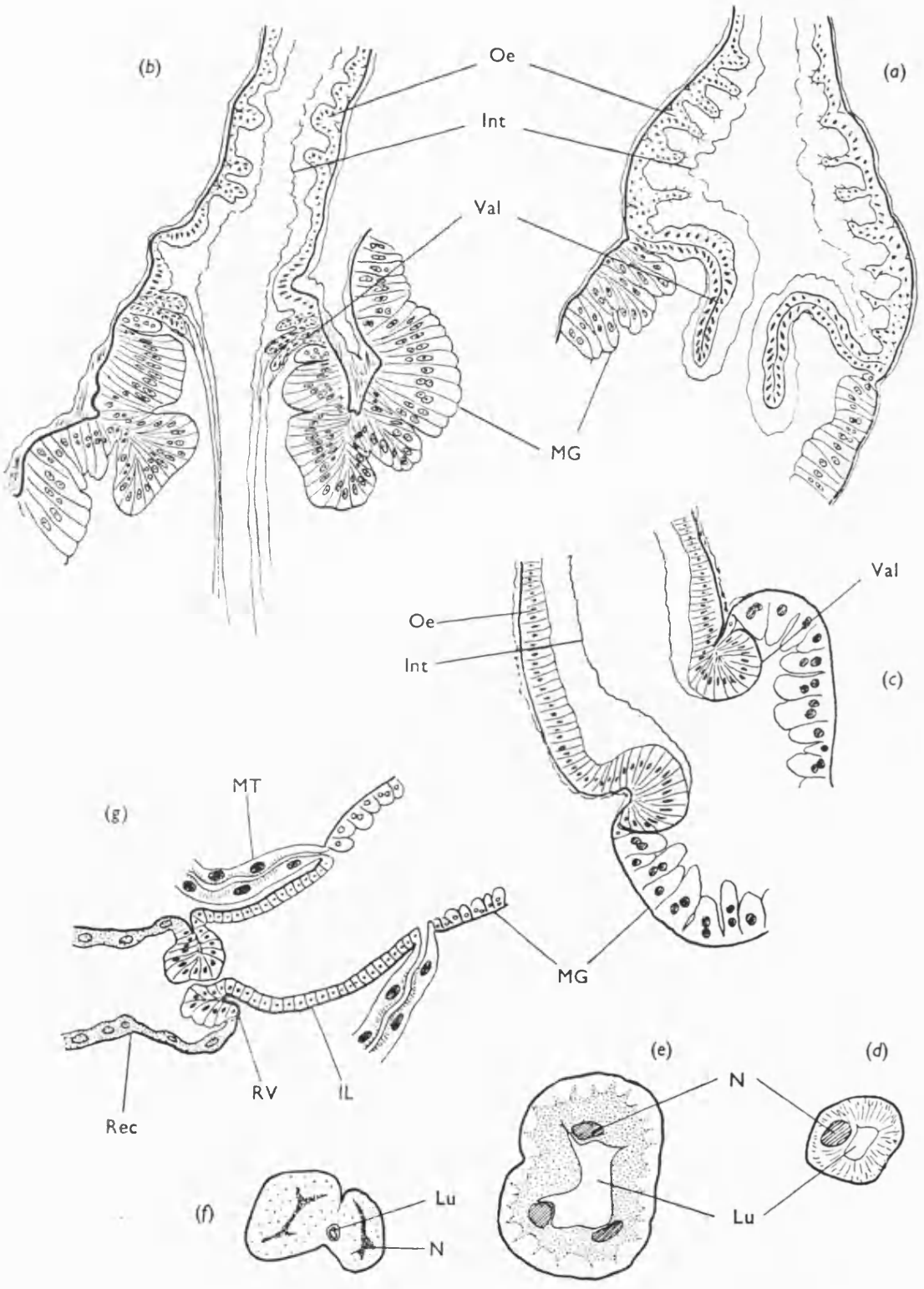


Fig. 1. *a*, Longitudinal section of oesophageal valve of *Mygdonia tuberculosa* Sign., Coreidae; *b*, the same, of *Piezosternum calidum* Fab., Pentatomidae; *c*, the same, of *Gyarina nigritarsis* Karsch, Flatidae; *d*, transverse section of Malpighian tubule of *Ptyelus flavescens* Fab., Cercopidae; *e*, the same, from glandular region in the same insect; *f*, the same, of *Pulvinaria jacksoni* Newst., Coccidae; *g*, longitudinal section of pyloric region of intestine of *Gyarina nigritarsis*. IL, ileum; Int, chitinous intima; Lu, lumen; MG, mid-gut; MT, Malpighian tubule; N, nucleus; Oe, oesophagus; Rec, rectum; RV, rectal valve; Val, oesophageal valve.

Vodjdani, 1954) the oesophagus is slightly expanded posteriorly and its lining epithelium folded into transverse ridges (Fig. 1*a*). The very small chamber thus formed is the nearest approach to a stomodaeal crop which is found in Hemiptera. In place of such a crop, these insects have, in most families, a stomach-like expansion of the mid-gut. Malouf (1933) interpreted this region in *Nezara viridula* (Linn.) as a chitin-lined crop, but Hamner (1936) showed this to be erroneous and all subsequent studies have confirmed this. In the Homoptera, certain authors refer to the anterior expansion of the mid-gut as the 'crop' (Dobroscky, 1931; Hickernell, 1920; Kershaw, 1913*b*), but it is clear that they did not intend to claim it as a stomodaeal derivative. Mukharji (1961) described the cells of this region in the jassid *Idiocerus clypealis* Leth. as possessing an intima and supported this claim with a photomicrograph. This intima was not observed by Qadri (1949) in the same species, nor was one found by Willis (1949) in another species of Typhlocybinae. The latter author gave a detailed account and illustration of the anterior mid-gut cells, showing a typical striated border. The intima photographed by Mukharji was detached from the cells and may possibly be a fixation artifact of some kind. The resemblance of the oesophageal valve to the same structure in other orders of insects in which it is preceded by a fully developed stomodaeal crop, as well as the failure of the majority of investigators to detect an intima in the anterior expansion of the gut of Hemiptera, is sufficient evidence that this region belongs to the mid-gut. It is less easy to define the beginning of the hind-gut so as to cover all the cases encountered. In many groups (e.g. Fulgoroidea, fig. 7, Amphibicorisae, fig. 9), a distinct change in epithelial type occurs at the level of the Malpighian tubule openings, while in others (e.g. Coccoidea, Pentatomomorpha) the typical mid-gut epithelium continues beyond this point to the pyloric valve. The cells of the posterior mid-gut of Miridae Bryocorinae have a border which appears to consist of a cuticle, while on the other hand the cells of the tubular hind-gut of Cicadoidea have a very poorly defined intima.

The oesophagus opens into the mid-gut by way of a valve, which consists of a hollow cone with columnar fore-gut cells on both inner and outer surfaces, projecting into the mid-gut lumen. The free end of this cone may be divided into lobes. It is covered with a chitinous intima and is well developed in most groups. In some cases, the chitinous membrane may be separated from the cells by a 'fluid secretion' (Weber, 1930, in *Aphis fabae* Scop.) or may form a long tubular fold (or *entonnoir*) containing blood (Corixidae, Sutton, 1951; Parsons, 1957*b*). In this family the valve is greatly elongated and the cellular layer thin, but in other Cryptocerata the invagination is shorter, with well-developed cell layers and an adherent intima. Parsons claims that in Belostomatidae the outer wall of the valve is mostly of mid-gut origin. In the Pentatomidae the cell layers tend to fuse and become degenerate (Harris, 1938; Bocharova-Messner, 1960; Goodchild, 1963*b*), so that in some cases only a tube of chitinous material projects into the mid-gut (Fig. 1*b*). In the Homoptera Auchenorrhyncha the valve is usually well defined but does not project far into the mid-gut, and the intima is always adherent to the cell layers (Fig. 1*c*). Mukharji (1961) describes poorly developed valves in species of Membracidae and Cercopidae. In some species the valve cells appear to resemble those of the mid-gut (e.g. *Phalix*

titan Fennah, Tettigometridae, Goodchild, 1963*a*), but usually they are distinct. Folds of mid-gut cells posterior to the valve may form additional valve-like structures (Fig. 1*b*). The oesophageal valve is poorly developed in seed-sucking Heteroptera and in Homoptera Sternorrhyncha (except Aphidoidea). The valve is not concerned with the formation of a peritrophic membrane in Hemiptera, so that it must mainly function in preventing regurgitation of food. Many Hemiptera become engorged with their food and so swell the body, and hence the distended gut wall exerts a back-pressure. These include aphids, blood-sucking bugs and many predatory and mesophyll-feeding species. Sap-sucking species, with the specialized adaptations which they possess, dispose of the watery part of the ingesta so rapidly that their mid-gut does not become distended. Thus, the poorly developed valves of Pentatomidae and many Homoptera can be easily understood. It has been suggested that the long chitinous introvert of Corixidae and Pentatomidae may help to prevent blood dilution in the region of the main locomotory muscles and nerve ganglia (Goodchild, 1963*b*). Both families ingest watery food, and of the aquatic Heteroptera, the Corixidae, with their detritus feeding habits, must take in more water with their food than their predatory relatives (Staddon, 1964).

The mid-gut is the region in which the specialized structures of different groups are developed, and these will be described below. The cells of which this region is composed typically have bulbous tips, joined to neighbouring cells only near the base, with a narrow striated border, and with two nuclei. Their secretory processes, as seen in stained sections, show a variety of forms, including extrusion of droplets, nipping off of cell tips and breakdown of complete cells. Mukharji (1961) has described these in certain Homoptera. It is likely that in many cases these appearances are not of true secretion, but of cellular degeneration. Rafiq Khan & Ford (1962) found that extrusion of droplets by the digestive epithelium of *Dysdercus fasciatus* Sign. (Pyrrhocoridae) was at a maximum during starvation, when enzyme activity was least, and that during active feeding the cells appeared as if in a resting phase. Similar results were obtained with cacao capsid bugs (Goodchild, 1952), when observation of living tissues showed that a viscid, finely granular material was secreted through the intact cell wall during the feeding cycle, and that swelling and detachment from the epithelium was associated with cell senescence at the end of such a cycle. Evidence of cyclical secretion can be found in the mid-guts of many sap-sucking Hemiptera, although digestive enzymes should not be required by these insects. The bulk of the secretion, as seen in the cacao capsids for instance, is out of proportion to its strength of enzyme activity, and it is likely that it has other functions such as providing a buffer zone against osmotic flow.

A proportion of the mid-gut cells in many species of Heteroptera can be seen to be uninucleate. Yanai (1952) and Yanai & Iga (1956) studied this on a quantitative basis, and found that the frequency of uninucleate cells was correlated with the abundance of nidi of regenerative cells. In the highly carnivorous Cryptocerata, nearly all the cells are uninucleate and nidi are very abundant, while in the phytophagous Pentatomidae there are few of either uninucleate or regenerative cells. It is probable that the uninucleate cells of Cryptocerata secrete by total cell breakdown

(holocrine), and thus require constant replacement, and the binucleate cells of terrestrial Hemiptera secrete without self-destruction (merocrine) and are longer lived. Pradhan (1940) found a similar state of affairs in comparing carnivorous and phytophagous Coccinellidae, with holocrine secretion in the former and merocrine secretion in the latter. There is, however, no correlation between cell type and food habit in the terrestrial Heteroptera, and in these the much-less abundant uninucleate cells may be stages in the replacement of the binucleate epithelium. Apart from the secreting cells, other cell types, probably of an absorptive nature, occur in the mid-gut of many phytophagous Hemiptera. These may have a border extended into the lumen as a long lobe, over which there is a dense mass of fine filaments (brush-like border), or the cell base may give rise to one or a few large filaments (Fulgoridae, Licent, 1912; Goodchild, 1963*a*; Miridae Bryocorinae, Goodchild, 1952).

Although it has generally been held that a peritrophic membrane is absent from Hemiptera, some authors (e.g. Kershaw, 1913*a*; Sutton, 1951; Woolley, 1949) claim to have detected rudiments of such a structure. Parsons (1957*a*) was unable to confirm Sutton's observations on Corixidae, but detected a fragmentary membrane in some individuals of *Ranatra fusca* P.B. A small proportion of her samples gave positive results to tests for chitin in the mid-gut, but she concluded that the positive results to such tests which were found by Sutton were due to chitin fragments in the food mass. The appearance of a membrane surrounding the food mass is not uncommon, but probably results from the precipitation of a layer of freshly secreted digestive enzymes close to the epithelial surface. In the course of numerous studies on both stained sections and teased fresh tissues, I have frequently observed an apparent peritrophic membrane in the former but never in the latter. This view is also held by Kurup (1961–1962) as a result of his studies on Cryptocerata and by Mukharji (1961) from work on Homoptera.

The Malpighian tubules primitively open into the extreme posterior end of the mid-gut in Hemiptera. They are four in number, except for Coccoidea and Aleyrodoidea which have two (three in *Icerya purchasi* Mask. (Pesson, 1936)) and the Aphidoidea which have none. Miyamoto (1961) noted the presence of six tubules in the male of a primitive Heteropteran, *Kokeshia esakii* Miya. (Schizopteridae). Confusion as to the number of tubules has arisen in certain cases where the distal ends fuse in pairs (Locy, 1884). These have been regarded as two tubules with their distal ends attached to the gut near to the origin of the proximal ends. This condition occurs in the Cicadoid Homoptera and the Cryptoceratan Heteroptera, and in both groups there are also species on which all four tubules are fused together at the distal ends, the lumen being continuous across the junction. Licent (1912) found this in 19 out of 40 species of Cicadoidea which he examined. The situation in Cryptocerata is discussed by Bahadur (1961). Miyamoto (1961) finds fusion of all four apices only in some Belostomatidae and Nepidae, but records fusion in pairs not only in these families and in Naucoridae, but among terrestrial Heteroptera in certain Berytidae, Lygaeidae (*Cymus*) and Reduviidae. The junction of the Malpighian tubules with the intestine is by separate openings for each tubule in most Heteroptera and Fulgoroidea, but in both groups there are species in which the

proximal regions fuse in pairs to form two ureters. The two tubules of Coccoidea also fuse just before they join the mid-gut, but in Cicadoidea, Licent (1912) found the most common condition to be one pair fused and the other pair with separate openings. Most of the other species studied (7 out of 40) showed complete fusion into one ureter, and only one cicadoid species (*Ulopa reticulata* Fabr.) was found to have the proximal regions fused in pairs. In the Psyllidae (Brittain, 1923), the short, blunt-ended tubules open at intervals along the length of the mid-gut (Fig. 5*c*). The normal histological picture of the Malpighian tubules shows a lumen of about half the overall diameter of the tubule, with a brush or honeycomb internal border (Fig. 1*c*), but in most Cicadoidea a portion of each tubule forms a so-called 'glandular segment' of enlarged diameter and with a relatively thick wall (Fig. 1*e*). The whole tubule in Coccoidea is thick walled and with an extremely narrow lumen (Fig. 1*f*). Licent (1912) suggested that the glandular segment may be concerned with the secretion of froth-stabilizing substances in Cercopidae, but similar regions were described by him in the tubules of families which do not form froth.

In the Homoptera, the region of the mid-gut which receives the openings of the Malpighian tubules is not differentiated from the rest of the mid-gut in any way, whereas in Heteroptera the tubules open into a region which is more or less distinct from the remainder of the intestine, being marked off from the mid-gut anterior to it by a valve-like constriction, as well as by the pyloric valve leading into the hind-gut, on its posterior end. In the Pentatomomorpha this region becomes a well developed sac, which is modified in various ways in different families (Goodchild, 1963*b*), but it remains small in Cimicomorpha and Amphibicorisae, and is almost indistinguishable in Cryptocerata. There is some confusion as to the nomenclature of this region in different groups. Many workers, studying pentatomomorph species, saw it as the most anterior part of a twofold hind-gut, and named it 'ileum', while others, noting that it received the openings of the Malpighian tubules, adopted the term 'pylorus'. In the Cryptocerata, in which a pyloric region is not distinct, the tubular anterior part of the hind-gut is properly termed the 'ileum'. The use of this term in Heteroptera is discussed by Rastogi (1962*a*). Although some authors (e.g. Harris, 1938) claimed to have detected a chitinous intima in the pentatomomorph ileum, it is now generally accepted (e.g. Srivastava & Bahadur, 1961) as belonging to the mid-gut, the hind-gut commencing at the ileo-rectal (pyloric) valve. Miyamoto (1961) observed the presence of a distinct pylorus in the most primitive Heteroptera, but gives no details of its histology. A clue as to the derivation of this heteropteran structure from the homopteran condition may be found in some Fulgoroidea (e.g. *Gyarina nigritarsis* Karsch), where the pyloric valve is separated from the Malpighian tubule openings by a short length of gut histologically similar to the valve itself (Fig. 1*g*). This strikingly resembles the same region in *Saldula* sp. as illustrated by Miyamoto and reproduced here (Fig. 2*a*) for comparison. In the Amphibicorisae the small pyloric region (Fig. 2*b*) has the very narrow Malpighian tubule openings just posterior to the widest part, and the lining cells anterior to this position are clearly of mid-gut type, while those posterior to it resemble the hind-gut cells of the pyloric valve. Miyamoto describes and illustrates an inflated pylorus in some *Gerris*

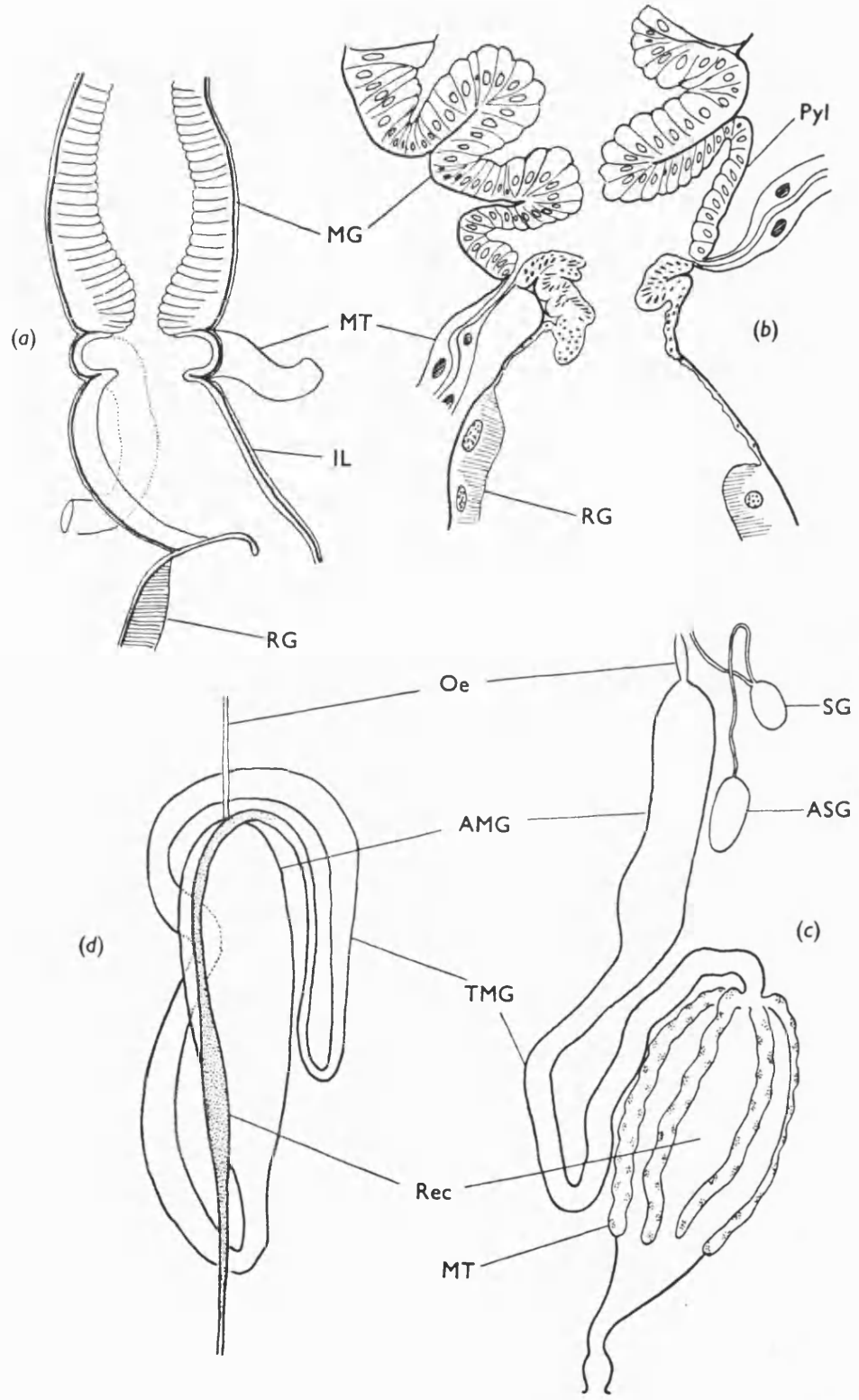


Fig. 2. *a*, Longitudinal section of pyloric region of *Saldula* sp., Saldidae (from Miyamoto, 1961, with acknowledgements); *b*, the same, of *Gerris gibbifer* Schum., Gerridae; *c*, alimentary canal and salivary glands of *Hemiodoecus veitchi* Hacker, Peloridiidae (from Pendergrast, 1962, with acknowledgements); *d*, alimentary canal of *Schizoneura lanigera* Haus., Aphididae (after Davidson, 1913). AMG, anterior sac of mid-gut; ASG, accessory salivary gland; Pyl, pylorus; RG, rectal gland; SG, main salivary gland; TMG, tubular portion of mid-gut; others as Fig. 1.

species, but his illustrations suggest that the point of junction of the Malpighian tubules has been misinterpreted, and that his supposed pylorus is the anterior part of the rectum. It is probable that the pylorus of the primitive Heteroptera is mainly of proctodaeal origin, and that the transition to a more completely mesenteric organ in the Pentatomomorpha is the result of the great increase in volume of this region and of the widening of the Malpighian tubule openings, which occur in this group.

In the epithelium of the hind-gut of Hemiptera there are two main cell types, one being a thin syncytium with many small scattered nuclei, the other being large domed or cuboid cells, with large coarsely granular nuclei and eosinophil cytoplasm. The latter are the cells of the rectal glands or 'pads'. In contrast to the mainly binucleate cells of the mid-gut, the rectal gland cells are uninucleate, though their boundaries may be indistinct. The sac-like rectum in Pentatomidae, Plataspidae, Rhopalidae, and many Fulgoroidea, is lined almost entirely with flattened gland-type cells, with the eosinophil striated borders only weakly developed, while in Coreidae, Lygaeidae, Pyrrhocoridae and Amphibicorisae the gland cells are cuboid, with well-developed borders, and are restricted to the dorsal wall of the rectum. In the Cimicomorpha the rectal gland area is at the anterior end of the rectum, around the pyloric valve opening, and in Cryptocerata the glandular region forms the tubular ileum, the rectal sac being lined with a thin syncytium.

About the function and homologies of rectal glands in Heteroptera there has been much confusion, since many authors (e.g. Hood, 1937; Rastogi, 1960; Woolley, 1949) have described the rectum as lacking glands because the gland cells were not in the form of a well-defined 'pad'. The homology of the gland cells throughout the Heteroptera has been asserted by Bahadur (1963) and myself (Goodchild, 1963*b*), but Miyamoto (1961) has failed to recognize them in Pentatomomorpha and, not taking the ileum into account, has denied their presence in Cryptocerata. In the Homoptera the hind-gut is usually lined entirely with domed glandular cells, though restriction to lateral strips occurs in some Fulgoroidea (Goodchild, 1963*a*). In many of the small forms, such as aphids or coccids (Davidson, 1913; Hough, 1925; Willis, 1949), the rectal nuclei are large but the cytoplasm is thin, so that the nuclei bulge out into the rectal lumen. The most primitive of existing Hemiptera, the Coleorrhyncha, have the rectum lined with 'a very thin epithelium' (Pendergrast, 1962), but in the absence of an illustration it is not clear whether this is glandular or not. The sac-like rectum of many Coccoidea appears to lack gland cells, but these may be present elsewhere in the hind-gut. The Miridae Bryocorinae are the only Hemiptera definitely known to lack rectal gland cells (Goodchild, 1952).

Observation of the histology of the gland cells, with a consideration of their probable function, leads to the conclusion that where the eosinophil material is distributed through the depth of the cell, as in the domed cells of the hind-gut of Cicadoidea or the tall columnar cells of the ileum of Cryptocerata, a solute-absorbing activity may be supposed to take place, while a well marked eosinophil striated border is associated with a probable water-absorbing function. It is interesting to compare this conclusion with that of Ramsay (1950), who studied osmoregulation in the larvae of

the mosquitoes *Aedes detritus* Edw. and *A. aegypti* L. In the latter species, which can only form hypotonic excreta, the rectal gland is thin, with a narrow striated border, while that of *A. detritus*, which can osmoregulate in both fresh and sea water, and forms hypertonic excreta in the latter, is thicker and divided into two distinct regions. The posterior region of the rectum resembles that of *A. aegypti* in having a conspicuous striated border, but the anterior region has cells which lack such a border, their nuclei being found at all levels, and their cytoplasm generally denser towards the base. Ramsay tentatively identified the anterior region as the site of water reabsorption in the formation of hypertonic excreta, since cells of this type were not found in *A. aegypti*. Although this is opposite to the conclusion reached from the study of rectal histology in Hemiptera, it should be noted that final proof is lacking. The two cell types do not occur together in any species of Hemiptera.

IV. SIMPLE TYPES OF INTESTINE

In all the different subdivisions of the Hemiptera there are families in which the intestine is simple, in that elaborate structures such as filter chambers are not developed. Except for zoophagous or seed-sucking Heteroptera, these insects are all of small size. Many are mesophyll feeders and are not in need of water-disposal mechanisms, but their smallness, and therefore increased area-to-volume ratio, may make it possible for them to lose a significant amount of water by cuticular transpiration, and enable them to feed upon sap. Where species with simple intestines are members of families in which larger forms possess specialized features of the alimentary canal, their simplicity may be due solely to this size factor, and they may even have evolved from ancestors with more complex intestines. Such groups as the Typhlocybinae (Cicadoidea), Delphacidae (Fulgoroidea) and certain subfamilies of Lygaeidae (Pentatomomorpha) can best be considered along with their specialized relatives.

There are, however, major groups of Hemiptera in which only simple intestines are found. These are the Coleorrhyncha (Pendergrast, 1962), the Aphidoidea except for Lachnidae and a few genera of Aphididae (Davidson, 1913; Borner, 1938; Weber, 1928), the Cimicomorpha and the Amphibicorisae (Miyamoto, 1961; Goodchild, 1963*b*). In the Coleorrhyncha (Fig. 2*c*) the oesophagus is short and joins the mid-gut well forward in the thorax. The anterior part of the mid-gut is slightly dilated. The mid-gut forms a simple loop in the abdomen, where it gradually narrows, is bent upon itself and passes forwards to join the hind-gut near the anterior end of the abdomen. The hind-gut consists of a dilated sac, on the outside of which lie the short simple Malpighian tubules. The tubules open into the mid-gut just before its junction with the hind-gut. Along with many other features of internal and external anatomy of this group, the alimentary canal is more primitive than that of any other living hemipteran and strikingly resembles that of some Thysanoptera (Sharga, 1933).

The alimentary canal of aphids (Fig. 2*c*) departs from the primitive condition in the more marked dilatation of the anterior mid-gut into a 'stomach' and in the absence of Malpighian tubules. The latter feature is also found in Collembola and

certain Thysanura, in which forms it may be primitive, since cephalic excretory organs analogous to those of Crustacea are present. No alternative excretory organ has been found in aphids, so it must be assumed that the cells of the gut itself effect this function. The mid-gut joins the hind-gut at a point well forward in the body cavity, near to the oesophageal valve. The hind-gut is evidently capable of considerable distension, being sometimes observed in dissections as a thin-walled sac, but it contracts to a tube when empty. Notwithstanding the lack of special water-excreting intestinal structures, the aphids are nearly all sap-sucking insects. In the aphids, however, attention must be drawn, not to the presence of additional structures, but to the absence, unique among higher insects, of the Malpighian tubules. It may be argued that in the aphids excessive dilution of the blood by water imbibed from the ingesta is prevented by the development of a turgor pressure in the insect's body. Were Malpighian tubules present, the internal pressure might cause an increased passage through them of fluid isotonic with the blood (Ramsay, 1950), with a resulting loss of useful solutes. They have therefore been suppressed in the course of evolution. It is probable that in many cases sap is ingested passively under the influence of the turgor pressure in the plant (Mittler, 1957), but this must be limited by a reverse pressure in the insect, except in so far as this is released by ejection of honeydew from the anus or evaporation of water from the cuticle and tracheal system. Production of honeydew has been measured at 2·0–3·6% body weight/hr. (Auclair, 1958), and evaporation at 1·0% in flight (Cockbain, 1961) and 0·35–0·40% in still air (Lamb, 1963). The last author found that this was doubled in an atmosphere containing 5% of carbon dioxide, as a result of relaxation of the spiracles. It would be interesting to discover whether the spiracles also respond to changes in osmotic pressure of the blood, or tension in the cuticle.

One of the problems involved in extracting nourishment from a flow of sap forced through the alimentary canal concerns the very different proportions of sugars and amino acids in plant sap and insect blood. Unless actively controlled in some way, this would result in a loss of amino acids and an excess of sugars in the blood. The activity of intestinal enzymes in forming trisaccharides and oligosaccharides from disaccharides (Auclair, 1963) may help to slow down diffusion of sugars into the body. Apart from cutaneous and respiratory water loss, absorption of nutrients will be governed by the capacity of the rectal epithelium to extract solutes and form hypotonic excreta. Excreta accumulate in the rectum for periods of 25–60 min. (Smith, 1937; Broadbent, 1951), mainly to allow the formation of droplets large enough to be expelled clear of the aphid colony, but this may also permit the rectum to absorb useful solutes. The nutrients in the sap are not very efficiently utilized. Of the 0·03–0·13% of amino acids in willow sap, about 55% was absorbed by *Tuberolachnus salignus* (Gmelin), and of the 5–10% of sugars only 5% was removed by this species (Mittler, 1958*b*). The high concentration of useful substances in the excreta of aphids has been the subject of comment since it was first recognized, and was supposed to indicate a need for some essential nutrient present only in trace amounts. It is more likely to be the result of inefficient extraction of solutes due to a low capacity for osmotic work in the hind-gut.

The specialized anatomical adaptation of aphids to sap-sucking habits may therefore be the absence of Malpighian tubules. This theory accounts for the subglobular shape of the aphid body as being due to cuticular tension resisting any increase in blood volume; for the well developed oesophageal valve, if sap is ingested against an internal pressure; and for the small size, since cuticular transpiration is an important route of water disposal, and since the sluggish movements imposed by a turgid body would render a large insect more vulnerable to predators. This restriction on size and activity has resulted in the evolution of extremely high rates of reproduction, facilitated by parthenogenesis and viviparity, in order to compensate for the depredations of natural enemies. It is significant that those aphids which have acquired a simple filter chamber (Lachnidae and some Aphididae, Knowlton, 1925) are generally among the largest of the aphids, and have higher rates of sap consumption (Mittler, 1958*a*) than aphids lacking this modification.

The most primitive Heteroptera are found in the families Dipsocoridae and Schizopteridae. In their alimentary canals (Miyamoto, 1961), short, simple Malpighian tubules are present. They differ from the Coleorrhyncha mainly in the more marked anterior dilatation of the mid-gut, in the constriction of the mid-gut anterior to the Malpighian tubule junction, forming a distinct pylorus, and in the restriction of gland cells in the hind-gut to a well defined pad on the dorsal wall of the rectum (Fig. 3*a*). It would appear that some Saldidae show a more primitive condition of the Malpighian tubule junction, with the pylorus not clearly defined (Fig. 2*a*), and that the Cryptocerata have retained this level of organization. While China (1955) places the Saldidae near the origin of Amphibicorisae (in which, however, the pylorus is distinct), Miyamoto (1961) reveals details of salivary-gland structure and Malpighian tubule arrangement which link this family with the terrestrial Heteroptera. They thus occupy a rather isolated position, and their particular combination of structural features cannot be regarded as lying on the main line of evolution of the suborder. The apparent separation of a tubular ileum from a sac-like rectum in Saldidae led Miyamoto to regard this as the primitive condition in Heteroptera. Although this author compared the ileum of Saldidae to that of Cryptocerata, the rectal gland cells of the former are found mainly in the sac-like region, extending to the supposed ileum only in *Saldoida armata* Horv., but not in *Saldula* sp. It seems more likely that the hind-gut of Heteroptera was primitively sac-like, as seen in Dipsocoridae, and it is important to note, because this is relevant to the discussion of the evolution of water-disposal mechanisms, that in Heteroptera the rectum and Malpighian tubule junction are situated posteriorly in the body cavity, at some distance from the oesophageal valve.

While the salivary glands in both Coleorrhyncha and Aphidoidea are in the form of two pairs of simple compact glands, those of Heteroptera consist of a pair of basically two-lobed main glands, and a pair of accessory glands terminating in thin-walled vesicles lying close to the anterior mid-gut, connected to the main glands by long ducts. This type of accessory gland is found in all zoophagous families, and its function may be that of recirculating water from the gut to ensure a copious flow of saliva to wash out the dissolved internal organs of the prey. The reduction of the

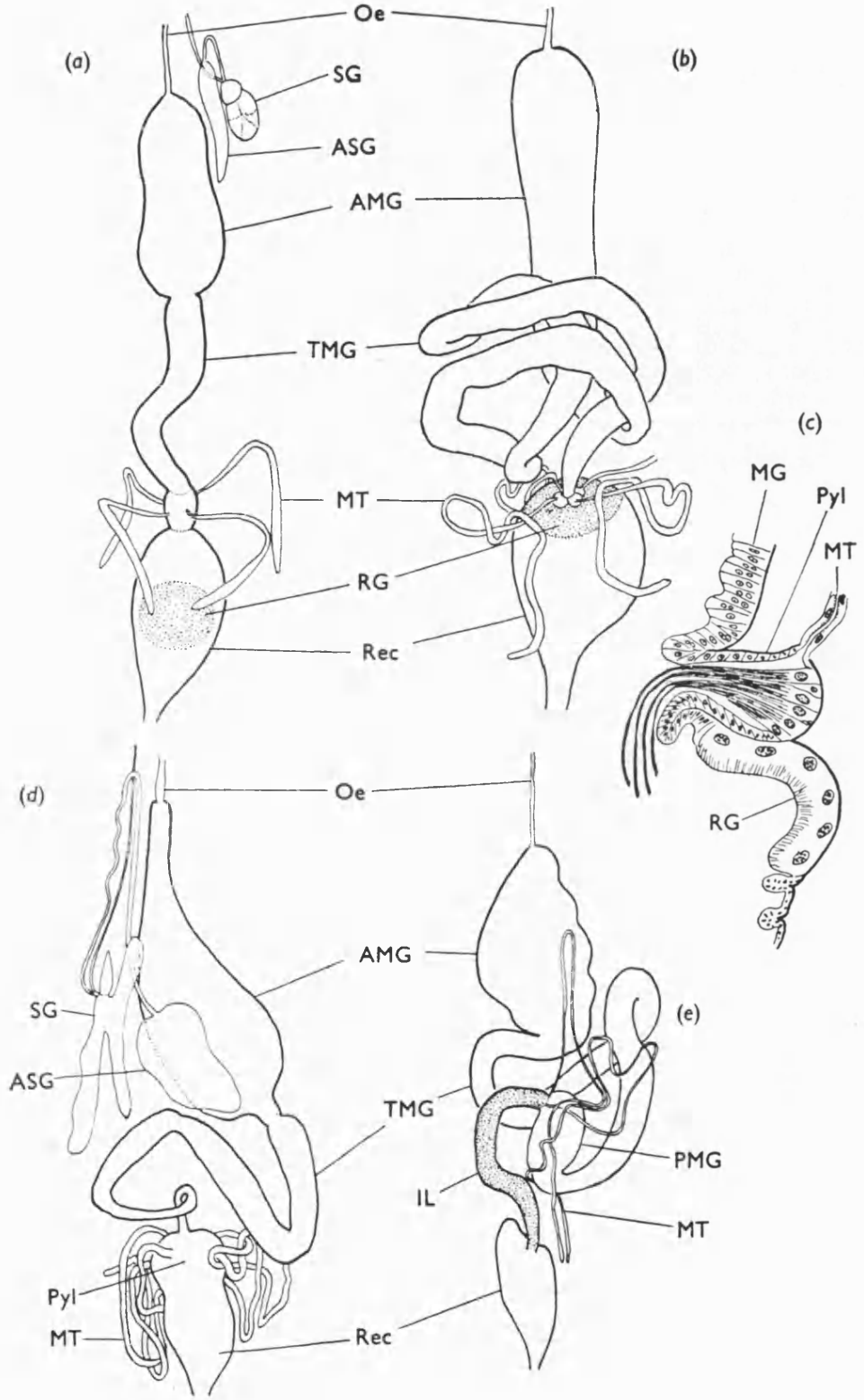


Fig. 3. *a*, Alimentary canal and salivary glands of *Hypselosoma hirashimai* Esaki & Miya., Schizopteridae; *b*, alimentary canal of *Stephanitis pyrioides* Scott, Tingidae; *c*, longitudinal section of pyloric ampulla of *Sphedanolestes* sp., Reduviidae; *d*, alimentary canal and salivary glands of *Sahlbergella singularis* Hagl., Bryocorinae; *e*, alimentary canal of *Anisops genji* Hutch., Notonectidae. (*a*, *b* and *c* from Miyamoto, 1961, with acknowledgements.). PMG, posterior swelling of mid-gut; others as Figs. 1 and 2.

vesicles in blood-sucking forms, and their absence from *Rhodnius prolixus* Stål, a highly specialized blood-sucker (Goodchild, 1955*b*), tends to support this interpretation.

The alimentary canal of Amphibicorisae does not differ greatly from that of Dipsocoridae. The pylorus is small (Fig. 2*b*), and may indeed have diminished in size from the primitive heteropteran condition, by reduction of the region posterior to the Malpighian tubule junction. The rectal gland is well developed, extending down the lateral walls of the rectum and in some cases (e.g. *Gerris* spp.) forming deep longitudinal folds in the rectal lumen. In addition to the anterior dilatation, the mid-gut tends to form a bulbous enlargement at its posterior end, and its tubular middle portion may form more than one loop. The posterior swelling of the mid-gut is variable in size in different species, and usually smaller than the anterior one. In some species the posterior swelling is only temporarily formed as food residues accumulate, but in others it seems to be permanent. The Malpighian tubules of Amphibicorisae tend to be longer and more convoluted than the primitive type, with the dorsal pair forming anterior loops. The Hydrometridae (Sprague, 1956; Miyamoto, 1961) are unusual in having a narrow tubular mid-gut, and the hind-gut divided into tubular ileum and sac-like rectum. The glandular area is confined to the former region, and the rectum has a narrow anterior diverticulum. This is essentially the pattern in Cryptocerata, but on external characters the Hydrometridae are in no sense a connecting link between the groups, but arise high up on the Amphibicorid stem (China, 1955). Possibly the internal structure is necessitated by the extremely narrow body of these insects.

The characteristic feature of the intestines of the Cimicomorpha is the reduction of the pyloric region almost to vanishing point, though with well defined valves both anteriorly and posteriorly (Cragg, 1915; Painter, 1930; Goodchild, 1963*b*). The Malpighian tubules open into the pylorus through flask-shaped ampullae (Fig. 3*b*), which contain long filamentous cells projecting into the rectal lumen (Fig. 3*c*). The rectal gland forms a pad surrounding the pyloric valve. The posterior swelling of the mid-gut is not very evident, and in most species it is absent. In the phytophagous families Miridae and Tingidae the tubular intestine is divided into two regions by a slight constriction, and the anterior one of these is lined with cells of a type not found in zoophagous families. These cells have a deep brush-like border, which is partly destroyed by fixation and does not take up histological stains. In certain large tropical Miridae of the subfamily Bryocorinae a unique intestinal pattern is found (Goodchild, 1952, 1963*b*) in which a large sac-like pyloric region opens by a wide and poorly developed valve into a rectum in which gland cells are totally lacking (Fig. 3*d*). The tubular intestine is twofold, but the posterior part, which in other Miridae is lined with the bulbous-tipped binucleate cells typical of the mid-gut of Hemiptera, is in these Bryocorinae furnished with a lining of cells bearing long filamentous processes. These absorb vital dyes intensely *in vitro*, and in the living insect may also absorb water strongly, since insoluble particles in the ingesta accumulate in this region. The pyloric ampullae are lacking in these species, but their unusual filamentous cells may be represented by the cells of this terminal part of the

mid-gut. These large Bryocorinae feed upon succulent shoots and fruits, destroying areas of plant tissue with their toxic saliva (the latter feature being typical of the mainly carnivorous character of the Cimicomorpha). The organs by which Cimicomorpha conserve water (rectal glands, pyloric ampullae) are absent, and it would seem that the tropical Bryocorinae, having a diet of high water content, have to dispose of an excess. Their excreta are always a transparent fluid, and they may also dispose of water through the accessory salivary-gland vesicles, since a number of clear droplets are exuded from the mouthparts on the completion of a feed. This small group of species must represent the nearest approach to sap suckers of all the Cimicomorpha, and this is accompanied, as in other groups, by alimentary modifications and by increased size compared with related mesophyll feeders. It should be noted that the small temperate representatives of the subfamily Bryocorinae are not thus modified, but have typical cimicomorph intestines (Miyamoto, 1961; Goodchild, unpublished).

V. THE CRYPTOCERATA (HYDROCORISAE)

The insects in this group agree in many respects with the Amphibicorisae. In both groups the main salivary gland has a narrow lumen (except in Corixidae) and in both the anterior loop of the Malpighian tubules is from the dorsal pair. The mid-gut (Fig. 3*e*) is expanded anteriorly in Cryptocerata, but the posterior bulb is usually only slightly, if at all, developed. There is no distinct pylorus, and the Malpighian tubules open into the posterior end of the mid-gut. The hind-gut consists of a tubular ileum, the lining of which is thick and of glandular nature except for a narrow ventral strip, and a thin-walled rectal sac having a well developed anterior diverticulum. The ileum may reach a considerable length (e.g. in Belostomatidae). Studies on the intestinal structure of this group are numerous (Hamilton, 1931; Rawat, 1939; Sutton, 1951; Parsons, 1959; Rastogi, 1961, 1962*b*; Kurup, 1961–62).The significance of the strongly glandular ileum and its tubular shape has been suggested to be solute absorption (Goodchild, 1963*b*). Bahadur (1963), while correctly noting the homology of this region with rectal pads in other Heteroptera, tried to interpret it as a water-absorbing organ as it is in terrestrial forms. His argument was that these insects had a body of low water content and therefore needed to conserve water. It is clear, however, from the studies of Holdgate (1956) and Staddon (1963, 1964) that the aquatic Heteroptera experience a steady osmotic inflow through the cuticle, which necessitates the excretion of a hypotonic urine.

VI. THE FULGOROIDEA

This large assemblage of species is of very varied external appearance and is difficult to define taxonomically. Their alimentary canal has been recognized as contrasting in its simplicity with that of Cicadoidea (Dufour, 1833; Licent, 1912; Kershaw, 1910, 1913*a*; Mukharji, 1961), but no other author has provided a convincing explanation of how the Fulgoroidea are able to feed upon sap while lacking an anatomically obvious filter mechanism. The mid-gut (Fig. 4*a*) is a narrow tube throughout its length and is coiled into a knot-like cluster of loops, from which the

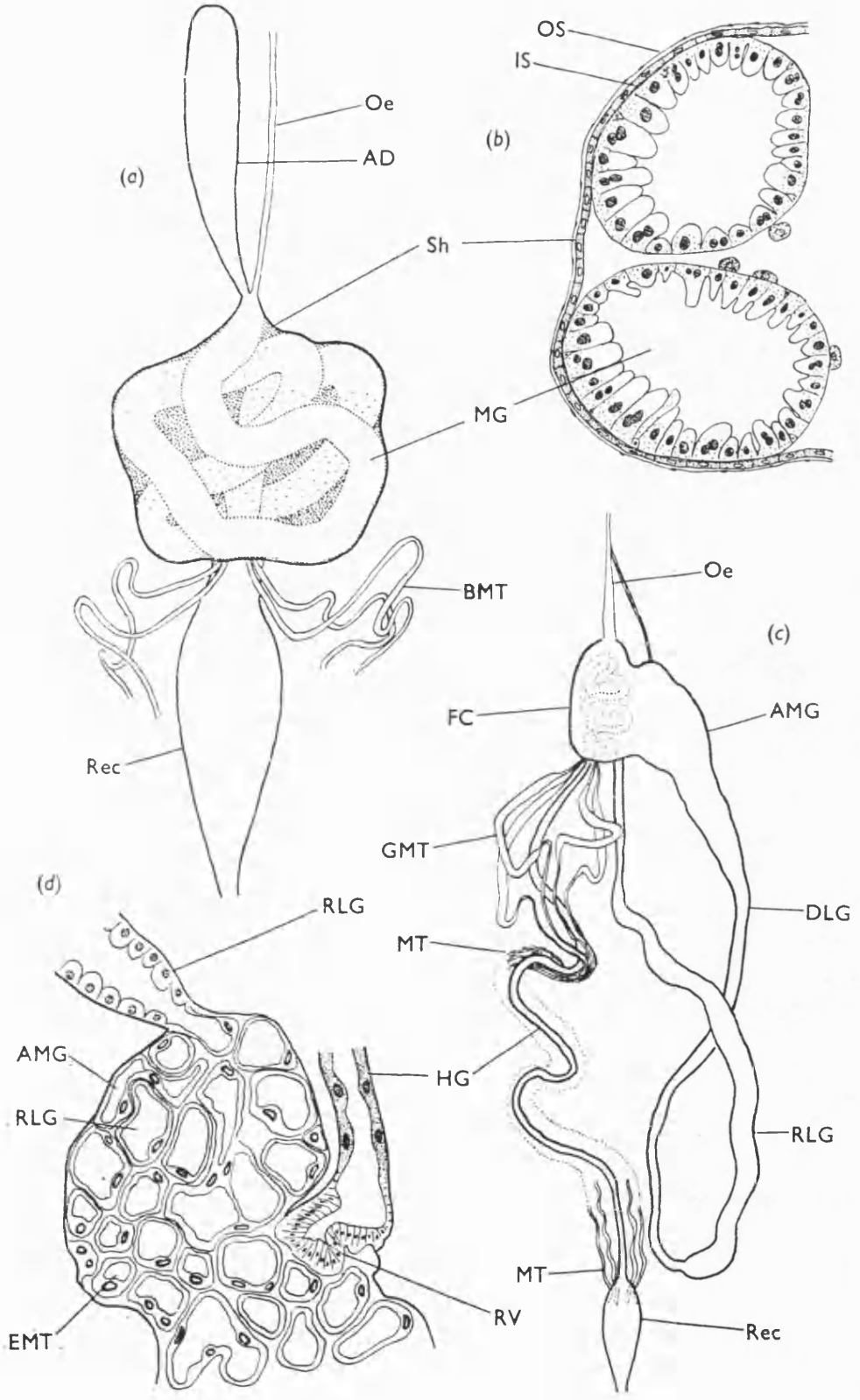


Fig. 4. *a*, Alimentary canal of fulgoroid type (based on *Pyrops tenebrosus* Fab., Fulgoridae); *b*, transverse section of part of ensheathed mid-gut of *Phalix titan* Fennah, Tettigometridae; *c*, alimentary canal of cicadoid type (based on *Tettigoniella mitrata* Gerst., Jassidae, middle portion of Malpighian tubules omitted); *d*, transverse section of filter chamber of *T. mitrata*. AD, anterior diverticulum of mid-gut; BMT, base of Malpighian tubule, distal part omitted for clarity; DLG, descending loop of mid-gut; EMT, Malpighian tubule enclosed in filter chamber; FC, filter chamber; GMT, glandular portion of Malpighian tubule; HG, hind-gut; IS, inner layer of intestine sheath; OS, outer layer of sheath; RLG, returning loop of mid-gut; Sh, intestine sheath; others as Figs. 1 and 2.

oesophagus emerges anteriorly and the sac-like rectum posteriorly. The rectal lining is of rather flattened gland-type cells, very like that of some Pentatomidae. Alongside the oesophagus there extends into the thorax, and in a number of species into the head, a diverticulum from the anterior end of the mid-gut. The Malpighian tubules join the mid-gut at its posterior end through very narrow openings, and are not fused at their distal ends. Between the oesophageal valve and the junction of the Malpighian tubules, the cluster of mid-gut loops is enclosed in a membranous sheath. This sheath has hitherto been regarded as of little significance, but its structure in a fairly primitive type, *Phalix titan* (Tettigometridae), shows features which provide a valuable clue to the mechanism of water control in the Fulgoroidea. In this species the sheath is two-layered (Fig. 4*b*), the inner cells being large, eosinophil and uninucleate, while the outer cells form a thin pavement epithelium with small dark-staining nuclei (Goodchild, 1963*a*). Among the tracheal branches which penetrate the sheath are scattered large eosinophil cells, of appearance similar to the cells termed oenocytes. Though organized as a layer of contiguous cells, those of the inner sheath are also remarkably like oenocytes in histological appearance. In Fulgoridae (e.g. *Pyrops tenebrosus* Fab.) the sheath is formed entirely of smaller pavement-like cells, but there is a continuous layer of oenocyte-like cells on the outer side of the mid-gut tube itself. Licent (1912) seems to have observed this in other Fulgoridae, but regarded it as a strong muscular coat. The cells lining the mid-gut may be entirely of simple bulbous type (e.g. in Flatidae), or the posterior mid-gut may contain cells with apical lobes having a deep brush-like border (e.g. in *Phalix titan*), while in Fulgoridae the mid-gut cells are produced into long apical filaments (Licent, 1912; Goodchild, 1963*a*). Licent observed in *Cixius* and *Issus* spp. that a free loop of the mid-gut emerged from the central knot, and suggested that this might be the equivalent of the mid-gut loop of Cicadoidea, with filtration of excess water taking place within the knot. The arrangement in Cicadoidea is, however, quite different, and the circumstances which lead to filtration in that group do not obtain in the Fulgoroidea. The mechanism by which this intestinal pattern is adapted to sap-sucking seems likely to involve the sheath, since this structure is confined to the group. It might be that the oenocyte-like cells are able to resist the inflow of water to the blood, while the mid-gut cells absorb solutes from the ingesta. Thus the contents of the intestine would become more dilute during passage along the mid-gut, leaving the sac-like and weakly glandular rectum with little osmotic work to perform. Oenocytes are usually associated with the insect hypodermis, where they may be concerned with waterproofing (Kramer & Wigglesworth, 1950), and the intestinal sheath may have originated as a subepidermal layer growing inward at front and rear to bridge the region of osmotic entry of water between the chitin-lined oesophagus and rectum. On this theory, it is not functionally important whether the oenocytic layer is in the sheath (which is probably the primitive position), as in *Phalix titan*, or on the outside of the mid-gut, as in *Pyrops tenebrosus*. Although Licent does not give histological details of the guts of *Cixius* and *Issus*, their emergence from the sheath would suggest that the waterproofing layer, if present, is around the gut wall. On the other hand, there are cases in which the sheath is incomplete or

absent, both as an enclosing membrane and on the outer surface of the mid-gut (Licent, 1912; Mukharji, 1961; Goodchild, 1963*a*). The small-sized Delphacidae are probably mesophyll feeders with no water-control problem. In the case of adult Flatidae (Goodchild, 1963*a*), there was evidence that active feeding upon sap was no longer taking place. Even in the simple gut of Delphacidae, the group characteristics of uniform mid-gut diameter and anterior diverticulum are present, so that it is not primitively simple.

The anterior diverticulum of the mid-gut has attracted a great deal more attention in the past than the other aspects of the fulgoroid intestine. In many species, and particularly in the lantern-flies with their remarkable elongation of the head, it extends into the anterior regions of the body in an elaborate manner. It is usually filled with air bubbles, especially after a moult, but its cells may show signs of active secretion (Mukharji, 1961). In *Gyarina nigritarsis* (Flatidae; Goodchild, 1963*a*) the cell tips are distended with an eosinophil material not found elsewhere in the mid-gut, and unlike the usual alimentary secretions of Hemiptera (though in staining reaction resembling salivary secretion). The diverticulum may sometimes contain fluid (Kershaw, 1913*a*; Mukharji, 1961) and was interpreted by Kershaw as a food reservoir (inaccurately termed 'crop'), or waxy material, supposedly separated from the food (Kershaw, 1910). Licent (1912) found the diverticulum in *Lycorma delicatula* White always to contain many large air bubbles bound by a viscid secretion, and interpreted the organ as a means of reducing the weight of the anterior parts of the body. Mukharji (1961) disagrees with this on the grounds that when the diverticulum contains fluid it will have the opposite effect. Kershaw (1914) also suggests that the diverticulum serves to separate air from the food before it passes down the mid-gut. Against this it may be said that air is commonly present in the alimentary canal of other plant-sucking Hemiptera, without apparent ill effect. The interpretation which I have placed upon this structure (Goodchild, 1963*a*) is that it is a corollary of the ensheathed mid-gut, in that it is used to inflate the thorax at the time of moulting, a function which the specialized mid-gut is no longer able to perform. This would account for the frequency with which it is found to contain air. During active sap-sucking, the diverticulum would be a liability on account of its permeable wall, and the secretions observed therein, which Licent described as binding the air bubbles, Kershaw regarded as 'waxy', and which are clearly of unusual character in *Gyarina nigritarsis*, may be adapted to combat this tendency. The evolution of the enormous degree of forward extension in some forms must be regarded as a secondary development, similar to the tracheal air sacs of many other insects (including among Hemiptera the Cicadidae, Plataspidae, and Pentatomidae), enabling the Fulgoridae to possess elaborate cephalic processes of cryptic or warning value, without excessive weight penalty. It may be that the tracheal system of the head is less able to respond in this way. Against Mukharji's objection, the views of Wigglesworth (1963) on the function of air sacs as a means of restricting the volume of blood in circulation may be relevant. The suggestion by Mukharji (1961) that the anterior diverticulum assists in the process of sucking in food, by the contraction of its wall, is based on very inadequate evidence.

VII. THE CICADOIDEA

It is in this group that the classic example of the filter chamber is found, and intestines of members of this superfamily have been described by several authors (Licent, 1912; Kershaw, 1913*b*, 1914; Hickernell, 1920; Cecil, 1930; Dobroscky, 1931; Mukharji, 1961). The mid-gut (Fig. 4*c*) has a clearly defined anterior sac, posterior to which a narrow tubular intestine passes towards the rear of the abdominal cavity, and then returns to a point near the oesophageal valve. The lining cells of the first part of this loop are typical mid-gut cells, but the returning part is lined with enormously hypertrophied cells which practically obliterate the lumen. Licent regarded these as excretory cells, packed with crystals of calcium carbonate, while Cecil thought they were engorged with bacteria and succeeded in obtaining cultures from them. At the anterior end of the abdominal cavity, the returning loop of the mid-gut, together with the proximal ends of the Malpighian tubules, passes beneath the peritoneal covering of the anterior mid-gut sac, near the oesophageal valve. In the subperitoneal space, the posterior end of the mid-gut follows a sinuous course forward and back again, to emerge not far from its point of entry. The Malpighian tubules are also convoluted in this region and join the mid-gut before it emerges. The transition to the hind-gut is at the point where the intestine emerges through the peritoneal covering and the lining cells here form a shallow valve-like collar. The lining epithelium of the anterior mid-gut is pushed out among the convoluted tubes of the filter complex in an elaborate series of folds (Fig. 4*d*). The whole filter system forms a smooth rounded capsule on the right side of the mid-gut near the oesophageal valve, with the opening into the rest of the mid-gut (anterior sac) rather constricted. In some Cicadidae the filter chamber is separated from the main sac by a distinct tube. Suspensory muscles run forward from the mid-gut sac and the filter chamber to join the oesophagus. The epithelia of all the parts concerned in the filter chamber, namely the basal parts of the Malpighian tubules and both extremities of the mid-gut, are of a uniform pattern, with thin flattened cells, large nuclei and weakly staining cytoplasm. In some of the smaller Cicadoidea (Jassidae, Licent, 1912; Membracidae, Mukharji, 1961) the filter arrangements are less complex, and the epithelium of the posterior mid-gut is thin only on the side facing the anterior mid-gut epithelium, the outer side, towards the peritoneal covering, being of normal columnar cells. In these simpler forms also, the cells of the second part of the tubular mid-gut are not greatly hypertrophied, but the lumen is usually occluded by a pasty mass, or 'coagulum'. In the subfamily Typhlocybinae of the Jassidae, a true filter chamber is not formed (Licent, 1912; Saxena, 1955; Willis, 1949). The posterior end of the mid-gut, just posterior to the junction with the Malpighian tubules, is closely applied to the slightly dilated anterior end of the mid-gut, and bound thereto by delicate strands of muscle. With the small area of contact, transfer of water must be slight, but Willis observed that the epithelium of the tubular intestine at this point is thin and vacuolated, and that pumping movements occur in it. In all other respects the alimentary canal is typically cicadoid, and may be secondarily simplified.

The hind-gut of Cicadoidea is very long and narrow and follows a more or less

sinuous path backwards from the filter chamber to the anus, closely accompanied by the Malpighian tubules. Its lining is entirely composed of domed, eosinophil, gland-type cells. There is usually a slight expansion, or rectum, at the posterior end, and in this region the ends of the Malpighian tubules come into close contact with the rectal wall, being sunk into pockets of the wall and enclosed by a delicate peritoneal membrane. In some cases this peritoneal covering is more strongly developed, and Mukharji (1961) describes the distal ends of the tubules in *Platypleura capitata* Oliv. (Cicadidae) as enclosed in a peritoneal sheath together with the convoluted posterior end of the tubular hind-gut, anterior to the rectal expansion. The terminations of the Malpighian tubules are separate in Cercopidae, but fused in pairs or all four together in other families.

A rational interpretation of the function of the cicadoid filter chamber owes much to Ramsay's (1950) concept of rectal absorption of solutes. Earlier approaches were inclined to regard it as a filtration under an applied pressure and Licent (1912) believed the occlusion of the mid-gut by hypertrophied cells to function as a 'filter bed' in restricting flow along the lumen, thereby directing it through the filter chamber. This view suffers from two disadvantages. One is that dilute ingesta under pressure in the mid-gut would lose water to the blood, which is undesirable, and the other is that the thin-walled tubes of the filter complex would collapse under a pressure applied to their outer sides. We can discount the possibility of active secretion of water by the posterior end of the mid-gut, since its epithelium, in the filter chamber, is thin and shows no signs of physiological activity. The only remaining possibility is that water is extracted from the ingesta by passive osmosis to the contents of the Malpighian tubules, which are isotonic with the blood. Effectively, this is the same osmotic gradient as that which tends to draw water through the mid-gut wall into the blood, but the Malpighian tubule fluid is exposed to the ingesta over a very large area of thin epithelium, before that ingesta can pass on into the rest of the mid-gut. By that time it will have lost its excess water, and since most of the material in it is assimilable, the more posterior region of the mid-gut will carry only a weak flow. Thus its cells may become modified for storage, or a precipitate of less soluble waste will accumulate. The Malpighian tubule fluid, diluted by absorbed water, will pass down the long tubular hind-gut, the glandular cells of which will absorb useful solutes so as to produce excreta which are hypotonic to the ingesta. This process is very efficient. Gruner (quoted by Licent, 1912) found that the excreta of the cercopids *Aphrophora* and *Philaenus* contained only 3·6% of the organic matter present in the ingesta. The shape of the hind-gut, as a long narrow tube, is admirably adapted to this function, by reason of its high surface-to-volume ratio. The physiological significance of the association of the distal ends of the Malpighian tubules with the rectum is not obvious. In comparison with the cryptonephridial systems of many Coleoptera, and of larvae of Lepidoptera and Myrmeleonidae, there is no apparent modification of the tubules in this region in Cicadoidea. In the other insects in which it occurs, this arrangement would seem to be a water-conversing mechanism (Wigglesworth, 1950). On this assumption, I have suggested that the filter chamber may be so effective in excluding excess water from the

haemolymph that the cryptonephridial arrangement is necessary in order to maintain a flow along the tubules (Goodchild, 1963*a*).

VIII. THE COCCOIDEA

In this group, as in the Cicadoidea, the hind-gut takes its origin from the mid-gut far forward in the abdominal cavity, where its proximity to the oesophageal opening makes a true filter system possible. Alimentary canals of Coccoidea have been described by Berlese (1893), Hough (1925), Misra (1931), Negi (1934), and Pesson (1933, 1935, 1936, 1941). The Malpighian tubules are two in number in most species, and are relatively short and not convoluted, but irregular in diameter like a string of beads. Their lumen is thread-like (Childs, 1914). The region of contact of the extremities of the mid-gut is sunk into an invagination in the anterior end of the sac-like, thin-walled rectum, which appears to be lined entirely with non-glandular cells. The connexion between the posterior end of the intestine and the rectum is by a delicate tube (*colo-rectum* of some authors), which emerges from the filter complex to enter the side of the rectum. Berlese failed to observe this tube and thought that the intestine ended blindly in the invagination of the rectal wall. To judge from published illustrations, the colo-rectum may be composed of rectal gland cells, but in the most advanced forms it is fused with the rectal wall and is non-glandular in nature. The thin syncytium forming the rectal lining bears a chitinous intima on its inner surface, which is produced into numerous minute peg-like or hair-like processes. The anatomy of *Pulvinaria* spp. (Pesson, 1935; Goodchild, unpub.) represents the highest degree of specialization of the coccoid filter apparatus (Fig. 5*a*). The extremities of the intestine, in the filter complex, are so fused together as to resemble superficially a single tube, which forms a helix of two or three turns in the rectal invagination. The dual nature of this tube was missed by some investigators (Hough, 1925; Misra, 1931), but in fact the outer side of the helix is the posterior end of the intestine and the inner side is the anterior end. A thin, apparently structureless, membrane which divides the lumen of this helical tube into inner and outer compartments represents the fused region of contact between the intestinal extremities (Fig. 5*b*). The inner wall of the helix is composed of tall columnar cells with pale-staining cytoplasm, while the outer wall consists of shallow domed cells with darker cytoplasm. Since the outer tube is posterior to the Malpighian tubule junction, Negi (1934) regards it as belonging to the hind-gut, in which case its cells may be of rectal gland nature. The fused tubes of the filter complex separate at the opening of the rectal invagination, and connect with the ends of the free loop of the mid-gut, which lies posterior to the filter chamber. From the anterior end of this loop there is a long tubular diverticulum on one side of the body cavity. Both the mid-gut loop and its diverticulum are lined with simple bulbous cells with rather basophil cytoplasm. The Malpighian tubules lie alongside the rectum on either side of the body and fuse into a short, narrow ureter before opening into the mid-gut near its posterior end. Normal mid-gut epithelium continues from that point until the tube joins the filter complex. At the inner end of the rectal invagination, the inner part of

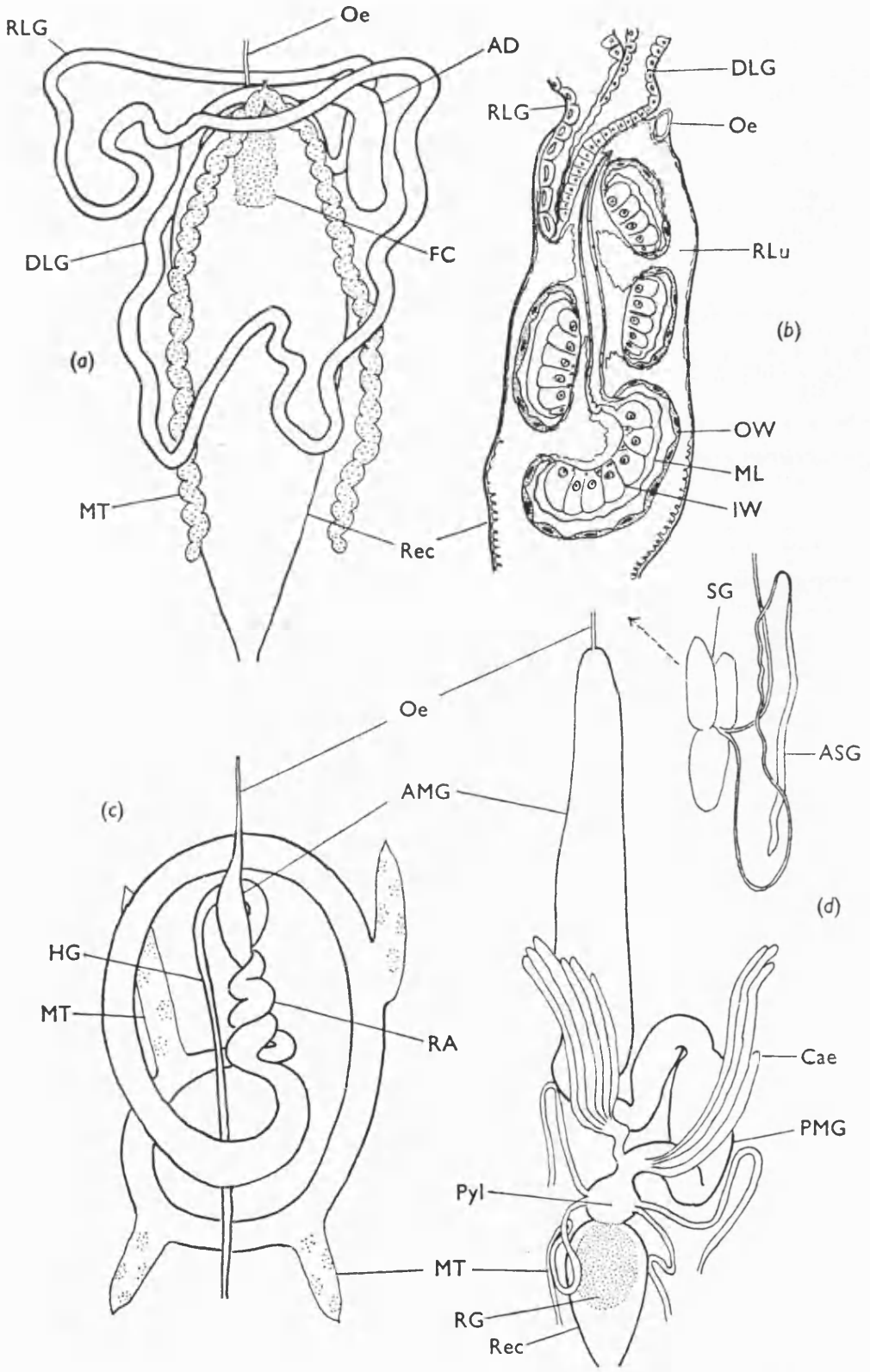


Fig. 5. *a*, Alimentary canal of *Pulvinaria jacksoni*, Coccidae; *b*, longitudinal section of filter of *P. jacksoni*; *c*, alimentary canal of *Psylla mali* Schmidt, Psyllidae (after Brittain, 1923); *d*, alimentary canal and salivary glands of *Yemma exilis* Horv., Berytidae (from Miyamoto, 1961, with acknowledgements). Cae, gastric caeca; IW, inner wall of filter tube; ML, middle lamella of filter tube; OW, outer wall of filter tube; RA, region of association of mid-gut extremities; RLu, lumen of rectum, others as Fig. 4.

the helix joins the very narrow, thin-walled oesophagus, which passes forwards along the axis of the helix to the cibarial pump. The outer part becomes the wider, but also thin-walled, colo-rectum, which passes forwards outside the helix. In *Pulvinaria* it would appear that the colo-rectum passes through the wall of the rectal invagination, across the rectal lumen, and then turns backwards to a wide opening into the rectum.

In less highly developed forms the contact region between the ends of the intestine is simpler. In Pseudococcidae (Pesson, 1933) the filter tubes are not twisted together, but a convoluted posterior end lies against a simple anterior end. In the Margarodidae (e.g. *Icerya purchasi*) there is a simple region of contact, which is only slightly sunk into the side of the rectum. There are three Malpighian tubules in this species, which appear from Pesson's (1936) illustration to be more normal in structure than in other Sternorrhyncha. The mid-gut of the Diaspididae is reduced to a blind sac, there is no filter apparatus, and the common ureter of the two Malpighian tubules opens direct into the rectum (Pesson, 1941). Filtering mechanisms are also found in other Sternorrhyncha, in the form of simple contact between parallel lengths of intestine, ensheathed by the peritoneal layer of the posterior end of the mid-gut (Aleyrodoidea and some Aphidoidea), or with the ends of the mid-gut twisted together (Psylloidea (Fig. 5*c*)). The hind-gut is a narrow tube in Aleyrodoidea and Psylloidea, but probably capable of distension like that of the Aphidoidea.

The manner of functioning of the coccoid filter chamber must be different from that postulated in Cicadoidea, since the rectum is mostly non-glandular, and the modified Malpighian tubules, with their thick wall and narrow lumen, in which the presence of a brush or honeycomb border has not been detected, probably do not pass a large volume of fluid. Instead of an osmotic withdrawal of water from the ingesta, followed by absorption of solutes in the hind-gut, it is likely that in Coccoidea the transfer of water from the inner to the outer part of the filter tube takes place under the influence of hydrostatic pressure. Unlike that of Cicadoidea, the structure of the parts in Coccoidea is well adapted to such a mechanism. The suggestion of Berlese (1893) that water might pass into the rectum through the wall of the invagination can be rejected, not only because the alimentary canal is now known to be continuous, but because it is unlikely that sufficient pressure would be available in the lumen of the invagination. The functional reason for the invagination of the filter complex into the rectum might be to isolate it from the blood, and to restrict any water which exudes from the inner part of the helix. The depth of the columnar epithelium on the inner wall would also help to slow down the escape of water in this direction. If the cells of the outer wall of the helix are functional in absorbing solutes, such materials will be transferred into the surrounding cavity, where they will raise the osmotic pressure of any leakage from the inner part before it mixes with the blood.

IX. THE PENTATOMOMORPHA

In this group of Heteroptera the most notable change from the primitive intestinal pattern is the enlargement of the pyloric region into a conspicuous sac, the so-called

ileum (Goodchild, 1963*b*). This region is lined with bulbous cuboid or columnar cells similar to those of the anterior mid-gut, and in living tissues the cell tips can be seen to be inflated with a clear fluid and occasionally to break away from the epithelium and pass into the rectum. This is possibly a method of water excretion, since this group is essentially phytophagous. The openings of the Malpighian tubules into the ileum are much wider than in other Hemiptera and lined with epithelium similar to that of the ileum for a short distance into the tubules. In the family Pyrrhocoridae, which have adopted a diet of dry seeds, the ileum lining is thicker, and its cells tall and narrow, and the tubule openings are restricted. In the families of Pentatomomorpha in which partly or wholly sap-sucking habits have been evolved, a second characteristic feature appears. This is the development, on the section of the mid-gut immediately anterior to the ileum, of numerous tubular or pouch-like diverticula, the gastric caeca. Altogether, the pattern of the alimentary canal is as follows (Whitfield, 1929; Breakey, 1936; Harris, 1938; Yanai, 1952; Bocharova-Messner, 1961). A large anterior expansion, lined with typical bulbous mid-gut cells, is followed by a tubular intestine lined with columnar cells having a narrow brush-like border. The length of this region is variable, being long and forming several loops on the ventral side of the anterior sac in Scutellerinae and Phyllocephalinae (Pentatomidae), and in Acanthosomatidae and Largidae, but being greatly abbreviated in Urostylidae, Plataspidae, Tessaratominae and Dinidorinae (Pentatomidae). In others it is of moderate length. Posterior to the tubular region there is a bulb-like swelling, histologically similar to the preceding region, and smaller than the anterior sac. It is a permanent feature in most species, but would appear to be temporary in many Pentatomidae. The gastric caeca are borne on a tubular region which follows the posterior bulb, the length of which varies from a small fraction to as much as three-quarters of the total mid-gut. The caeca are irregularly bunched long tubes in some Lygaeidae (Rhyparochrominae and Blissinae), form two opposing longitudinal rows of finger-shaped caeca in Largidae and Berytidae (Fig. 5*d*), and in other families form long series of pouch-like caeca, the rows of which twist in a gentle helix around the central tube of the caeca-bearing region, except in Pyrrhocoridae, Rhopalidae, Lygaeinae and some other Lygaeidae, and Asopinae (Pentatomidae), in which the caeca are secondarily vestigial or absent.

In the most advanced forms the caeca are flattened in the longitudinal direction by pressure from adjacent caeca, and the connexion with the central tube is through a narrow tube, with a lumen only a few microns wide. In the Pentatomidae there are four rows of caeca and the caeca are laterally expanded so as to touch those of neighbouring rows, so that the central tube is hidden (Fig. 6*a*). The other families have only two rows of caeca, and the transparency of the connecting tube zone in fresh material may give the impression that the caeca are isolated from the mid-gut (Rosenkranz, 1940). In its natural position in the insect, the caeca-bearing region of the mid-gut lies closely against the posterior dorsal part of the anterior sac. Where the caeca are long tubes they are forwardly directed to lie in contact with this sac, and where they are short, the longer section of intestine which bears them is looped around the dorsal part of the sac. The caeca, in the living state, contain tall domed

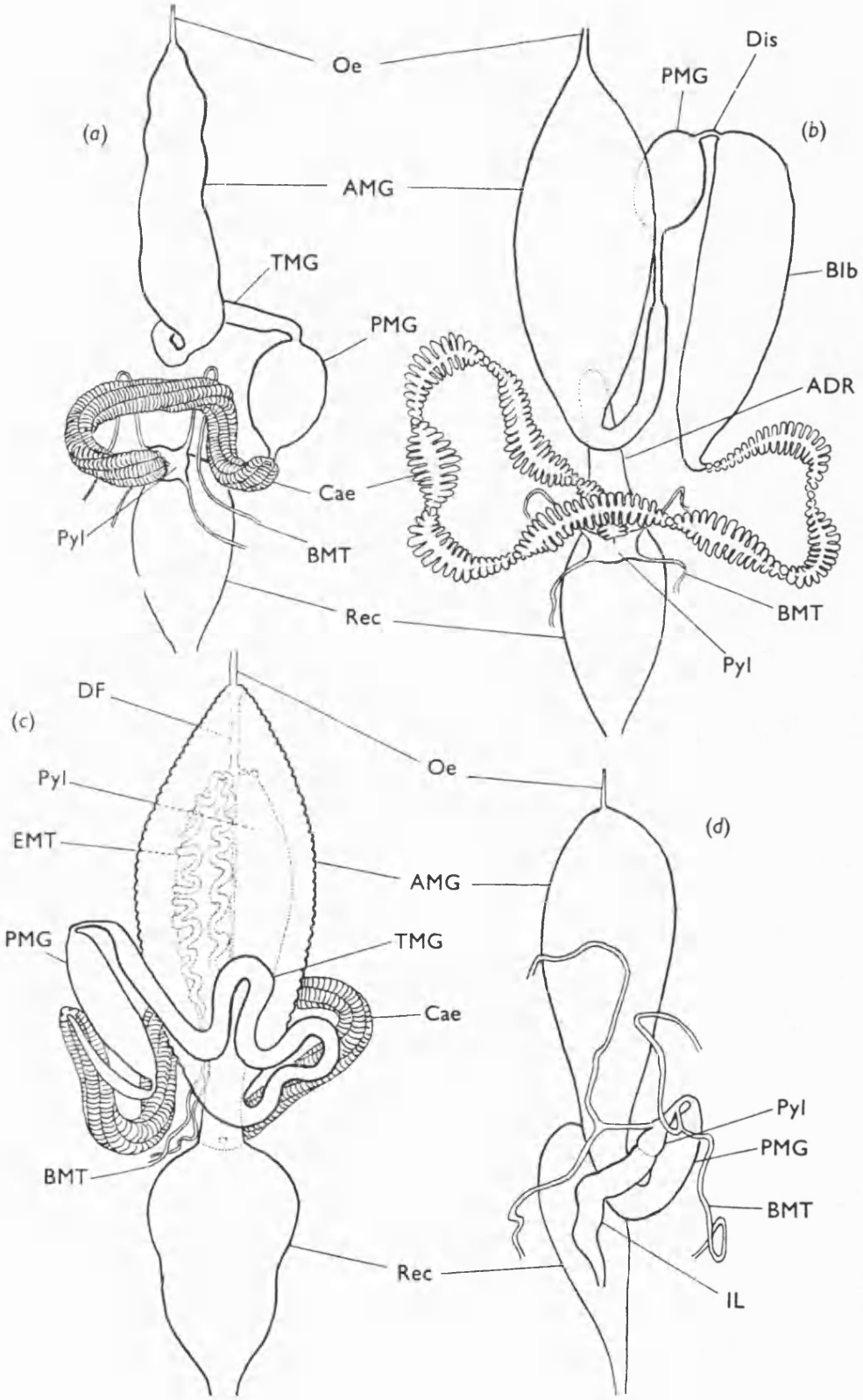


Fig. 6. *a*, Alimentary canal of unspecialized pentatomid type (based on *Agonoscelis versicolor* Fab.); *b*, the same, of *Acanthocoris obscuricornis* Dall., an advanced type of Coreidae; *c*, the same, of *Dalsira bohndorffi* Dist., Phyllocephalinae, ventral view; *d*, the same, of *Mezira scabrosa* Scott, Aradidae (from Miyamoto, 1961, with acknowledgements). ADR, anterior diverticulum of rectum; Blb, bulb at anterior end of caecal region; DF, position of dorsal folds of mid-gut sac; Dis, region of discontinuity of intestinal lumen; others as Figs. 3, 4, 5.

cells, each of which may have a large clear vacuole at its tip. In the caecal lumen there may be found transparent spheres, formed by the nipping off of the vacuolated cell tips. These discharge their contents into the connecting tube and do not leave the caecum in an intact state. Unfortunately, the vacuoles and spheres are destroyed by fixation and in histological sections the epithelium of the caeca is almost pavement-like in appearance. The caeca also contain, in most cases, large numbers of bacteria, which are readily seen in sections. In Pentatomidae these micro-organisms are distorted thread-like forms, which can neither be cultured artificially nor identified with any known species of bacteria. In the other families the caecal organisms can be cultured (Glasgow, 1914; Steinhaus, Batey & Boerke, 1956) and resemble the free-living soil bacteria of the genus *Pseudomonas*. The gastric caeca have in the past been regarded as organs developed solely for the harbourage of symbiotic bacteria, although the nature of the relationship has not been clearly established. It is possible, however, to consider the caeca as having been evolved originally for purposes of water excretion, even though in a number of cases they have undoubtedly become mycetome-like organs. The evidence for this view has been presented in full (Goodchild, 1963*b*) and may be summarized as follows. The unusual type of secretory process seen in living caeca may be a means of actively discharging a hypotonic fluid from the body, and does not appear to be in any way concerned with ordinary processes of digestion. The sac-like rectum, being lined with a chitinous intima, can safely accumulate a hypotonic excreta, but the ileum is not so lined and water might re-enter the blood through its permeable walls. It is therefore significant that in the most advanced sap-sucking groups, the Pentatomidae and Coreidae, the position of the opening of the mid-gut into the ileum has changed so as to bring it closer to the ileo-rectal (pyloric) valve, the ileum becoming a diverticulum receiving only the fluid from the Malpighian tubules. Possible osmotic flow through the wall of the central tube is minimized by its thick wall of deep columnar cells, or in Pentatomidae by surrounding it entirely by the caecal rows, and flow anteriorly along the axis of the gut is restricted by a valve-like narrowing of the lumen, which in a number of species is carried to the point of complete discontinuity (some Blissinae, larval stages of many Pentatomidae, some Urostylidae and Cydnidae, of the less specialized forms). The close anatomical association of the caeca with the anterior mid-gut is also favourable to the water-excreting hypothesis.

The essential nature of the bacterial inhabitants may also be queried, since they are variable in specific identity within a single insect species (Steinhaus *et al.* 1956) and in some cases their presence in the caeca is not constant (Goodchild, 1963*b*; Slater & Carayon, 1963). The resemblance of the bacteria to common soil types, which may be accidentally ingested (Miles, 1958; Srivastava & Rouatt, 1963), suggests that the association may be fortuitous, the purity of the caecal cultures being explained by competition between different strains of organisms (as in *Triatoma infestans* (Klug.) (Goodchild, 1955*a*)). Both Bonnemaison (1946) and Müller (1956) have succeeded in rearing bacteria-free specimens of Pentatomidae and Coptosomatidae (Plataspidae) respectively. Organisms which are known to be symbiotic, in synthesizing essential growth factors, can be harboured in intestines not having

gastric caeca, as they are in *Rhodnius prolixus* (Brecher & Wigglesworth, 1944). The possibility that the association of the insect with bacteria may not be the primary reason for the evolution of gastric caeca does not mean that the bacteria are of no value to their hosts. Indeed, in the Pentatomidae the specialized nature of the bacteria, and the development of organs in the female reproductive system which smear the eggs with a culture of infective forms, to ensure the transmission of a bacterial flora to the offspring (Rosenkranz, 1940), shows that their presence must be advantageous. In their ability to grow in the caeca, the bacteria show that the material secreted into them must contain useful materials such as amino acids and sugars, which would mostly be lost to the insect if not locked up in the bacteria. It has been observed (Miyamoto, 1961) that in those species in which the intestine is discontinuous between the third and fourth mid-gut regions, the anterior end of the latter (caeca-bearing) region is enlarged into an additional bulb (Fig. 6*b*) which is in communication with the caecal region. The size of this bulb varies from a diameter only slightly greater than the mid-gut tube, in most Pentatominae, to as large as, or larger than, the third region (posterior bulb) of the mid-gut in Dinidorinae and Tessaratominae (Pentatomidae) and some Cydnidae and Coreidae. In Pentatominae the intestine is discontinuous in the larval stages, but becomes continuous when the adult stage is reached, and the additional bulb disappears. There is a strong histological evidence from the contents of the pre-caecal bulb, that the bacteria from the caeca are being digested therein (Goodchild, 1963*b*). The contents have a fibrous hyaline appearance but grade into typical bacteria at the end nearest the caeca. Material of this kind has also been seen in the mid-gut, between the posterior bulb and the caecal rows, of less specialized Coreidae with continuous intestines and no additional bulb. There is therefore a process by which the insect can recover useful substances from the bacteria, so that they can correctly be referred to as symbiotic, but in a manner which does not contradict the water-excreting theory of gastric caeca.

In certain extreme cases, in Plataspidae, Dinidorinae, and a few Coreidae and Lygaeidae (Blissinae), the mid-gut is also discontinuous posterior to the caecal region, which then becomes an isolated mycetome-like organ, still retaining its additional bulb. Although these insects are truly sap-sucking and should therefore show the maximum development of water disposal mechanisms, the gastric caeca can no longer function in this way. The ileum, however, the water-excreting possibilities of which have already been mentioned, is in these forms well developed. In Dinidorinae its epithelial lining is folded into numerous crypts and strongly vacuolated, while in Plataspidae the whole organ is invaginated into the posterior end of the anterior mid-gut sac. It must be supposed that symbiosis with the bacteria enters a new phase in these insects and a certain amount of circumstantial evidence exists in favour of a possible nitrogen-fixing function (Goodchild, 1963*b*). Whatever may be the case, the rate of sap intake must be such that the ileum can maintain osmotic equilibrium by itself. It is noteworthy that the caeca of these insects are not seen to contain large vacuoles or transparent spheres in the living state, and in Plataspidae and Dinidorinae the caeca are shallow and tend to merge with the central tube, which is no longer thick-walled.

The subfamily Phyllocephalinae of the Pentatomidae is unique in the extreme development of the ileum (Miyamoto, 1961; Goodchild, 1963*a*), which extends forwards from the rectum, dorsal to the anterior mid-gut sac, to the anterior end of the abdominal cavity. The Malpighian tubules join it at its anterior end, and both the ileum and the proximal parts of the tubules are enclosed by folds of the anterior sac which extend upwards on each side, leaving only a narrow dorsal gap in which the heart is situated (Fig. 6*c*). This anatomical specialization can only be regarded as a kind of filter chamber, and it is interesting to note that it has the characteristic feature of the cicadoid filter chamber in the histological resemblance of the different layers. The mode of operation cannot be the same as in Cicadoidea, however, since the rectum is sac-like and weakly glandular as in other Pentatomidae, and it is probable that active secretion of hypotonic fluid takes place in the ileum. Unfortunately no studies have yet been made on fresh tissues, and in the fixed condition the ileum cells are flattened and no vacuole formation can be detected. The gastric caecal system of this subfamily is of a rather primitive pentatomid type, with neither intestinal discontinuity nor pre-caecal bulb, although the histology suggests that bacterial digestion may be taking place in the third region (posterior bulb) of the mid-gut. The opening of the mid-gut into the ileum, near its junction with the rectum, is wide in *Gonopsis affinis* Uhler (Miyamoto, 1961) and *Dalsira distinctus* Sign. (Goodchild, unpub.), but is a very narrow capillary in *D. bohndorffi* Dist. (Goodchild, 1963*a*). Unless this opening is closed during larval life, which is at present unknown, it is difficult to see why the ileum should have developed in this way. The presence of four rows of caeca shows that these insects are of pentatomid stock, but their line of evolution has clearly been separate from an early stage.

There is a high degree of correlation between the presence of caeca and preference for feeding on soft plant tissues. It is true that the Miridae and Tingidae do not possess caeca, but they have a different evolutionary background and feed upon mesophyll cells with the aid of a toxic saliva. Some caeca-bearing species have a preference for drier foods, such as developing ovules (e.g. Podopinae, Scutellerinae, some Pentatominae (Pentatomidae), Alydinae (Coreidae), Acanthosomatidae), but their caecal regions are short and in many cases it is likely that the caeca are not in communication with the lumen of the mid-gut. However, even among species feeding upon ovules with a water content as low as 50%, such as the wheat pest *Eurygaster integriceps* Put., watery excreta collect in the rectum. This was studied in some detail by Bocharova-Messner (1960), who showed that ingested water passed rapidly through the wall of the anterior mid-gut sac, and described its subsequent appearance in the rectum but without suggesting any mechanism by which this might be achieved. In a diagram, a direct movement of water through the rectal wall is indicated. Huber-Schneider (1957) also suggested that water might enter the rectum in this way, regarding the anterior diverticulum of the coreid rectum as a rudimentary filter chamber. In view of the impermeability of the rectal lining and the absence of any obvious secretory activity in its epithelium this route does not seem likely. Bocharova-Messner illustrates a section through a caecum in which circular gaps in the mass of bacteria must represent the positions of the secretory vacuoles,

but does not make any mention of this phenomenon. In the Pentatomomorpha which lack gastric caeca (Pyrrhocoridae, etc.) the diet is of dry seeds or is zoophagous, though they may pierce plants in search of water. This habit can cause confusion as to their true source of food (Puchkov, 1956). Saxena & Bhatnagar (1958) claimed to have shown that the cotton-seed bug, *Oxycaraenus hyalinipennis* (Costa), preferred succulent parts of the plant, but Bhatnagar (1963) has shown that this species only seeks such material when in a state of dessication. Saxena (1962), quoting a Ph.D. thesis of Kumari, states that *Dysdercus koenigii* Fabr. failed to reach maturity on succulent tissues alone, but grew normally on seeds. Most of the Pentatomomorpha which lack caeca, or in which only vestiges are present as in many Pyrrhocoridae, have lost them secondarily and retain a distinct thick-walled tubular region between the third mid-gut region and the ileum (Hood, 1937; Woolley, 1949; Rastogi, 1960). In some Lygaeidae (Geocorinae, Chauliopinae) and in Aradidae, the posterior bulb joins the ileum directly and it is possible that they are primitively without caeca. No histological details are available for Aradidae. In gross structure, the alimentary canal of Aradinae is much like that of primitive Lygaeidae, but that of Aneurinae and Mezirinae has a small cone-shaped ileum, connected to the rectum by a long thin-walled tube (Fig. 6*d*). Miyamoto (1961) states that this has no thick glandular wall and is not equivalent to the ileum of Cryptocerata. It may be conjectured that it is merely an elongated ileo-rectal valve. This valve tends towards a tubular shape in Coreidae (Goodchild, 1963*b*), and the exaggeration of this to the degree found in these Aradidae may have some connexion with the arrangements of the internal organs in their extremely flattened bodies. All Aradidae have a discontinuity of the mid-gut lumen between the posterior bulb and the ileum, the purpose of which is not readily apparent.

The utilization of symbiotic micro-organisms which have absorbed nutrients that would otherwise have been lost to the insect, as has been postulated for the gastric caecal system of Pentatomomorpha, may possibly be paralleled in some Fulgoroidea. It was found (Goodchild, 1963*a*) in *Gyarina nigritarsis* (Flatidae) that in newly moulted adults the rectal organ, a rounded body found on the dorsal side of the rectum in the female and consisting of a dense mass of micro-organisms, had disintegrated and its contents could be recognized not only in the rectum but in the lumen of the mid-gut throughout its length.

X. CONCLUSIONS

It will be seen from the foregoing account that there exists in the Hemiptera a considerable variety of patterns of alimentary canal structure. The most complex of these are associated with the utilization of plant sap as a source of food, which provides a strong *a priori* inference that they are concerned with disposal of excess water. Another moderately complex intestine, that of the water bugs (Cryptocerata), is also concerned with the excretion of water, which suggests that a predisposition towards this kind of adaptation in the order has contributed to their evolutionary success in that habitat. There are five different types of intestine found in sap-

sucking Hemiptera, namely those of Aphidoidea, simple and lacking Malpighian tubules; of Coccoidea, with a helically twisted filter tube invaginated into the sac-like, thin-walled rectum; of Cicadoidea, with the convoluted proximal ends of the Malpighian tubules inserted beneath the peritoneum of the anterior mid-gut to form the filter chamber, and a long, narrow, glandular hind-gut; of Fulgoroidea, with a sheath of oenocytic cells enclosing the mid-gut; and of the Pentatomomorpha, with their actively pumping cells in the ileum and gastric caeca. The specialized filter chamber of Phyllocephalinae must have arisen in the pentatomomorph line after that group had begun its adaptation to sap-sucking, and differs mainly in degree, rather than in kind, from other members of the group. Likewise, the aphidoid and coccoid patterns could be derived from a single line which had begun to exploit sap. Bearing these points in mind, it is still possible to distinguish four kinds of sap-sucking alimentary structure which must have arisen separately in evolution from a basic, mesophyll-feeding stock with simple intestines.

Some of these types have been analysed in terms of probable physiological mechanism in relation to histological structure (Goodchild, 1963*a*, *b*). It was pointed out that where water transfer between adjacent parts of the alimentary canal is concerned, there are only three possible mechanisms, these being hydrostatic pressure in the donor region, osmotic movement due to higher concentration of solutes in the receptor region, or active secretion of hypotonic fluid by the cells of the receptor region. All of these may be found in Hemiptera, the first in Coccoidea, the second in Cicadoidea, and the third in Pentatomomorpha. In addition to these, there are the Fulgoroidea and Aphidoidea which, in different ways, have solved the problem by resisting altogether the ingress of water into the haemocoel. Repeated evolution of sap-sucking might have been expected to produce a series of convergently similar alimentary structures, but the differences which are found can be shown to be a necessary consequence of the evolutionary pathways which they have followed.

It is necessary to postulate a basic mesophyll-feeding stock in which the mouth-parts had already evolved their peculiar pattern. There is no doubt that the advantage to be gained from the exploitation of deeper cell layers would be sufficient to stimulate such a development from an earlier, thrips-like surface-scratching type of mechanism. It is notable that there is a gradation in size according to the amount of nourishment available at one feeding site, from the minute Thysanoptera through the small mesophyll-feeding Jassidae, Miridae, etc., to the large, often very large, sap-sucking Cicadidae, Coreidae, Pentatomidae, and so on. It is therefore reasonable to assume that an early evolutionary radiation would have occurred at the mesophyll-feeding level, bringing with it sufficient variability for selection to operate upon, in the further development of sap-sucking adaptations.

There are among living Hemiptera certain insects, the Peloridiidae, which also form the series Coleorrhyncha of the Homoptera, that are judged to be the most primitive members of the order. China (1962) has suggested that they may indeed be living members of the extinct family Ipsviciidae of Upper Triassic date. Their basal position in the Homoptera is shown by the derivation (Müller, 1962) of symbiont types and symbiont-harbouring organs in the Auchenorrhyncha from the condition

in Coleorrhyncha. Although now associated with the Homoptera (China, 1924), the Peloridiidae were formerly allied with the Heteroptera (Reuter, 1910). They have been regarded as intermediate between the two suborders, and Pendergrast (1962) regards the salivary glands as showing resemblance to Heteroptera in their simplicity, but since they consist of two pairs of compact glands they are in fact nearer to the aphid pattern.

The ancestral population of peloridiid-like Hemiptera, feeding upon cell contents, would occasionally penetrate phloem bundles and imbibe sap, or have it forced into them by the turgor pressure of the sieve tubes (Mittler, 1957). In the absence of any mechanism for handling it, surplus water would rapidly pass into the blood, the increase in pressure and volume of which would initiate a flow through the Malpighian tubules. The unadapted rectum would not be able to extract sufficient solutes from the unaccustomed increase in flow, so that a serious loss of materials such as amino acids (which are in much lower concentration in sap than in insect blood) would ensue. Thus, most of these chance encounters with the plant vascular system would be disastrous to these insects. Of the insects which formed this primitive stock, those in which the hind-gut was more tubular, and commenced well forward in the body cavity, would be able to evolve by-pass pathways which gradually became the filter chambers of Cicadoidea and Coccoidea, though in the latter there had already begun a trend to resist the wasteful outflow of Malpighian tubule fluid by suppression of the tubules. It has been shown by Maddrell (1963) that in *Rhodnius prolixus* the secretion of the urine after a blood meal is initiated in response to neural and endocrine stimuli. Perhaps in the early Sternorrhyncha the release mechanism was first suppressed and the Malpighian tubules became anatomically modified subsequently. In the Aphidoidea the tubules have been completely lost, though in the other Sternorrhyncha a filtering arrangement in the alimentary canal was evolved before this extreme state was reached. The reduction which had taken place, however, was enough to render impossible an osmotic reabsorption mechanism as in Cicadoidea and the system became adapted to operate on hydrostatic pressure, which in turn reduced the need for a glandular hind-gut. On present evidence we may assume that the aphid filter chamber has evolved separately from that of other Sternorrhyncha, as a means of escape from the limitations on size, activity and rate of sap utilization which a turgid body imposes.

The line of evolution from the basal stock which led to the Fulgoroidea and Heteroptera must have been one in which the hind-gut was shorter and sac-like, with the Malpighian tubule junction not adjacent to the anterior end of the mid-gut, thus precluding the development of a true filter chamber. Such a rectum might have arisen as an adaptation to the formation of drier, semi-solid excreta, and in both of the groups concerned, at any rate in less specialized forms, glandular cells in the rectum are restricted to limited areas. The dorsal location in Heteroptera might be supposed to be well adapted to water absorption, the solid residues accumulating ventrally and not tending to smother the gland area. One may visualize the evolution of the fulgoroid pattern as resulting from accidental encounters with plant vascular tissue as in the previous case, until a mutation occurred which favoured the separation of an

oenocyte-rich inner layer of the epidermis to become the intestine sheath, leaving the Malpighian tubules in a normal blood environment, to remain unmodified.

All the homopteran lines of evolution remained feeders on tissues or sap of growing plants, but in the transition to the Heteroptera this habitat was deserted for the forest floor litter. The diet in this situation would necessarily be mixed, with little in the way of plant material but seeds and fungi, but with a good supply of animal life—mites, springtails, molluscs, worms and so on. Thus were laid the foundations of the present-day families of Heteroptera, the majority of which are carnivorous, and yet from among which have arisen, not merely families of plant suckers, but another dynasty of sap-suckers. The assumption of a litter habitat is borne out, on the one hand by the fact that the most primitive living Heteroptera, the Enicocephalidae and Dipsocoridae (Dipsocorimorpha of Miyamoto, 1961), are to be found in this situation, and on the other by the appropriateness of heteropteran structure to such a life. The articulation of the rostrum at the front of the head enables it to be extended in front of the insect, a position which is adopted by most seed-sucking or zoophagous Heteroptera, and also by the haematophagous forms. It is appropriate to feeding in confined spaces in litter, though the more advanced predators, such as Reduviidae and Cryptocerata, no longer living in cramped situations, use the fore limbs in capturing active prey and suck it from above. In the plant-sucking Heteroptera, the stylets are inserted into the food below or even behind the head, which further demonstrates that the change in position of articulation could not have occurred in a purely phytophagous line. The antennae, which in Homoptera follow a general trend towards shortening and reduction in number of joints as in most of the more advanced insects, with specialized chemoreceptors replacing the tactile receptors of filiform antennae, are secondarily elongated (as the small number of joints indicates) in Heteroptera. This is no doubt important in enabling them to palpate possible food objects in an environment where, unlike the broad expanses of leaf and stem, most of the objects encountered are inedible. The modifications of the wings of Heteroptera, in toughness of the fore wing and in manner of folding, would provide a flatter and smoother dorsum for penetrating piles of plant debris, and the walking habit, as contrasted with the leaping habit common in most Homoptera, must also be a consequence of life in this situation. The difficulty of escape from enemies (spiders, centipedes, other insects) in the narrow crevices in litter would have encouraged the evolution of the repugnatorial glands, which have now been clearly shown (Remold, 1963) to have this function.

A formidable assemblage of internal features has been listed by Miyamoto (1961) as characterizing different groups of Heteroptera. In addition to the variations in the pylorus region and the disposition of the rectal gland cells, which have been described earlier, the main salivary gland has a wide lumen in Pentatomomorpha, micomorpha, Saldidae and Corixidae, and a narrow one (the more primitive condition) in Dipsocorimorpha, Amphibicorisae and most Cryptocerata; the accessory salivary gland is tubular in Pentatomomorpha, but terminates in a thin-walled vesicle in all other forms (though that of Enicocephalidae is a narrow tube); and the Malpighian tubules make an anterior loop from the ventral pair in Pentatomomorpha, Cimico-

morpha and Saldidae, from the dorsal pair in Amphibicorisae and Cryptocerata, and are short, without anterior loop, in Dipsocorimorpha.

The adaptive significance of the Malpighian tubule and main salivary gland variations is not clear. The vesicular accessory gland is strongly correlated with the zoophagous habit, and its presence in primitive forms such as Dipsocoridae is not surprising. Miyamoto regards the tubular accessory gland of the Pentatomomorpha as being derived from the vesicular form. It is noticeable in the cimicomorph families which have adopted a phytophagous habit that the accessory gland duct is thickened and may be regarded as glandular, and the origin of the tubular form in this way would account for its shape, since the duct must have become elongated in order to bring the terminal vesicle into contact with the anterior mid-gut (Goodchild, 1952) so as to absorb water from that region. Those members of the Pentatomomorpha which have a zoophagous feeding habit (notably Pentatomidae Asopinae and Lygaeidae Geocorinae) have not retained a vesicular accessory gland, even in Lygaeidae, which are near to the base of the evolutionary tree of this group, nor has it been redeveloped. Many Pentatomomorpha, and particularly predatory species, have a long and sinuous accessory gland duct closely applied to the anterior mid-gut, which may to some extent functionally replace the lost vesicle. The possible value of the expanded pylorus region (ileum) in performing a limited amount of water excretion in phytophagous forms has already been mentioned. Its presence in predatory Lygaeidae is further support for the view that they have returned secondarily to the zoophagous habit. The Lygaeidae are a family in which a great variety of dietary habits may be found and the exact source of food of many species is obscure (Sweet, 1960; Eyles, 1964). Some species have even become suckers of vertebrate blood (Slater & Carayon, 1963). As a means of water excretion, the ileum seems to have had limitations, which have only been overcome in the aberrant Phyllocephalinae. Additional organs, the gastric caeca, developed for this purpose, perhaps through an intermediate stage in which the wall of the posterior mid-gut itself was strongly vacuolated (Goodchild, 1963*b*). The family Lygaeidae exhibits in its various members most of the possible shapes which the caeca could assume. Although long tubular caeca can enfold the anterior mid-gut effectively, the shape which was adopted by the families that arose out of the early lygaeid stock was that of numerous short caeca. It may be that this facilitates the discharge of their secretion into the mid-gut. The invasion of the caeca by bacteria has been advantageous to the insects, probably because useful substances are locked up in the bacterial organisms which would otherwise be lost, and thus mechanisms have evolved which enable the insect to draw on this store by digesting the bacteria. Mechanisms have also evolved, especially in the pentatomoid line, which ensure the transmission of bacteria to the next generation. This symbiosis with bacteria suggests that the degree of hypotonicity of the caecal secretion is low. Such an inefficient process may be the reason for the inadequacy of the ileum.

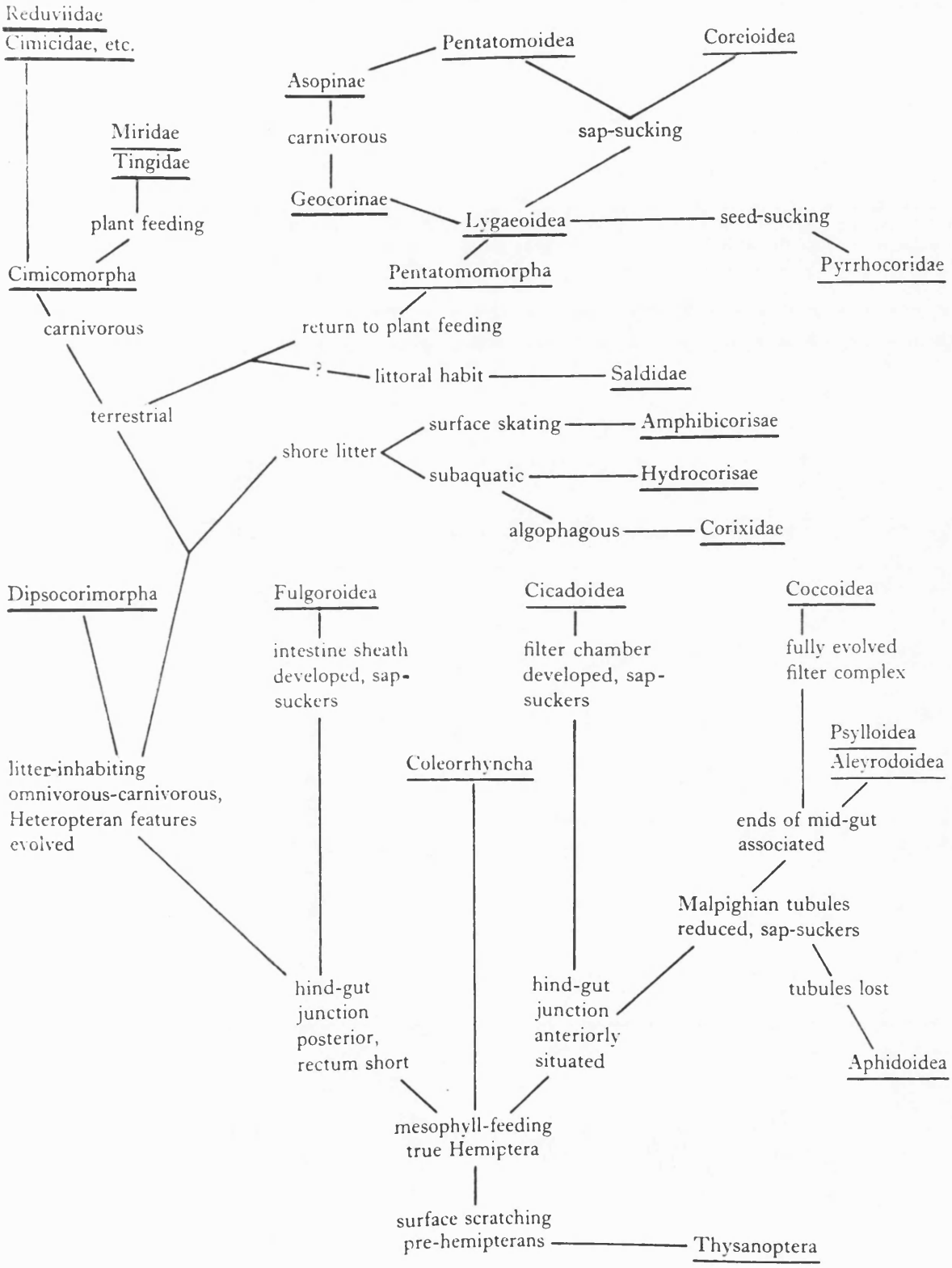
The Cimicomorpha appear, from the structure of their pylorus and rectal gland, to have followed a separate line of evolution from an early period and have become adapted to the water-conserving existence of purely terrestrial predators, the ampullae

of the pyloric region absorbing water strongly (Wigglesworth, 1931). Phytophagous habits have arisen in the families Miridae and Tingidae, and have reached an extreme in the tropical Bryocorinae, which have almost become sap-suckers and show alimentary-canal modifications. Nevertheless, the fact that their representatives in the temperate zone feed upon geologically ancient plants such as ferns must not be regarded as showing a continuity of phytophagous feeding from early times.

Many of the litter-inhabiting ancestral Heteroptera would have invaded the accumulations of debris on the shores of lakes and streams, from which it is a short step to the occupation of the water surface or subaquatic habitats. The resemblances of salivary gland and Malpighian tubule characteristics shows that the Amphibicorisae and Cryptocerata are related, and have departed from each other in adaptation of bodily shape to locomotion under different conditions. The long tubular hind-gut of Cryptocerata is clearly an adaptation to osmotic regulation, and the fusion of the apices of the Malpighian tubules and the reduction of the pylorus are probably also involved in this adaptation, the resemblance to Cicadoidea being convergent rather than an indication of a closer evolutionary connexion. In spite of some apparently primitive features of the gut and mouthparts (Sutton, 1951), the Corixidae are generally regarded (China, 1955; Marks, 1957) as arising high up on the cryptoceratan stem, so that the wide lumen of the main salivary gland must have evolved separately from that of terrestrial Heteroptera, perhaps as an adaptation to their peculiar algophagous diet. The internal features of the Saldidae strongly suggest that they are a group which has arisen near the base of the Pentatomomorpha, and in some species have evolved a slight resemblance to Cryptocerata in their hind-gut, as a response to the osmotic problems of occasional submersion. Pendergrast (1957) places them in the Geocorisae on the basis of reproductive organ structure and Parsons (1962) finds no links with either Amphibicorisae or Cryptocerata in the anatomy of the head. The dissociation of Saldidae from the ancestral line of either of the two major aquatic groups resolves the problem encountered by Brown (1948), who observed that the aquatic habit was most strongly developed in Saldids of seashore habitats (where the osmotic situation calls for water conservation, as on land), and that Saldids remain quiescent while submerged. The probable sequence of events in the evolution of Hemiptera can be represented graphically as shown in Table I.

In reviewing the patterns of alimentary canal structure in the Hemiptera, it can be seen that the basic assumptions that haemolymph dilution is an occupational hazard of sap-suckers, and that the Malpighian tubules are not adequate to maintain osmotic equilibrium in these circumstances, provides a consistent basis for the interpretation of the different types of intestine. This includes the correlation of simple types of intestine with feeding habits not involving osmotic stress. The specialized structures associated with sap-sucking have been shown to include, in addition to the previously recognized filter chambers, the gastric caeca of Heteroptera, the intestinal sheath of Fulgoroidea and the absence of Malpighian tubules in Aphidoidea. The reason why this order of insects has been so prolific of sap-sucking adaptations, in contrast to their absence in the Diptera (for example), is the absence of a stomodaeal crop,

Table 1. *Evolutionary interrelationships between divisions of Hemiptera, based on alimentary structure and function*



which must have rendered the occasional piercing of phloem bundles more frequently fatal to the insect, and intensified the selective advantage of the first mutations in the direction of water-disposal mechanisms.

XI. SUMMARY

1. Insects of the order Hemiptera are characterized by the possession of highly specialized suctorial mouthparts, which are essentially similar in structure throughout the order. In contrast to this, their alimentary canals show great variety, and, in some groups, great complexity.

2. The sources of food taken by hemipteran species range from plant sap, with a high water content, through plant cell contents, tissues of invertebrates and vertebrate blood, with moderate water content, to dry seeds. The Hemiptera are unique in the ability of many species to utilize plant sap as their only source of nourishment.

3. The presence of specialized structures in the alimentary canal is correlated with the existence of a sap-sucking habit, which necessitates the production of excreta hypotonic to the blood and to the ingesta. A modified alimentary-canal pattern is also found in the Cryptocerata, where it is concerned with the osmotic control associated with the freshwater habitat.

4. Although the existence of the various alimentary-canal specializations has in most cases been known for many years, only those complex associations of the extremities of the mid-gut which form the filter chambers of Cicadoidea and Coccoidea have hitherto been regarded as water-excreting mechanisms.

5. Following recent researches of Ramsay which suggest that the Malpighian tubules are not the site of osmoregulation in insects, but that this is effected by the glandular areas of the hind-gut, the special features of the alimentary canals of Fulgoroidea and Pentatomomorpha have been examined for anatomical and histological evidence of a water-controlling function. It has been found that the structures concerned can reasonably be interpreted in this way.

6. The general structure of the hemipteran alimentary canal has been reviewed, with particular reference to features about which there exist differences of interpretation or of nomenclature.

7. Among the Hemiptera with simple types of alimentary canal (of which the most significant feature, compared with other orders, is the absence of a stomodaeal crop), the Coleorrhyncha (Peloridiidae) are primitively simple and probably feed on plant cells. The Cimicomorpha and Amphibicorisae have developed from an omnivorous or carnivorous basal heteropteran stock, into terrestrial and water-surface inhabiting carnivores respectively. Of the Cimicomorpha, many Miridae and Tingidae have returned to a plant-feeding habit, but in a carnivore-like manner, using a toxic saliva to destroy patches of cells. The tropical Miridae Bryocorinae feed on plant tissues of higher water-content and their alimentary canal is modified by the lack of water-absorbing structures.

8. The Aphidoidea, although they have a simple type of alimentary canal, are mostly sap-suckers. Since the function of specialized structures in other sap-sucking groups appears to be that of minimizing dilution of the blood by osmotically imbibed

water (as well as facilitating a rapid throughput of sap), it is suggested that such dilution is prevented in aphids by the development of a turgor pressure in the insect's body. This theory might account for the absence of Malpighian tubules in this group, as an adaptation to prevent leakage of solutes under the increased internal pressure, and for the subglobular shape, small size and slow movements characteristic of aphids.

9. The structure of the filter chambers of Cicadoidea and Coccoidea has been reviewed and an analysis of their function has been made. The anatomy and histology of the parts in Cicadoidea suggests that water is extracted from the ingesta by passive osmosis to the fluid passing down the Malpighian tubules, solutes being subsequently absorbed in the hind-gut so as to form hypotonic excreta. In Coccoidea it would seem that hydrostatic pressure in the anterior part of the mid-gut is the operative factor.

10. The structure of the alimentary canal of Fulgoroidea has been discussed and it is suggested that the membranous sheath which surrounds the intestinal coils may be physiologically significant in preventing dilution of the blood. This interpretation also provides a reasonable explanation for the air-filled anterior diverticulum of the mid-gut of these insects.

11. In the Pentatomomorpha the alimentary canal departs from the primitive condition in two ways: the presence of an enlarged, sac-like, pyloric region, and the development of a series of hollow outgrowths of the posterior mid-gut, the gastric caeca. These features are well known, but have not previously been regarded as organs of water excretion. The evidence for this interpretation is presented and it is shown to be compatible with earlier theories of the gastric caeca as harbourages for symbiotic bacteria, since the bacteria may absorb materials which would otherwise be lost, and are themselves digested and absorbed. In the most highly evolved species, which occur in several different families, the gastric caecal system is isolated by interruption of the mid-gut both anteriorly and posteriorly. There is evidence to suggest that the bacteria in these insects actively supplement the source of nutrition, possibly by nitrogen fixation. The remarkable structure of the Phyllocephalinae (Pentatomidae), where the pyloric expansion is arranged in the manner of a filter chamber, is in need of further study on living insects, but confirms the idea that the pylorus of Pentatomomorpha is concerned with water excretion.

12. Altogether, five different types of alimentary canal are associated with feeding on plant sap, namely those of Aphidoidea, Coccoidea, Cicadoidea, Fulgoroidea, and Pentatomomorpha. Although convergent in food habit, these groups show no convergent resemblance in alimentary structure. This is the result of the operation of Dollo's Law, concerning the failure of organisms to regenerate structures lost in the course of evolution. The first three groups named must have arisen from an ancestry in which the hind-gut extended far forward in the abdominal cavity, so that the extremities of the mid-gut were close together. This would make possible the by-passing of the mid-gut by means of a filter chamber. In the ancestors of the Cicadoidea the Malpighian tubules were fully developed, but in the line leading to Coccoidea suppression of the tubules and restriction of their outflow had begun. A different filtration mechanism, and, correspondingly, a different anatomical arrangement, was evolved. In the Aphidoidea the suppression of the tubules was complete,

and in the majority of species no filter chamber has been evolved. The Fulgoroidea and Heteroptera are probably derived from a line in which the hind-gut was short, sac-like, and confined to the posterior part of the abdomen. This may have evolved as an adaptation to the production of semi-solid excreta, perhaps in an arid environment which encouraged water retention. This type of alimentary canal could not develop a filter chamber, owing to the separation of the mid-gut extremities. The Fulgoroidea remained phytophagous, and with the evolution of the intestine sheath were able to become sap-suckers. The Heteroptera evolved probably in a litter habitat and their characteristic features can be regarded as adaptations to this life. It also resulted in the adoption of carnivorous habits, which have been retained by most families. The Pentatomomorpha separated from the basal heteropteran stock as a line which had returned to plant feeding, first as mesophyll feeders but in their most highly evolved forms as true sap-suckers.

XII. REFERENCES

ABDEL-MALEK, A. A. & BALDWIN, W. F. (1961). Specificity of plant feeding in mosquitoes as determined by radioactive phosphorus. *Nature, Lond.*, **192**, 178–9.

AUCLAIR, J. L. (1958). Honeydew excretion in the pea aphid, *Acyrthosiphon pisum* (Harr.). *J. Insect Physiol.* **2**, 330–7.

AUCLAIR, J. L. (1963). Aphid feeding and nutrition. *A. Rev. Ent.* **8**, 439–90.

BAHADUR, J. (1961). The Malpighian tubules of certain aquatic Heteroptera; Hemiptera. *Ann. Mag. nat. Hist.* (13) **4**, 341–40.

BAHADUR, J. (1963). Rectal pads in the Heteroptera. *Proc. R. ent. Soc. Lond.* (A), **38**, 59–69.

BERLESE, A. (1893). Le cocciniglie italiani viventi sugli agrumi. *Riv. Patol veg., Padova*, **3**, 107–201.

BHATNAGAR, P. L. (1963). Certain factors influencing the establishment of *Oxycaraenus hyalinipennis Costa* (Heteroptera, Lygaeidae) on various plants. I. Ingestion of food. *Indian J. Ent.* **25**, 48–62.

Бочарова-Месснер О. М. (Bocharova-Messner, O. M.) (1960). Пищеварительный аппарат вредной черепашки (*Eurygaster integriceps* Put.) и функциональная специфика его отделов. *Вредная Черепашка, Vred. Cherep.* **4**, 6–32.

BONNEMAISON, L. (1946). Remarques sur la symbiose chez les Pentatomidae (Hem.). *Bull. Soc. ent. Fr.* **51**, 40–2.

BORNER, C. (1938). Neuer Beitrag zur Systematik und Stammesgeschichte der Blattläuse. *Abh. naturw. Ver. Bremen*, **30**, 167–79.

BREAKEY, E. P. (1936). Histological studies of the digestive system of the Squash Bug (*Anasa tristis* De G. Hemiptera, Coreidae). *Ann. ent. Soc. Am.* **26**, 561–77.

BRECHER, G. & WIGGLESWORTH, V. B. (1944). The transmission of *Actinomyces rhodnii* Erikson in *Rhodnius prolixus* Stål (Hemiptera) and its influence on the growth of the host. *Parasitology*, **35**, 220–4.

BRITTAIN, W. H. (1923). The morphology and synonymy of *Psylla mali* Schmidt. *Proc. Acadian ent. Soc.* **8**, 1–16.

BROADBENT, L. (1951). Aphid excretion. *Proc. R. ent. Soc. Lond.* (A), **26**, 97–103.

BROWN, E. S. (1948). The ecology of Saldidae (Hemiptera-Heteroptera) inhabiting a salt-marsh, with observations on the evolution of aquatic habits in insects. *J. Anim. Ecol.* **17**, 180–8.

CECIL, R. (1930). The alimentary canal of *Philaenus leucopthalmus*. *Ohio J. Sci.* **30**, 120–8.

CHILDS, L. (1914). The anatomy of the diaspine scale insect, *Epidiaspis piricola*. *Ann. ent. Soc. Am.* **7**, 47–57.

CHINA, W. E. (1924). A new genus of Peloridiidae (Hemiptera) from Tasmania. *Ent. mon. Mag.* **60**, 199–203.

CHINA, W. E. (1955). The evolution of the water bugs. *Bull. Symp. org. Evol., Natn Inst. Sci. India*, **7**, 91–103.

CHINA, W. E. (1962). South American Peloridiidae (Hemiptera-Homoptera: Coleorrhyncha). *Trans. R. ent. Soc. Lond.* **114**, 131–61.

COCKBAIN, A. J. (1961). Water relationships of *Aphis fabae* Scop. during tethered flight. *J. exp. Biol.* **38**, 175–80.

CRAGG, F. W. (1915). The alimentary tract of *Cimex*. *Ind. J. med. Res.* **2**, 706–20.
DAVIDSON, J. (1913). The structure and biology of *Schizoneura lanigera* Hausmann, or woolly aphis of the apple tree. I. The apterous viviparous female. *Q. Jl. microsc. Sci.* **58**, 653–701.
DOBROSCKY, I. D. (1931). Morphological and cytological studies on the salivary glands and alimentary tract of *Cicadula sexnotata* (Fallen), the carrier of aster yellows virus. *Contr. Boyce Thomson Inst. Pl. Res.* **3**, 39–58.
DUFOUR, L. (1833). Recherches anatomiques et physiologiques sur les Hémiptères. *Mem. Sav. étr. Acad. Sci., Paris*, **4**, 129–462.
EYLES, A. C. (1964). Feeding habits of some Rhyparochrominae (Heteroptera: Lygaeidae) with particular reference to the value of natural foods. *Trans. R. ent. Soc. Lond.* **116**, 89–114.
GLASGOW, H. (1914). The gastric caeca and caecal bacteria of Heteroptera. *Biol. Bull. mar. biol. Lab., Woods Hole*, **26**, 101–70.
GOODCHILD, A. J. P. (1952). A study of the digestive system of the West African cacao capsid bugs (Hemiptera, Miridae). *Proc. zool. Soc. Lond.* **122**, 543–72.
GOODCHILD, A. J. P. (1955*a*). The bacteria associated with *Triatoma infestans* and some other species of Reduviidae. *Parasitology*, **45**, 441–8.
GOODCHILD, A. J. P. (1955*b*). Some observations on growth and egg production of the blood-sucking Reduviids, *Rhodnius prolixus* and *Triatoma infestans*. *Proc. R. ent. Soc. Lond.* (A), **30**, 136–44.
GOODCHILD, A. J. P. (1963*a*). Some new observations on the internal structures concerned with water disposal in sap-sucking Hemiptera. *Trans. R. ent. Soc. Lond.* **115**, 217–37.
GOODCHILD, A. J. P. (1963*b*). Studies on the functional anatomy of the intestine in the Heteroptera. *Proc. zool. Soc. Lond.* **141**, 851–910.
GRASSÉ, P. P. (ed.) (1951). *Traité de Zoologie*, **10** (2). Paris.
HAMILTON, M. A. (1931). The morphology of the water scorpion, *Nepa cinerea* L. (Rhynchota, Heteroptera). *Proc. zool. Soc. Lond.* **101**, 1067–136.
HAMNER, A. L. (1936). The gross anatomy of the alimentary canal of *Solubea pugnax* (Fab.) (Heteroptera, Pentatomidae). *Ohio J. Sci*, **36**, 157–60.
HARRIS, C. S. (1938). The anatomy and histology of the alimentary system of the Harlequin Cabbage Bug, *Murgantia histrionica* Hahn. (Hemiptera, Pentatomidae). *Ohio J. Sci.* **38**, 316–31.
HICKERNELL, L. M. (1920). The digestive system of the Periodical Cicada, *Tibicen septendecim* L. *Ann. ent. Soc. Am.* **13**, 223–48.
HOLDGATE, M. W. (1956). Transpiration through the cuticles of some aquatic insects. *J. exp. Biol.* **33**, 107–18.
HOOD, C. W. (1937). The anatomy of the digestive system of *Oncopeltus fasciatus* Dall. (Hemiptera, Lygaeidae). *Ohio J. Sci.* **37**, 151–60.
HOUGH, W. S. (1925). The internal anatomy of the Clover Root Mealy-bug, *Trionymus trifolii* Forbes (Homoptera, Coccidae). *Bull. ent. Res.* **16**, 25–9.
HUBER-SCHNEIDER, L. (1957). Morphologische und physiologische Untersuchungen an der Wanze *Mesocerus marginatus* L. und ihren Symbionten. *Z. Morph. Ökol. Tiere*, **46**, 433–80.
IMMS, A. D. (1937). *Recent advances in entomology*. London.
KERSHAW, J. C. W. (1910). A memoir on the anatomy and life history of the homopterous insect *Pyrops candelaria* (or 'candle-fly'). *Zool. Jb.* (Syst.), **29**, 105–24.
KERSHAW, J. C. W. (1913*a*). The alimentary canal of *Flata* and other Homoptera. *Psyche, Camb.* **20**, 175–88.
KERSHAW, J. C. W. (1913*b*). Anatomical notes on a Membracid. *Annls. Soc. ent. Belg.* **57**, 191–201.
KERSHAW, J. C. W. (1914). The alimentary canal of a Cercopid. *Psyche. Camb.* **21**, 65–72.
KNOWLTON, G. F. (1925). The digestive tract of *Longistigma caryae*. *Ohio. J. Sci.* **25**, 244–50.
KRAMER, S. & WIGGLESWORTH, V. B. (1950). The outer layers of the cuticle in the cockroach *Periplaneta americana* and the function of the oenocytes. *Quart. J. micr. Sci.* **91**, 63–72.
KURUP, N. G. (1961–62). On the digestive tract of some Cryptocerata. I, II, III. *J. zool. Soc. India*, **13**, 16–23, 128–35; **14**, 73–87.
KUSKOP, M. (1923). Bakteriensymbiosen bei Wanzen. *Arch. Protistenk.* **47**, 350–85.
LAMB, K. P. (1963). Water loss from aphids at moderate temperatures. *J. Insect Physiol.* **9**, 703–11.
LICENT, P. E. (1912). Recherches d'anatomie et de physiologie comparée sur le tube digestif des Homoptères supérieurs. *Cellule*, **28**, 1–161.
LOCY, W. A. (1884). Anatomy and physiology of the family Nepidae. *Am. Nat.* **18**, 250–5.
MADDRELL, S. H. P. (1963). Excretion in the blood-sucking bug, *Rhodnius prolixus* Stål. I. The control of diuresis. *J. exp. Biol.* **40**, 247–56.
MALOUF, N. S. R. (1933). Studies on the internal anatomy of the stink bug, *Nezara viridula* L. *Bull. Soc. ent. Egypte*, **17**, 96–119.
MARKS, E. P. (1957). The food pump of *Pelocoris* and comparative studies on other aquatic Hemiptera. *Psyche, Camb.* **64**, 123–34.

MILES, P. W. (1958). Contact chemoreception in some Heteroptera, including chemoreception internal to the stylet food canal. *J. Insect Physiol.* **2**, 338–47.

MISRA, A. B. (1931). On the internal anatomy of the female lac insect, *Laccifera lacca* Kerr (Homoptera, Coccidae). *Proc. zool. Soc. Lond.* **101**, 303–8.

MITTLER, T. E. (1957). Studies on the feeding and nutrition of *Tuberolachnus salignus* (Gmelin). I. The uptake of phloem sap. *J. exp. Biol.* **34**, 334–41.

MITTLER, T. E. (1958*a*). The excretion of honeydew by *Tuberolachnus salignus* (Gmelin). *Proc. R. ent. Soc. Lond.* (A), **33**, 49–55.

MITTLER, T. E. (1958*b*). Studies on the feeding and nutrition of *Tuberolachnus salignus* (Gmelin). II. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. *J. exp. Biol.* **35**, 74–84.

MIYAMOTO, S. (1961). Comparative morphology of alimentary organs of Heteroptera, with the phylogenetic consideration. *Sieboldia*, **2**, 197–259.

MUKHARJI, S. P. (1961). The structure and histology of the alimentary canal of the Homoptera (Hemiptera). *Indian J. Zootomy*, **2**, 56–121.

MÜLLER, H. J. (1956). Experimentelle Studien an der Symbiose von *Coptosoma scutellatum* Geoffr. (Hem., Heteropt.). *Z. Morph. Ökol. Tiere*, **44**, 459–82.

MÜLLER, H. J. (1962). Neuere Vorstellungen über Verbreitung und Phylogenie der Endosymbiosen der Zikaden. *Z. Morph. Ökol. Tiere*, **51**, 190–210.

NEGI, P. S. (1934). The alimentary canal, its appendages, salivary glands, and the nervous system of the adult female lac insect, *Laccifera lacca* Kerr (Coccidae). *Bull. ent. Res.* **25**, 541–50.

NUORTEVA, P. (1958). Die Rolle der Speichelsekrete im Wechsel-Verhältnis zwischen Tier und Nahrungspflanze bei Homopteren und Heteropteren. *Ent. exp. & appl.* **1**, 41–9.

PAINTER, R. H. (1930). A study of the cotton flea hopper, *Psallus seriatus* Reut., with especial reference to its effect on cotton plant tissues. *J. agric. Res.* **40**, 485–516.

PARSONS, M. C. (1957*a*). The presence of a peritrophic membrane in some aquatic Hemiptera. *Psyche, Camb.* **64**, 117–22.

PARSONS, M. C. (1957*b*). The oesophageal valves of some aquatic Hemiptera. *Psyche, Camb.* **64**, 135–48.

PARSONS, M. C. (1959). The mid-gut of aquatic Hemiptera. *J. Morph.* **104**, 479–525.

PARSONS, M. C. (1962). Skeleton and musculature of the head of *Saldula pallipes* (F.) (Heteroptera, Saldidae). *Trans R. ent. Soc. Lond.* **114**, 97–130.

PENDERGRAST, J. G. (1957). Studies on the reproductive organs of the Heteroptera with a consideration of their bearing on classification. *Trans. R. ent. Soc. Lond.* **109**, 1–63.

PENDERGRAST, J. G. (1962). The internal anatomy of the Peloridiidae (Homoptera: Coleorrhyncha). *Trans. R. ent. Soc. Lond.* **114**, 49–65.

PESSON, P. (1933). Contributions à l'étude du tube digestif des Coccides. I. Pseudococcinae. *Bull. Soc. zool. Fr.* **58**, 404–22.

PESSON, P. (1935). II. Lecaniinae. *Bull. biol. Fr. Belg.* **69**, 138–52.

PESSON, P. (1936). III. Monophlebinae. *Bull. Soc. zool. Fr.* **61**, 282–93.

PESSON, P. (1941). IV. Diaspinae. *Bull. Soc. zool. Fr.* **66**, 230–8.

POISSON, R. (1924). Contribution à l'étude des Hémiptères aquatiques. *Bull. biol. Fr. Belg.* **58**, 49–305

PRADHAN, S. (1940). The alimentary canal and pro-epithelial regeneration in *Coccinella septempunctata*, with a comparison of carnivorous and herbivorous coccinellids. *Q. Jl. microsc. Sci.* **81**, 451–78.

Пучков В. Г. (Puchkov, V. G.) (1956). Основные трофические группы растительноядных полужесткокрылых насекомых и изменение характера их питания в процессе развития. *Зоологический Журнал. Zool. Zh.* **35**, 32–44. (Summary in English.)

QADRI, M. A. H. (1949). On the digestive system and the skeleto-muscular structure of the head capsule in the mango-hoppers, *Idiocerus niveosparsus* Leth. and *Idioceris clypealis* Leth. (Jassidae). *Proc. zool. Soc. Bengal*, **2**, 43–55.

RAFIQ KHAN, M. & FORD, J. B. (1962). Studies on digestive enzyme production and its relationship to the cytology of the mid-gut epithelium in *Dysdercus fasciatus* Sign (Hemiptera, Pyrrhocoridae). *J. Insect Physiol.* **8**, 597–608.

RAMSAY, J. A. (1950). Osmotic regulation in mosquito larvae. *J. exp. Biol.* **27**, 145–57.

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

RASTOGI, S. C. (1960). The anatomy of the digestive system of *Lygaeus pandurus* Scop. (Heteroptera: Lygaeidae). *Proc. Rajasthan Acad. Sci.* **7**, 29–37.

RASTOGI, S. C. (1961). The anatomy of the digestive organs of *Sphaerodema rusticum* Fabr. (Heteroptera: Belostomatidae). *Proc. Rajasthan Acad. Sci.* **8**, 60–78.

RASTOGI, S. C. (1962*a*). The status of ileum in Heteroptera. *Sci. Cult.* **28**, 31–2.
RASTOGI, S. C. (1962*b*). Anatomy of the digestive organs of *Ranatra elongata* (Fabr.) (Heteroptera: Nepidae). *Proc. Rajasthan Acad. Sci.* **9**, 35–44.
RAWAT, B. L. (1929). Notes on the anatomy of *Naucoris cimicoides* L. (Hemiptera-Heteroptera). *Zool. Jb.* (Anat.), **65**, 535–600.
REMOLD, H. (1963). Scent-glands of land-bugs, their physiology and biological function. *Nature, Lond.*, **198**, 764–8.
REUTER, O. M. (1910). Neue Beiträge zur Phylogenie und Systematik der Miriden. *Acta Soc. Sci. fenn.* **37**, 1–172.
ROSENKRANZ, W. (1940). Die Symbiose der Pentatomiden (Hemiptera Heteroptera). *Z. Morph. Ökol. Tiere*, **36**, 279–309.
SAXENA, K. N. (1955). The anatomy and histology of the digestive organs and Malpighian tubules of the Jassidae (Homoptera). *J. zool. Soc. India*, **7**, 41–52.
SAXENA, K. N. (1962). Food intake in relation to the growth of *Dysdercus koenigii* Fabr. (Heteroptera). *Ann. ent. Soc. Am.* **55**, 218–25.
SAXENA, K. N. & BHATNAGAR, P. (1958). Physiological adaptations of Dusky Cotton Bug, *Oxycaraenus hyalinipennis* (Costa) (Heteroptera: Lygaeidae) to its host plant, cotton. *Proc. natn. Inst. Sci. India*, **24**, 245–57.
SCHNEIDER, G. (1940). Beiträge zur Kenntnis der symbiontischen Einrichtungen der Heteropteren. *Z. Morph. Ökol. Tiere*, **36**, 595–644.
SHARGA, U. S. (1933). On the internal anatomy of some Thysanoptera. *Trans. R. ent. Soc. Lond.* **81**, 185–204.
SLATER, J. A. & CARAYON, J. (1963). Ethiopian Lygaeidae. IV. A new predatory Lygaeid from Africa with a discussion of its biology and morphology (Hemiptera: Heteroptera). *Proc. R. ent. Soc. Lond.* (A), **38**, 1–11.
SMITH, L. M. (1937). Growth, reproduction, feeding and wing development of the Mealy Plum Aphid in relation to climatic factors. *J. agric. Res.* **54**, 345–64.
SPRAGUE, I. B. (1956). The biology and morphology of *Hydrometra martini* Kirkaldy. *Kans. Univ. Sci. Bull.* **38**, 579–693.
SRIVASTAVA, P. N. & ROUATT, J. W. (1963). Bacteria from the alimentary canal of the Pea Aphid, *Acyrtosiphon pisum* (Harr.) (Homoptera, Aphididae). *J. Insect Physiol.* **9**, 435–8.
SRIVASTAVA, U. S. & BAHADUR, I. (1961). The development of the Malpighian tubules in *Dysdercus koenigii* Fabr. (Hemiptera, Pyrrhocoridae). *Q. Jl. microsc. Sci.* **102**, 347–60.
STADDON, B. W. (1963). Water balance in the aquatic bugs *Notonecta glauca* L. and *Notonecta marmorea* Fabr. (Hemiptera; Heteroptera). *J. exp. Biol.* **40**, 563-71.
STADDON, B. W. (1964). Water balance in *Corixa dentipes* (Thoms.). *J. exp. Biol.* **41**, 609–19.
STEINHAUS, E. A., BATEY, M. M. & BOERKE, C. L. (1956). Bacterial symbiotes from the caeca of certain Heteroptera. *Hilgardia*, **24**, 495–518.
SUTTON, M. F. (1951). On the food, feeding mechanism, and alimentary canal of Corixidae (Hemiptera-Heteroptera). *Proc. zool. Soc. Lond.* **121**, 465–99.
SWEET, M. H. (1960). The seed bugs, a contribution to the feeding habits of the Lygaeidae (Hemiptera-Heteroptera). *Ann. ent. Soc. Am.* **53**, 317–21.
VODJDANI, S. (1954). Contribution à l'étude des Punaises des céréales et en particulier d'*Eurygaster integriceps* Put. (Hemiptera, Pentatomidae). *Annls. Epiphyt.* (Ser. C) **5**, 105–60.
WEBER, H. (1928). Skelett, Muskulatur und Darm der schwarzen Blattlause, *Aphis fabae* Scop., mit besondere Berücksichtigung der Funktion der Mundwerkzeuge und des Darmes. *Zoologica, Stuttg.* **28**, 5–118.
WEBER, H. (1930). *Biologie der Hemipteren.* Berlin.
WHITFIELD, F. G. SAREL- (1929). The Sudan millet bug, *Agonoscelis diversicolor* F. *Bull. ent. Res.* **20**, 209–24.
WIGGLESWORTH, V. B. (1931). The physiology of excretion in a blood-sucking insect, *Rhodnius prolixus* (Hemiptera, Reduviidae). *J. exp. Biol.* **8**, 411–51.
WIGGLESWORTH, V. B. (1950). *The principles of insect physiology.* London.
WIGGLESWORTH, V. B. (1963). A further function of the air sacs in some insects. *Nature, Lond.*, **198**, 106.
WILLIS, D. M. (1949). The anatomy and histology of the head, gut and associated structures of *Typhlocyba ulmi. Proc. zool. Soc. Lond.* **118**, 984–1001.
WOOLLEY, T. A. (1949). Studies on the internal anatomy of the box-elder bug (*Leptocoris trivittatus* (Say), Hemiptera, Coreidae). *Ann. ent. Soc. Am.* **42**, 203–26.
YANAI, T. (1952). Morphological and histological studies of the mid-intestine of heteropterans, with special reference to binucleated epithelial cells. *Annotnes zool. jap.* **25**, 427–33.
YANAI, T. & IGA, T. (1956). Further study on the binucleated epithelial cells in the mid-intestine of heteropterous insects. *Cytologia*, **21**, 183–7.

XIII. ADDENDUM

Although no paper on the Hemiptera which has been published, or has come to my attention, since this article went to press contains information which affects the validity of the views expressed above, it is worth mentioning some similar studies which have been pursued on a parallel course independently of my own. Bahadur (1963) has surveyed the structure of the so-called 'ileum' of eighteen species of terrestrial Heteroptera and suggested the name 'excretory vesicle' for this region of the gut, since it receives the fluid from the Malpighian tubules. While he accepts that the four ampullae of the Cimicomorpha are water-absorbing organs, he does not offer any explanation of the large sac-like vesicle of the Pentatomomorpha. Rastogi (1965*a*) has examined the oesophageal valves of six species of terrestrial and three species of aquatic Heteroptera, and arranged them into five types on essentially the same lines as indicated above. Although the structure of these valves is clearly capable of preventing regurgitation of alimentary contents, this author seems to be confused by the numerous records of pre- or post-feeding salivary exudation into supposing alimentary regurgitation to be common in Hemiptera. In fact, this is not the case. This confusion has led Rastogi to seem to cast doubt on my observations on cacao capsid bugs (Goodchild, 1952), but the salivary source of this 'regurgitation' was clear from several lines of morphological and experimental evidence. The suggestion is also made by this author that the elongated and largely chitinous valve of Corixidae and Pentatomidae is a primitive relic of the peritrophic membrane. However, in those insects where the oesophageal valve is concerned with the formation of the peritrophic membrane, by acting as an annular mould, the cell layers are well developed, and resemble the valves of Aphididae or Miridae. It would, perhaps, be true to say of the Corixidae and Pentatomidae that their oesophageal valve structure is a secondary return to a partial peritrophic membrane, for the reason which I have suggested above, and like all secondary re-developments of lost structures it does not reproduce the ancestral structure in detail, but adapts existing structures to a similar function. In another paper, the same author (Rastogi, 1965*b*) describes denticles in the epipharyngeal region of *Coridius janus* (Fabr.) (Dinidoridae), which he compares with those found in Corixidae and Naucoridae (Sutton, 1951), and again regards as possibly primitive. In view of the extremely small number of observations which have been made that might have revealed these structures in other species of Hemiptera, their apparent absence in those species is insufficient evidence for the phylogenetic significance of their presence. If they are regarded as functioning in the comminution of particulate food (as, for example, in Corixidae), it is important to note that the alimentary canal of *C. janus* is very unlike that of other Dinidoridae in lacking the gastric caeca and the interrupted lumen (Rastogi, personal communication, 15 March 1961), and a form of mesophyll feeding is to be expected.

REFERENCES

BAHADUR, J. (1963). On the so-called 'Ileum' of Heteroptera. *Zool. Anz.* **170**, 354–64.

RASTOGI, S. C. (1965*a*). The oesophageal valve of some terrestrial and aquatic Heteroptera. *Zool. Anz.* **174**, 337–47.

RASTOGI, S. C. (1965*b*). The food pump and associated structures in *Coridius janus* (Fabr.) (Heteroptera: Dinidoridae). *Proc. R. ent. Soc. Lond.* (A), **40**, 125–34.

Reprinted from the East African Agricultural and Forestry Journal, Vol. XXXIII, No. 2, October 1967

APPENDIX 5

SHIELD BUG (Piezosternum calidum Fab.) INFESTATION OF OYSTER NUT

By A. J. P. Goodchild, Bath University of Technology, England

(Received for publication on 6th March 1967)

During a visit to Makerere University College, Kampala, from July to September 1966, the writer had the opportunity of observing an intense infestation of a plant of the Oyster Nut (*Telfairea pedata* Hook. Cucurbitaceae), growing in the University Botanic Garden, by the shield bug *Piezosternum calidum* (Tessaratomidae). The effect on the plant was very severe. In the articles dealing with this crop, in earlier volumes of this journal, the only insect pests mentioned are those with generalized feeding habits, which also attack many other types of crop. Swynnerton (1937) mentions termite damage as shortening the useful life of the plants in the Tanganyikan plains, while Poppleton (1940) refers to attacks by grasshoppers on young plants. According to Greenway (1945) the plants are usually grown in a scattered fashion by native farmers, because in a close stand they are subject to pests and diseases, but this author does not mention any particular pests. Oysternut plants may also be affected by mammals (rodents or ungulates) and by the root nematode *Heterodera marioni*, which causes spongy swellings just below the soil (Nattrass, 1941). There does not appear to be any record of attack by a pest of the nature of *P. calidum*, that is, a sap-sucking insect with a restricted range of host plant species. In the writer's experience, and from records in the British Museum collection, this species occurs on species of *Momordica* (Cucurbitaceae) only, though the solitary immature specimen in the British Museum was collected on "passion fruit" in Tanganyika. This infestation represents a change of host plant species for this insect, and perhaps the severe reaction of the plant was partly due to lack of adaptation to the saliva injected by the pest. It may be that a race of insects is arising which can infest Oyster Nut, and possibly in due course other cultivated Cucurbitaceae, just as many other insect pests, the cacao capsid bugs for example, have turned from wild host plants to crop plants, in recent years. Although this is the first record of *P. calidum* breeding on a crop (and indeed the first record of any investigation into its breeding habits), there is an early record (Mayné, 1917) of adults causing mild damage to cacao pods, and a related species, *P. fallax*, has been seen on cacao in Nigeria (Golding, 1941). In both cases the pest status was of negligible importance.

The Insect

Piezosternum calidum is a moderately large insect, about $\frac{3}{4}$ inch long and $\frac{3}{8}$ inch broad, with the shield-like shape characteristic of this group (FIG. 1). The back is smooth, shiny, and dark green in colour, with dark blue-grey wing membranes covering the posterior part of the abdomen. The head, anterior margin of the thorax, and legs are yellow (the latter with green patches at the joints). The narrow antennae, $\frac{3}{8}$ inch long, and the proboscis (which encloses the sucking mouth parts) are brown. The edge of the abdomen, which can be seen outside the edges of the folded wings, is serrated, due to the prolongation of the posterior margin of each segment into a short spine.

Plate I—*Piezosternum calidum* adults feeding on stem of Oyster Nut

When the insect flies, the transparent dark-blue hind wings, and the iridescent dark-blue dorsal surface of the abdomen, can be seen. The underside is deep orange, with narrow transverse black lines at the margin of each segment of the abdomen, and a central longitudinal black line. The third thoracic segment bears, between the bases of the third pair of legs, a conspicuous sternal ridge, which extends forwards below the second thoracic segment, becoming narrower and deeper, and terminates abruptly at the posterior margin of the first segment, where the tip of the proboscis rests against it.

Examples of the last larval stage were found in the infestation. These are bright orange in colour, with a broad black band on each side, both dorsally and ventrally, in which is situated in each segment of the abdomen a large creamy-white spot. There are longitudinal black lines on either side of the middle of the thorax, dorsally, and on the outer side of the leg articulations, ventrally, and rectangular black spots around the openings of the scent glands on the dorsal surface of the abdomen. The final larval stage is $\frac{1}{2}$-$\frac{5}{8}$ inch long and $\frac{3}{8}$ inch broad. The edges of the abdomen are spiny, as in the adult.

P. calidum might possibly be confused with the common and widely distributed Green Shield Bug, *Nezara viridula* Linn. The latter insect may be distinguished, however, by its uniform green coloration, lighter in shade and less shiny than *P. calidum*, its colourless wing membranes and hind wings, and the absence of sternal ridge and spiny edges to the abdomen. The larval stages of *N. viridula* are dark brown, later green, with two rows of white spots on each side, and are not spiny edged.

The recorded distribution of *P. calidum* is in forest regions throughout tropical Africa.

The Infestation on Telfairea pedata

The plant of Oyster Nut on which these insects were found was a well-grown specimen, estabished nearly four years (the species is capable of bearing for as long as 20 years), and growing over the top of a mango tree about 25 feet high. The insects were on mature woody stems not far below the growing tips, which latter, when first seen by the writer, had died back almost completely. There were no healthy leaves on the plant at all, and in the infested region there was a mass of tangled dead leaves, tendrils, and shoots (FIG. 2). A plant of Oyster Nut at the University Farm, 11 miles distant, was in a state of vigorous growth, so it was concluded that climatic factors were not the cause of the die-back.

Plate II—The affected Oyster Nut plant

Most of the infested stems were out of reach of direct measurement, but a rough estimate indicated that a total of about 22 feet of stem was densely infested, and a few sample counts gave an average of 35 insects per foot. The total population was therefore in the region of 770 insects. Since these counts were made not long after the colony had been violently disturbed by shaking the vine, for the purpose of collecting specimens, the initial density may have been somewhat higher, perhaps as many as a thousand insects. At this stage in their development, however, most of the disturbed insects returned to the plant, and the degree of infestation seemed as great as before. On horizontal stems the insects were predominantly on the underside, though they occupied all sides of vertical or near-vertical stems, in which situation all feeding insects were orientated tail downwards. In fact the density of insects quoted above represented the maximum possible for insects of this size, almost in contact with one another.

In spite of the total absence of healthy leaves, the insects continued to feed actively and excrete normally, during the two-month

period of observation. Like all sap-sucking Hemiptera, they passed clear watery excreta. The volume excreted was much less than that passed by such forms as the familiar "froth bugs" (Cercopidae), of which some species can saturate the ground below their host plant with the rain of excreta, when present in comparable numbers. Based on a series of observations in which both numbers of droplets of excreta per excretion, and numbers of excretions per hour were recorded, it was calculated that the average number of drops per excretion was 7.6, and the average interval between excretions was 59.7 minutes. By catching droplets in a dish of liquid paraffin, it was possible to measure their diameter. Although these measurements lacked accuracy, it could be estimated that the modal diameter was between $1\frac{3}{4}$ and 2 mm., giving an approximation for volume of 3 cu.mm. The excretory output of the whole colony was therefore about 17.6 ml. per hour, or 422.4 ml. per day. The frequency of excretion of the whole colony would be about one per five seconds, which is in complete agreement with the impressions of an observer standing beneath! The fact that this flow was being maintained so long after the growing tips of the plant had died suggests that root damage, by nematodes or soil insects, was not the cause of the symptoms. The dead parts did not show any signs of fungal infection, nor were there visible lesions where the insects were feeding. Interference with translocation in the plant by blockage of the vessels with salivary secretion is conceivable, but against this it must be noted that the insects were distributed over considerable lengths of stem, and those farthest from the roots would be receiving sap which had passed the feeding sites of many others. It is probably a simple matter of competition for sap between the growing tips and the infesting insects, which the latter temporarily won. In confirmation of this, information has reached me that the insects ceased feeding and left the infested region during October, and that subsequently vigorous regeneration of the plant took place.

Insects of the same family are pests in other parts of the tropics. The "bronze-orange bug" (*Musgraveia sulciventris* (Stâl) Leston and Scudder) is an important pest of citrus orchards in Australia, causing die-back of shoot tips (Hely, 1938). *Tessaratoma papillosa* Thun. causes similar damage to lichee trees in India (Kershaw, 1907), and *T. Javanica* Thun. is a sporadic pest of "kusum", the host tree of the lac-insect, in Chota Nagpur (Mehra and Purakayastha, 1955). No other Tessaratomidae are reported as pests of Cucurbitaceae, but in a related family, Dinidoridae of similar feeding habits, *Megymenum brevicorne* Fab. is a minor pest of Cucurbitaceae in Malaya (Miller, 1929) and Queensland (May 1946), and *Coridius janus* (Fab.) is known in India as the "red pumpkin bug".

Notes on the biology of Piezosternum calidum

When the infestation was first observed, in mid-July, about one-tenth of the population were immature forms, and moulting to the adult stage was taking place. Later, very few young specimens were to be found, so that the period of close observation coincided largely with a period of adult maturation. This was still incomplete when observation ceased in mid-September, since feeding was continuing and none of the sample females dissected (about twenty) had mature eggs in their ovaries. Although I had observed a pair mating, and another attempted copulation, in early September, I am informed that no further mating was observed before the insects ceased feeding in late September, nor was any seen while they migrated up the vine and dispersed during October. The impression that mating takes place after the dispersal flight is supported by the fact that these insects possess the ability to stribulate. This was observed in the feeding colony as a brief "buzz" of the wings, in the half extended position, for about a second, repeated at five- to ten-second intervals for a few minutes. Only occasionally did individuals perform in this way, although often they seemed to be "answered" by other individuals. The proportion of insects stridulating during the hour-long observation sessions seemed to be increasing towards the end of the two months of study. This behaviour seemed to have no useful function in the feeding colony, although it appeared possible that it might serve to dislodge excretory droplets which had fallen on to the insect from others higher up, but it was not correlated with the presence of such contamination. A means of mutual recognition would be of value if mating took place after dispersal, since finding a mate would present no problems if it normally occurred before the colony dispersed.

Maturation of the eggs probably takes place after active feeding has ceased. It was observed in the dissected specimens that the

alimentary canal became progressively more filled with a pasty food mass while feeding was taking place, and this would presumably be digested to supply the needs of egg production. The insects evidently fed continuously at one spot for many hours at a time, but in the groups of 20 to 40 intensively studied, there were always two or three in the act of moving along the stem, and another one or two resting with their mouth parts withdrawn from the plant. Those which were feeding usually continued to do so during the hour of observation, and could be found in the same position many hours later. If we infer that, since five out of forty insects are not feeding at any one time, then on average each insect spends seven-eighths of its time feeding, and one-eighth resting or wandering, it is likely that the actual periods involved are of the order of hours (e.g. perhaps seven hours feeding and one hour not feeeding). A behaviour trait of constant occurrence but unknown function, observed usually three or four times in each observation period, was a vibration of the abdomen up and down relative to the substratum, with an amplitude of about one millimeter, five or six times in about 1½ seconds, the whole repeated about every five to ten seconds as with stridulation. The similarity of repetition to the stridulation suggests a similar function, either as low frequency vibration transmitted through the stem, or as sound of frequency too high for the human ear.

It is unusual to find so long a period of maturation in the adult stage, especially in a tropical insect. Assuming that most of the population had become adult in early July, and ceased feeding in late September, the adult feeding period is about 80 days. According to later information, ovipositing adults and young larvae were present again in mid-December. If the larvae are assumed to be the progeny of the earliest adults, and the period from oviposition to hatching is as usual for this type of insect, namely two to three weeks, it would appear that there is a non-feeding period of adult maturation of about 50 days (i.e. late September to mid-November), making a total pre-oviposition adult life of 130 days. In related species, such as the Bronze-Orange Bug in Australia, a long pre-oviposition life is associated with a dormant period during the hottest time of the year, December to February, when no feeding takes place. With *Tessaratoma javanica* in Chota Nagpur, Mehra and Purakayastha (1955) found the pre-oviposition period to vary from 23 to 72 days in summer to 111 to 279 days over the winter season.

Whatever may be the reason for this long period of adult maturation, there does not appear to be any serious mortality due to predatory natural enemies in the course of it. Although wasps of several kinds were seen occasionally hovering near the infested stems, no attempts to capture *P. calidum* individuals were observed. This might be due partly to the large size of the adult insect, but must also be connected with the possession, in common with other shield-bugs and related forms, of glands which secrete a pungent and irritating fluid. These glands are situated in the last thoracic segment of the adult, and the dorsal side of the abdomen of the larvae. Hely (1938) describes how the Bronze-Orange Bug will project jets of this fluid to a distance of two feet, even at persons passing by, and that it also causes unsightly spots on leaves and fruit of the infested citrus tree, apart from the damage caused by sap sucking. Kershaw (1907) states that *Tessaratoma papillosa* can eject its odoriferous fluid to a distance of six to ten inches. Although *P. calidum* secretes such a fluid when handled, and it is pungent and stains the skin yellow, it has not been seen to be forcibly expelled, and the insects are not disturbed by movements in their vicinity. If the stem itself is shaken, this stimulates the discharge of excreta from the anus, but this material has neither odour nor irritant properties.

The occurrence of this massive infestation of an insect which, in my experience of several years collecting in both East and West Africa, is usually confined to forest zones, and even there is only seen as an occasional specimen resting on vegetation within reach of the collector, raises the question of its origin. This species has been found in lakeside forest about five miles from Kampala, and as they seem to be strong flyers could, with assistance from storm winds, reach the site where the infestation was observed. The absence of any previously recorded occurrence of this type must mean that, since mating probably follows dispersal, the chance of a pair being accidentally transported to the same spot outside their normal habitat, or of a mated female being so transported, is vanishingly small. In addition, suitable host plants are widely scattered in an urban area. The possibility of transport among

botanical specimens cannot, in the present instance, be ruled out. It is of interest to note that the insects were breeding on a hitherto unrecorded host, while plants of *Momordica* spp. in the same Botanic Garden were not affected. Oyster Nut was evidently providing an adequate source of food, although a small proportion of the adult insects had deformed wings, due to failure to expand after moulting, and this might have been due to a dietary factor. The numbers present make it almost certain that it was the progeny of more than one female, even allowing for the possible freedom, in a strange habitat, from the parasitic Hymenoptera which destroy a proportion of the eggs of insects of this kind. With *Tessaratoma javanica*, the highest number of eggs from one female was 406 (Mehra and Purakayastha, 1955). One to two hundred would be a reasonable figure to expect as an average. Since the coincidence of accidental transport of several females is unlikely, it must be assumed that the infestation represents the second generation on this plant, the first having escaped notice. Mortality from egg parasites may reach quite high levels in other species of large Heteroptera. Mortality among the larval stages is mostly due to predators such as spiders, ants, lizards, birds, mantids, etc., in other species of Heteroptera, and is of variable and uncertain extent. In the adults of *P. calidum* dissected, two out of thirty contained parasites of the Order Strepsiptera, which would render them sterile. As was stated above, predation upon adults was not observed. Allowing for the effects of the Strepsipteran parasite, and assuming, for convenience, a total population of 840, the original female and the average first generation egg production need not be higher than 85 eggs to produce the observed population density, even with 50 per cent egg and larval mortality. With 80 per cent mortality, an average egg number of 150 would be required. These numbers are well within the range of what is known for closely related insects. It is hoped, if infestations continue to appear, to study more closely the bionomics of this insect, and obtain exact information as to fecundity and mortality.

Summary

An infestation of a plant of the Oyster Nut (*Telfairea pedata* Hook.) by a hitherto unrecorded pest, *Piezosternum calidum* Fab. (Heteroptera, Tessaratomidae), is described.

The main recognition features of the insect are described, and the effects of its feeding upon the plant. As a result of sap sucking by several hundred large insects, all the actively growing parts of the plant were desiccated and killed.

Some aspects of the biology of *P. calidum* are discussed, with reference to information available from related pests in other regions of the tropics.

Acknowledgements

I am indebted to Miss A. C. Tallantire, of the Botany Department, Makerere University College, for details of the history of this Oyster Nut plant, and for observations on the behaviour of the insects after September, and to Mr. M. C. Lubega for the information on appearance of adults and larvae in December. I wish also to acknowledge assistance from Mr. E. A. J. Duffy of the British Museum in identifying the parasite from the adult insects, and from Dr. M. S. K. Ghauri for permission to examine the British Museum collections.

References

[1] Golding, F. D. (1941). Capsid pests of Cacao in Nigeria. *Bull. ent. Res., 32,* 83-89.

[2] Greenway, P. J. (1945). Origins of some East African food plants. *E. Afr. agric. J., 10,* 251-256.

[3] Hely, P. C. (1938.) The bronze-orange bug. A pest in citrus orchards. *Agri. Gaz. N.S.W., 49,* 378-380.

[4] Kershaw, J. C. (1907). Life-history of *Tessaratoma papillosa* Thunberg. *Trans. ent. Soc. Lond., 1907* (2), 253-258.

[5] May, A W. S. (1946). Pests of Cucurbit crops. *Queensland agric. J., 62,* 137-150.

[6] Mayné, R. (1917). Insects et autres animaux attaquant le Cacaoyer au Congo Belge. *Etudes Biologie agricole, No. 3, Min. Colon. Serv. Agr. Roy. de Belg.*

[7] Mehra, B. P., and Purakayastha, B. K. (1955). Bionomics and control of *Tessaratoma javanica* (Thunberg, a sporadic pest of Kusum (*Schleichera oleosa*) in Chota Nagpur. *Indian J. Ent.. 17,* 76-88.

[8] Miller, N. C. E. (1929). *Megymenum brevicorne* F. (Pentatomidae), a minor pest of Cucurbitaceae and Passifloraceae. *Malay Agric. J., 17,* 421-436.

[9] Nattrass. R. M. (1941). Notes on plant diseases. Nematode disease of Oyster Nut. *E. Afr. agri. J., 6,* p. 73.

[10] Poppleton, W. J. (1940). The Oyster Nut, *Telfairea pedata. E. Afr. agric. J., 5,* 114-120.

[11] Swynnerton, R. J. M. (1937). The Oyster or Kweme Nut. *E. Afr. agric. J.. 2,* 444-446.

APPENDIX 6

Some unusual cell inclusions in the mid-gut of a moth bug, *Gyarina nigritarsis* Karsch (Homoptera : Flatidae), and their possible significance in nutrition

By A. J. P. GOODCHILD

School of Biological Sciences Bath, University of Technology

SYNOPSIS

An account is given of the histology of the anterior diverticulum, intestine and hind gut of *Gyarina nigritarsis*. The nature of rod-like inclusions in the cells of the diverticulum is discussed.

INTRODUCTION

IT is now well established that the typical alimentary canal of Fulgoroidea consists of a tubular mid-gut coiled within a membranous sheath, from the anterior end of which a diverticulum, often branched or irregularly shaped, emerges and extends forward into the thorax. There has been some diversity of opinion on the function of this diverticulum (reviewed by Goodchild, 1966), but whatever duties it has come to perform in different species, it is not unlikely that its evolutionary origin is connected with that of the sheath enclosing the intestine in order to provide a region capable of inflation in the process of ecdysis.

Fulgoroidea are also well provided with microbial symbionts contained within various special organs in the body cavity, or in a compact mass in the ventral wall of the rectum, or as yeast-like cells freely invading the extensive fat body, or in combinations of these situations (Ermisch, 1960).

On an earlier occasion, examination of a few specimens of the flatid bug *Gyarina nigritarsis* Karsch (Goodchild, 1963) revealed that the cells lining the mid-gut diverticulum were distended with an apparently homogeneous eosinophil material, similar to that observed by Kershaw (1913) in another Flatid, *Siphanta acuta* Walker. It was also observed that the mid-gut of adults contained much basophil matter, which was interpreted as being derived from the breakdown of a symbiont-containing rectal organ (the gut of sap-sucking Hemiptera being usually empty of solid material).

It was possible to collect more specimens of this species during a visit to Uganda in 1966, and a more rigorous examination of the alimentary canal has made it necessary to revise these interpretations.

MATERIAL AND METHODS

Adults and nymphs of various ages were collected from twining plants in a small grove of *Eucalyptus* and other trees on the campus of Makerere University College, Kampala. Nymphs were fixed in alcoholic Bouin's fluid after small ruptures had been made in the cuticle to assist penetration. Adults were pickled in Pampel's fluid. It was not at first intended to use the adults for histological study, but when so used, the fixation appeared to be entirely satisfactory. Nymphs were embedded whole in ordinary paraffin wax and, because of their soft cuticle, excellent sections were obtained without other treatment. Adults were embedded in Steedman's ester wax after excision of the hardest regions of the cuticle, the rostrum, leg and wing bases, and genitalia. Sections 8 μ thick were stained by triple staining techniques, the most satisfactory being a normal haematoxylin/eosin followed by a brief dip in Edicol pea green (1 per cent. in 90 per cent. alcohol), although Masson's iron haematoxylin/ponceau fuchsin/light green was also used.

Proc. R. ent. Soc. Lond. (A). 44 (1–3). Pp. 11–16, 1 fig., 2 plates. 1969.

Anterior Diverticulum of Mid-Gut

Sagittal sections of half-grown nymphs showed that the anterior diverticulum originates from the mid-gut just behind the oesophageal valve, in the second abdominal segment (fig. 1). From this point it travels forward, dorsal to the oesophagus, as a tube similar in diameter and cell type to that of the mid-gut proper, as far as the first abdominal segment, where it rapidly expands into a wide sac, filling the median region of the thorax and head and extending back above the connecting tube to the level of its origin. The cells lining this sac are mostly extremely large (up to 80 μ by 180 μ) compared with normal mid-gut cells (average 15 μ by 40 μ), their tips are swollen to near spherical proportions, and their dark-staining, coarsely granular nuclei also enlarged (33 μ by 22 μ, compared with the mid-gut nuclei of 12 μ by 8 μ). The

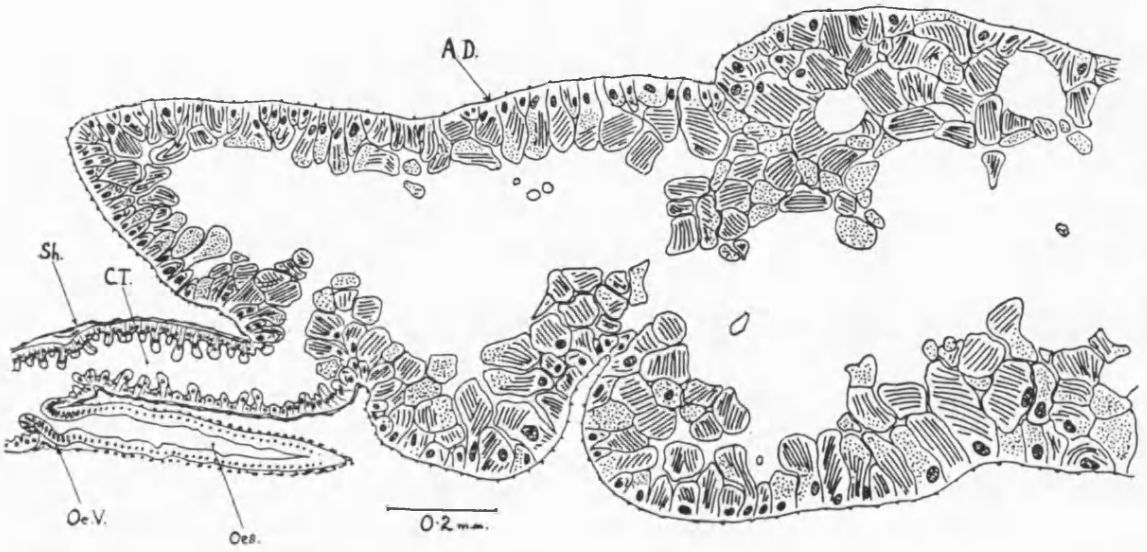


Fig. 1.—Sagittal section through posterior half of anterior mid-gut diverticulum of late nymph of *G. nigritarsis* (detail simplified for clarity). *A.D.*, anterior diverticulum, showing rod-like inclusions cut in various planes; *C.T.*, tube connecting diverticulum to mid-gut proper; *Oes*, oesophagus; *Oe.V*, oesophageal valve; *Sh*, cellular sheath of mid-gut.

cell border is a thin membrane, and the cytoplasm is completely occupied by a bundle of parallel rods, each about 5 μ in thickness and as long as the cell and sometimes longer, when they are accommodated by one or two flexures of the whole bundle, with the outside of the curve towards the lumen of the sac. The rods may be orientated parallel to the wall of the sac, or perpendicularly to it, or at slight angles to either of these directions, the orientation being generally the same in groups of several contiguous cells. In some cells there are two bundles of rods, with different orientations. Counts of transverse sections of bundles indicate that each may consist of as many as 50 rods. At the bases of some cells fragments of rods may be seen, and in the main bundles some rods may show clean transverse fractures, dividing the rod into two or more segments, which, however, retain their linear arrangement. The staining reaction of the rod substance is mildly eosinophil, that is to say, similar to that of the muscle fibres, which with eosin/pea green are pale orange, and unlike that of the storage granules of the fat body, which are bright pink with this stain. The rod structure is completely homogeneous, and neither differentiated internal structures nor distinct limiting membrane can be detected. The scanty cytoplasm around the rods is finely granular and distinctly basophil. Where the rods are crowded against the cell border they are more markedly eosinophil, or with Masson's stain a bright red (whereas deeper in the cell they are green with this stain). The rods show up extremely well under phase contrast illumination. Rods dissected from a whole preserved adult, and examined in water, seem to be of a transparent crystalline nature. Included

among the cells were abundant droplets of lipoid material, but the intact rods had no trace of affinity for fat stains.

Scattered among the bases of these large cells are other cells of all sizes down to about 20 μ by 25 μ (the nuclei of which are of normal mid-gut size). All cells contain the rod-like inclusions, running the full height of the cell distal to the nuclei or, in the smallest, on either side of the nucleus. Although fewer in number and shorter than the rods seen in the largest cells, these undeveloped rods are not much less thick (2 to 3 μ). In the region surrounding the opening of the connecting tube there is a gradual transition from normal mid-gut cells to the rod-containing cells. The anterior dorsal part of the sac wall lies close beneath the hypodermis of the head and first two thoracic nota, and its cells are obviously stretched by the expansion of the sac at ecdysis, being very wide and flattened (about 90 μ by 10–15 μ). Their cytoplasm is well stocked with rod fragments of various sizes, in some instances partly projecting through a torn cell border.

In the posterior part of the sac a delicate meshwork of muscle strands can be detected among the cell bases, and there is also a thin peritoneal membrane, with scattered small nuclei, around the whole sac.

In the smallest nymphs studied (possibly first instar), all the cells of the anterior mid-gut diverticulum were small, but most contained typical rod promordia. In males and immature females the posterior part of the sac is immensely inflated, the cells stretched into a pavement epithelium, and the organ extending far back into the abdomen, between the gonad and the mid-gut. It penetrates amongst, and is in immediate contact with, the ovarioles (Plate I, fig. A), but is wholly ventral to the testes, from which it is separated by a layer of fat body. The middle part of the sac, in the thorax, is restricted by the development of the longitudinal muscles, but in the head it remains expanded and there the cells are also flattened. The narrow neck of the sac in the thorax is filled with swollen cells, some of which are no longer attached to the epithelium and may also be enucleate. Among them and posteriorly towards the clearly defined spherical margin of the air bubble is a mass of finely granular basophil material enclosing clusters of rods (Plate II, figs. A, B). At the edge of the air space the rods are densely crowded, tangentially orientated and with indistinct outlines. Intermediate stages in the degenerative process can be found. From the typical cell with a bundle of long rods, there appears to be a change towards a break-up of the rods into lengths of 20 to 40 μ, with rounded ends, and an increase in the amount of normal cytoplasm present so that the rods are less closely packed. The whole mass may be liberated by the dissolution of the cell membrane. Also present to a greater or less degree are cell tips (no nuclei being visible), in which the rods remain long and closely packed but their outlines become less distinct; on the dissolution of the membrane a structureless eosinophil mass, containing poorly defined thread-like fibres, is released. The distinction between the two modes is not sharp, however. In some specimens bundles of eosinophil rods may be found among the debris, still within an enclosing membrane, and in one specimen such a bundle was seen in the anterior part of the mid-gut proper (Plate I, B). The nuclei in these degenerating cells may be swollen and misshapen, and often surrounded by a zone of normal basophil cytoplasm. It may be that the increase of cytoplasm only occurs when the nucleus is present, cells that lose their nuclei undergoing the second mode of break-down. Over the surface of the cells in the posterior part of the sac, and within the margin of the air bubble where it is in contact with the rod-laden debris, is a narrow zone of strongly eosinophil granules and strands, in many instances fusing into an eosinophil membrane on its inward side.

In the mature female a similar situation obtains in the anterior and thoracic region of the sac, but with the growth of the eggs the posterior region contracts and its epithelium returns to a cuboid or columnar appearance (although the cells are separate, except near their bases). Few of these cells contain rods, but many have frayed tips

indicating the loss of their distal part. The nuclei are of various sizes, densely granular and basophil. The cytoplasm is also basophil. The muscular layer is thick and conspicuous, and many of the nuclei appear as if trapped in it and distorted to a dumb-bell shape.

The Intestine and Hind Gut

The mid-gut proper is a sinuous tube of even diameter, which leads backwards from the oesophageal valve to near the posterior end of the abdomen on the ventral side, then returns forward and loops over its anterior end before turning back again to join the hind gut in the middle of the abdomen. As in other Fulgoroidea, it is enclosed in a sheath, which in this species takes the form of a thin pavement epithelium, better developed in the nymph than in the adult. In the latter it cannot be followed continuously around the gut coils, and is reduced in thickness to an eosinophil membrane with occasional nuclei. Close around the gut wall there is also a thin basement membrane with scattered small nuclei. The cells lining the mid-gut are essentially of a uniform nature, but appear to undergo cyclical changes, possibly in waves of activity passing along the length of the gut. This conclusion is based on the fact that such differences as can be seen are not constantly associated with particular sections of the gut. The cells are columnar, with bulbous tips projecting into the lumen, sometimes singly but more usually in groups of about six cells forming a single lobe. Around the circumference of the gut there may be six to ten such lobes, separated by much shallower cells. The cell tips contain finely granular basophil cytoplasm, usually with many tiny vacuoles, and have a well defined brush-like border. Particularly in the middle region of the mid-gut, the cell tip may discharge a large, thin-walled globule of cytoplasmic material into the lumen. The tallest cells are about 50 μ by 10 μ, and the free lumen between the tips is about equal to the cell height in nymphs and young adults. The lumen is empty except for a few eosinophil granules in immature forms, but in maturing adults it becomes filled and its width increases three or four times, the cells simultaneously being stretched into a wider and lower shape. The anterior half of the mid-gut of adults contains scattered eosinophil granules, which on close examination appear to be arranged in regular circular or part-circular patterns of about 20 μ diameter (Plate I, fig. A). The impression is given that these are relics of spherical shells of eosinophil material, irregular in thickness, which surrounded a globule of a substance leached out by the processes of histological preparation. Similar material can be traced forwards along the connecting tube and into the anterior diverticulum. In the posterior part of the mid-gut the shell-like structures are much less frequent, and the few remaining are embedded in a dense mass of finely granular basophil material with which the gut is noticeably distended. In the middle region of the gut a transition zone is present, with coarse granules of varying degrees of eosinophilia among the basophil contents. In some specimens the posterior part of the gut was empty, while the anterior part contained abundant eosinophil material, as if the digestive process was in an early stage. In some very mature females the gut was mostly empty throughout, and the anterior diverticulum shrunken and with little rod debris. This suggests that the gut contents are voided as the ovarian system expands.

The hind gut is a single sac-like rectum, the walls being composed entirely of rather flattened cells with nuclei somewhat larger than those in the mid-gut cells. It is thus more in the nature of rectal gland epithelium than the usual rectal syncytium. In all adults, and some larger nymphs, the rectum was strongly contracted and the lining thrown into complex folds, with little free lumen visible, and surrounded by a thick muscle layer. Some granular, neutral staining, debris was seen in the lumen or rectal valve region in a few specimens. No symbiont-containing rectal organ could be detected.

Discussion

When the results of this investigation are compared with the early account by Kershaw (1913), many points of similarity can be found. Although Kershaw did not observe rod-like inclusions in the anterior diverticulum ("reservoir" in his terminology) of *Siphanta acuta*, his finding of the epithelium as constantly in a state of degeneration and renewal suggests that, as in the present writer's earlier studies of *Gyarina*, the fixation methods were unsuitable. Kershaw gives no details of his procedure in this respect, but in the writer's experience dissected guts of small Hemiptera easily suffer from over-fixation or post-fixation damage, and vastly better results have been obtained from the fixation and embedding of whole animals. Furthermore, it does not seem that Kershaw used a triple staining technique, without which the structures in *Gyarina* are far less evident.

Whether or not Kershaw's material could have been rod-like, the remainder of his description of cell detachment, breakdown into a viscid fluid and passage along the mid-gut, is in full agreement with what appears to happen in *Gyarina*. On the other hand the situation in *Gyarina* does not lend itself to interpretation in the way that Kershaw put forward for *Siphanta*. He regarded the secretion of the reservoir as primarily a digestive secretion, with the possibility that the organ might aid in getting rid of the waxy matter secreted by the insects. The rod-like inclusions of *Gyarina* are totally unlike any known digestive secretion of insects, the normal appearance of secreting epithelium being that of the mid-gut proper in this species. The crystalline style of molluscs has some similarity, perhaps, but there seems to be no reason why such a structure should arise in an insect. As regards the waxy nature of the reservoir contents, the presence of lipoid droplets in the diverticulum of *Gyarina*, and the probability that the gut contents are partly of this nature, corroborates the observations on *Siphanta*, although the ingestion of wax secreted by the cuticle of the insect itself must be regarded as unlikely.

Rod-like intra-cellular structures, especially in such insects as Homoptera, must always be suspected of being a form of symbiotic micro-organism, but in *Gyarina* the large size reached, the lack of nuclear structures, and the brittle texture, make it difficult to accept such an hypothesis. Furthermore, the anatomical situation would be unique among Fulgoroid symbionts, and there is no evidence of a mechanism of transmission, whether transovarial or by spore formation.

Supposing, therefore, that the rod-like inclusions are formed by the cells themselves, they could be a form of storage, either of an excretory nature, or for subsequent utilisation. The breakdown and passage through the gut could be compatible with an excretory product, if there was need for space in the body for the maturing reproductive system, but the histological evidence suggests that a digestive process is taking place. As Kershaw pointed out, certain plants, and particularly the eucalyptids on which *Siphanta acuta* fed, contain an abundance of lipid or resinous substances, which may be disposed of by intra-cellular deposition in the insect. In many Cercopidae (Goodchild, 1966), the hinder part of the mid-gut is clogged with hypertrophied cells containing excretory matter. On the other hand, nymphs of the Flatidae secrete such an abundance of wax on their external surface that it is difficult to imagine there being a surplus for internal deposition. If it is stored for use at the time of maturation of the reproductive system, why is it not stored in the fat body, as in most insects? In *G. nigritarsis* the fat body is very densely infested with yeast-like symbionts, which seem to be undergoing continual destruction and absorption, judged by the variation in staining reaction and refractivity under phase-contrast illumination. It may be that this specialisation of the fat-body prevents its acting as a storage depot in the usual way. Incidentally, no other form of symbiont or symbiont-containing organ was found in this species. The rods may be a lipoid-protein complex, which separates in the breakdown phase into a lipoid droplet surrounded by a protein skin and is digested in the mid-gut in this form. The dense basophil granular contents of

the posterior mid-gut would then be the final stages in this process and would not, as previously suggested, be caused by the breakdown of a rectal symbiont organ.

It has been tentatively suggested (Goodchild, 1966) that the secretory activity of the cells of the anterior diverticulum might be a reaction to resist osmotic flow into the haemocoele in this region, which is not enclosed in the intestinal sheath. In *G. nigritarsis* the activity seems too exaggerated to be directed to this end alone, and the presence, in nymphs at any rate, of a definite constriction, with conspicuous muscle layers in the connecting tube, suggests that flow of the ingested plant sap into the diverticulum may be restricted, if not entirely prevented.

It is regrettable that this account is based on histological evidence alone, and there seems to be a case for more detailed examination of the nutrition of Flatidae by those in a position to study the living insects.

Summary

(1) In the anterior diverticulum of the mid-gut of *Gyarina nigritarsis* Karsch (Fulgoroidea, Flatidae), the cells are swollen to accommodate large rod-like inclusions.

(2) In maturing adults these rods break down and pass into the posterior mid-gut, where the contents appear as shells surrounding presumed lipoid droplets, and grade into finely granular basophil material in the hindmost part of the mid-gut.

(3) It is suggested that these inclusions are formed by the cells themselves for subsequent utilisation.

References

Ermisch, A., 1960, Vergleichend-anatomische Untersuchungen über die Endosymbiose der Fulgoroiden mit besonderer Berücksichtigung der Araeopiden. *Z. Morph. Ökol. Tiere* **49** : 47–138.

Goodchild, A. J. P., 1963, Some new observations on the intestinal structures concerned with water disposal in sap-sucking Hemiptera. *Trans. R. ent. Soc. Lond.* **115** : 217–37.

—— 1966, Evolution of the alimentary canal in the Hemiptera. *Biol. Rev.* **41** : 97–140.

Kershaw, J. C., 1913, The alimentary canal of *Flata* and other Homoptera. *Psyche, Camb.* **20** : 175–88.

(Manuscript received 28th February, 1968)

Plate I

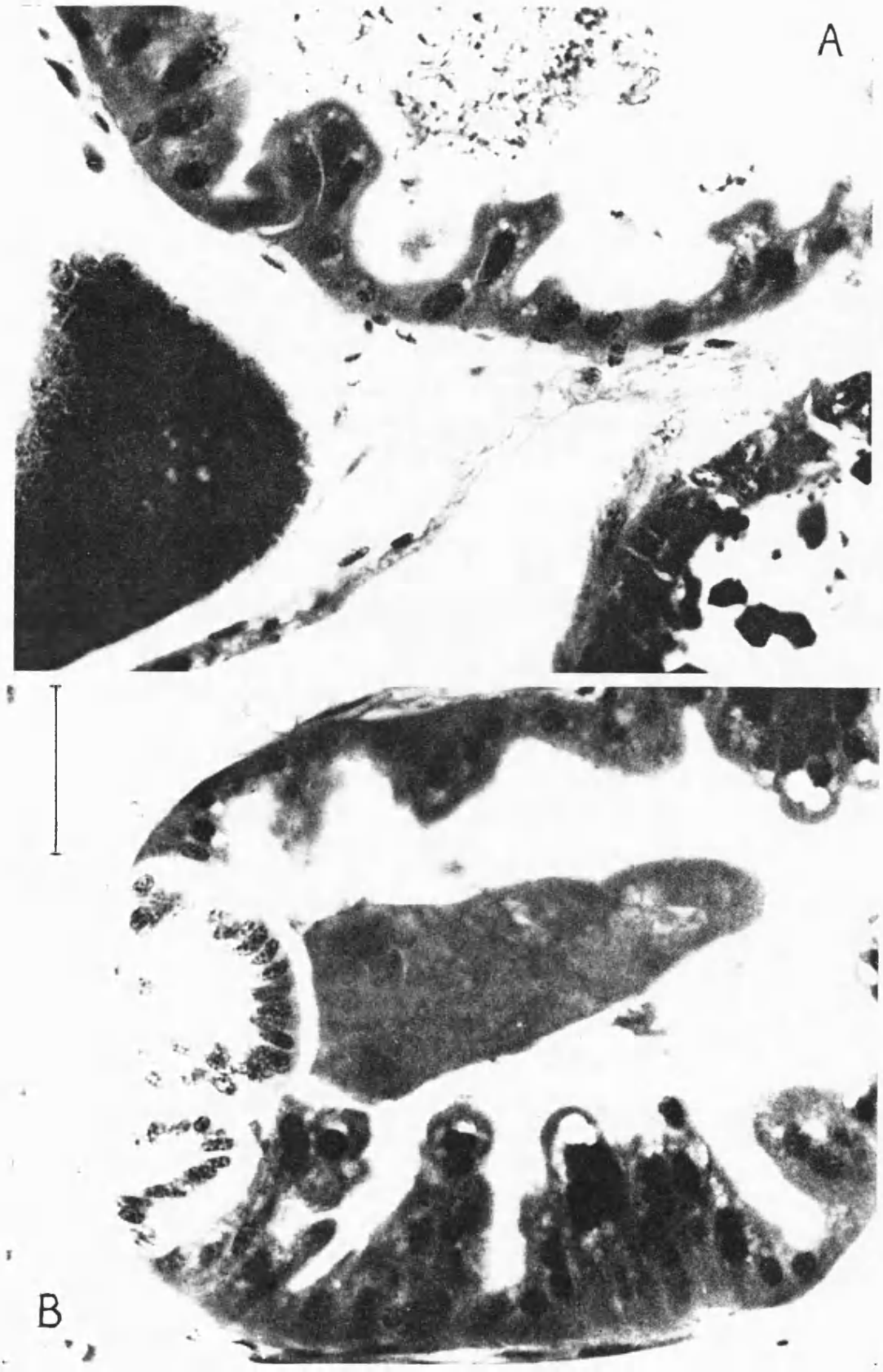
Fig. A.—Upper part: part of transverse section through mid-gut, showing lining cells and "eosinophil shell" contents. Lower part: fold of stretched anterior diverticulum between two ovarioles.

Fig. B.—Clump of rod debris from anterior diverticulum, in anterior end of mid-gut adjacent to oesophageal valve (left hand edge). Scale, 50 μ.

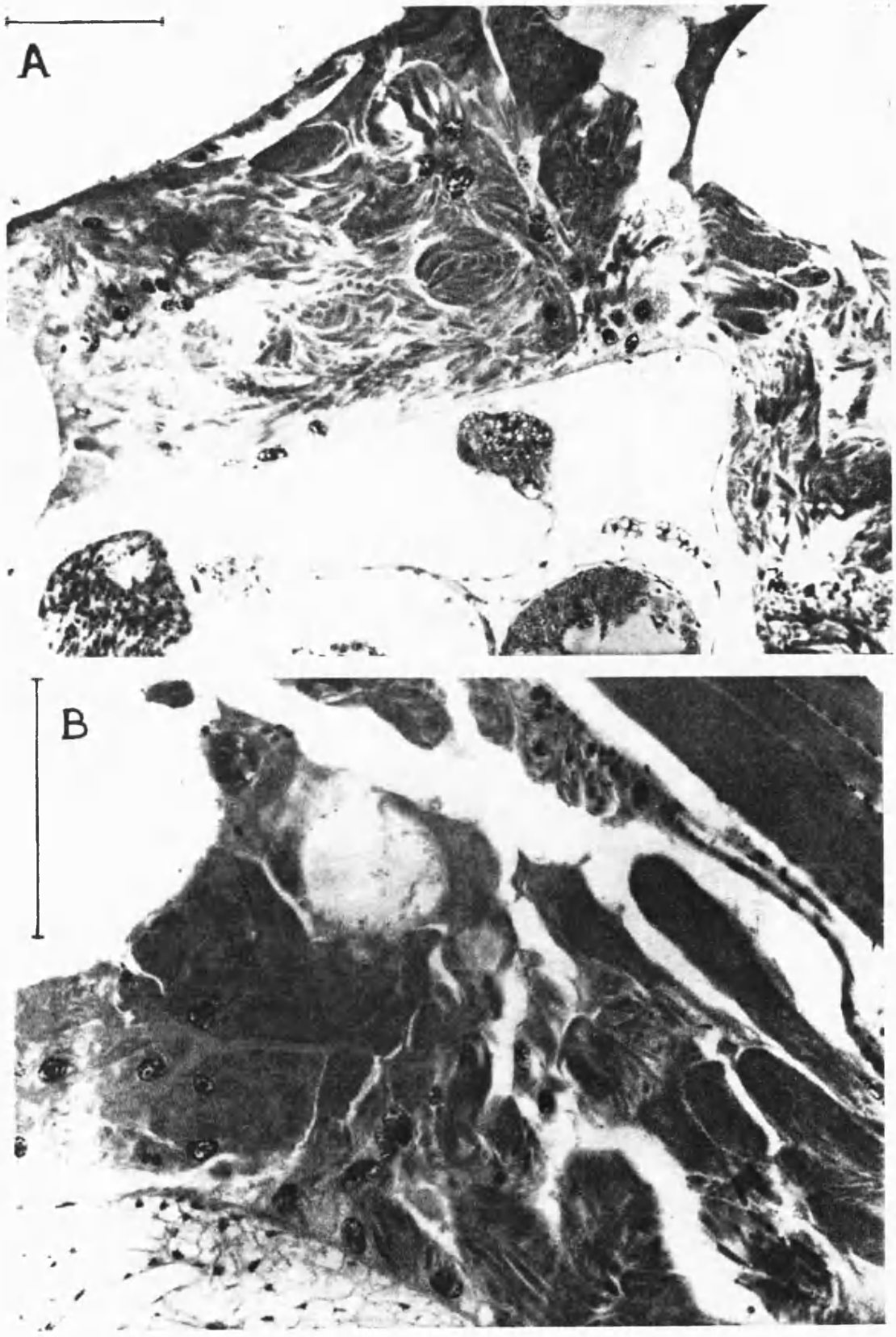
Plate II

Fig. A.—Sagittal section through head and thoracic region of the anterior diverticulum of a mature female, showing rod-containing cells in various stages of breakdown.

Fig. B.—Sagittal section of thoracic region of anterior diverticulum of a mature male, showing cell breakdown. Scale on each fig., 0·2 mm., anterior end to right.



Gyarina nigritarsis



Gyarina nigritarsis

APPENDIX 7

The rectal glands of *Halosalda lateralis* (Fallén) (Hemiptera : Saldidae) and *Hydrometra stagnorum* (L.) (Hemiptera : Hydrometridae)

By A. J. P. GOODCHILD

School of Biological Sciences, Bath University of Technology

SYNOPSIS

An account is given of the histology of the hind gut in *Halosalda lateralis* and *Hydrometra stagnorum*, and in larvae of two species of *Aedes*. The possible mechanisms of rectal gland function are discussed.

INTRODUCTION

Specialised regions of large columnar or cuboid cells are found in the hind gut of most kinds of insects. Usually referred to as "rectal glands" or "rectal pads", they have long been regarded as the main site of water absorption from the rectal contents. Physiological studies, such as those of Ramsay (1955, 1964), Sutcliffe (1960) and Phillips (1964), have confirmed the earlier suggestion by Wigglesworth (1932) that water is re-absorbed in the rectum of certain insect species against a steep osmotic gradient. The identification, as the tissues responsible for this, of the "gland" regions, rather than the thin syncytial layer that forms the remainder of the rectal wall rests essentially on the histological evidence, although it is supported by the finding of Berridge & Gupta (1967) that in the blow-fly *Calliphora* the ordinary rectal wall was not affected by injection of hypotonic fluid into the rectum, whereas marked changes were produced in the glandular papillae. Even in insects such as those species of Hemiptera studied by Bahadur (1963) and Berridge (1965), which can only re-absorb water from hypotonic rectal contents, extensive gland areas are present.

The mechanism by which this movement of water is effected remains uncertain. The concept of "active transport" of water is no longer accepted by those vertebrate physiologists such as Diamond (1965), who have found no evidence for water transport in the absence of a concomitant transport of ions. On the other hand, water is known to pass through the outer cuticle of insects against an osmotic gradient (Beament, 1965), and the rectum is lined with similar material. The complex rectal papillae of *Calliphora*, described in detail by Graham-Smith (1934) (who, incidentally, did not accept water absorption as their sole function), have been re-investigated with electron microscopy by Gupta & Berridge (1966*b*). In physiological experiments, the same authors find (Berridge & Gupta, 1967) evidence for a structure compatible with the "double-membrane" model for water transport proposed by Curran & McIntosh (1962).

A relationship between cell structure and function in the rectal gland was put forward by Ramsay (1950) as a result of his observations on the larvae of the mosquitoes *Aedes aegypti* L. and *Ae. detritus* Edwards. The first named species breeds in fresh water, and the larva maintains the osmotic pressure of its body fluids by passing hypotonic excreta, as well as by absorbing salts through its anal papillae (Wigglesworth, 1933). Ramsay showed that the urine flowing from the Malpighian tubules is isotonic with the haemolymph, and that it is during the passage through the rectum that the excreta become hypotonic. The other species, *Ae. detritus*, can develop in fresh, brackish or saline water, and the larvae therefore pass excreta that

Proc. R. ent. Soc. Lond. (A). **44** (4–6). Pp. 62–70, 1 fig., 2 plates. 1969.

are hypotonic or hypertonic to the haemolymph, according to the nature of the environment. In the larvae of these species, the rectum is lined for the posterior four-fifths of its length with thick gland cells. In *Ae. aegypti*, these cells are all of one kind, with a narrow striated border towards the lumen, but in *Ae. detritus* there are two kinds of gland cell, those of the anterior part of the gland having a greatly diminished striated border and being sharply distinguished from the cells of the posterior part, in which the striated border is very well developed. Ramsay concluded that the posterior part of the gland in *Ae. detritus* most greatly resembled that of *Ae. aegypti*, and that it was therefore this region in which solutes were absorbed, water being absorbed in the anterior gland.

Rectal glands in the Hemiptera present a considerable variety of form (Bahadur, 1963; Goodchild, 1963), but although they range from a small discoidal area to almost the entire lining of the rectum, or may be formed into a tubular sub-region of the hind gut, they always consist of a simple single layer of cells, not complex papillae as in adult Diptera. Within the order Hemiptera there are species, terrestrial as well as aquatic, that are like *Ae. aegypti* in passing hypotonic excreta. The best-known of these belong in two widely distinct groups, the Homoptera Cicadoidea (Licent, 1912) and the Heteroptera Hydrocorisae (Staddon, 1963, 1964). They share a common anatomical feature of a long tubular hind gut lined with gland-type cells, although the detailed histology of these differs considerably in the two groups. In terrestrial Heteroptera, a comparison of rectal gland structure over a range of species from the most water-conserving (*e.g.* Reduviidae) to the water-disposing sap suckers (*e.g.* Pentatomidae) suggested a positive correlation of depth and eosinophilia of the striated border with probable water absorption (Goodchild, 1963). This conclusion is strengthened by the discovery by Berridge (1965) that in the cotton-stainer (*Dysdercus fasciatus* Signoret) water is absorbed only from hypotonic rectal contents. Although this insect can feed on dry seeds, it requires water to drink, and its rectal gland (presumably inherited from plant-sucking ancestors) has a poorly developed striated border.

During the preparation of a review of the alimentary canal structure in Hemiptera, I realised that the conclusions reached by Ramsay (1950) on the relationship of development of striated cell border to cell function were opposite to those I had formed from studying Hemiptera (Goodchild, 1966). To resolve the contradiction, I decided to investigate a hemipteran insect from the same kind of habitat as *Ae. detritus*. *Halosalda lateralis* (Fallén) belongs to the family Saldidae, and since Miyamoto (1961) described the hind gut of some genera of this family as having two distinct glandular areas, on the tubular ileum and on the rectal sac, it was hoped that histological studies would reveal two cell types, a feature not hitherto recorded in Hemiptera. Another problem referred to in the above-mentioned review was the existence in Hydrometridae of a hind gut of the hydrocorisid pattern, with tubular ileum and rectal sac. Since the ileum of Hydrocorisae has been presumed to absorb solutes and form hypotonic excreta (Goodchild, 1963), whereas most Amphibicorisae have a simple sac-like hind gut with gland cells of presumed water-absorbing type, material of *Hydrometra stagnorum* (L.) (Hydrometridae) was examined to discover the histological nature of the ileum.

Material and Methods

Specimens of *Halosalda lateralis* were collected from a *Salicornia* marsh at Wyke Regis, Dorset, and *Hydrometra stagnorum* from ponds in the Bath area. For purposes of comparison with the latter species, specimens of Corixidae (*Sigara* sp.) were collected from local streams. Material of *Aedes* was also examined. Larvae of *Ae. detritus* were collected in the Severn estuary near Avonmouth, and those of *Ae.*

aegypti were reared from eggs kindly supplied by Mr. S. A. Smith of the London School of Hygiene and Tropical Medicine.

The alimentary canals were dissected out under saline, fixed in alcoholic Bouin's fluid, embedded in paraffin wax and sectioned at 8 μ. Stains used were Mayer's haemalum and alcoholic eosin.

Histology of the Hind Gut

Halosalda lateralis

The hind gut of *H. lateralis* consists of a narrow ileum of fusiform shape, joined by a short tubular region to a wide rectal sac (fig. 1). The mid-gut opens into the

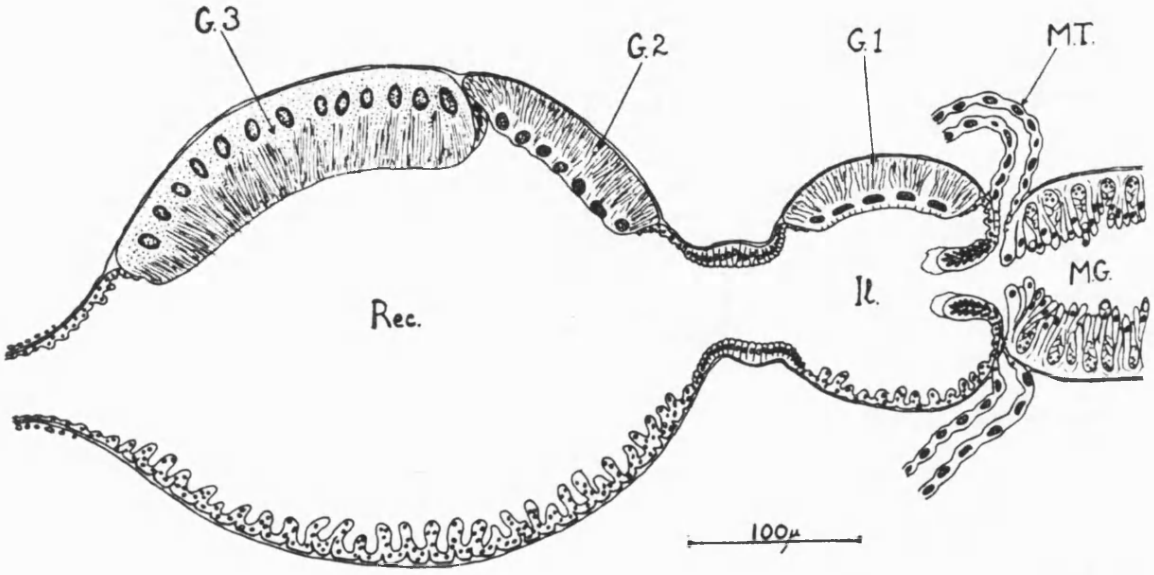


Fig. 1.—Semi-diagrammatic sagittal section through hind-gut of *H. lateralis*, showing disposition of gland-type cells. *G* 1, gland in ileum; *G* 2, anterior rectal gland; *G* 3, posterior rectal gland; *Il*, ileum; *MG*, midgut; *MT*, Malpighian tubule; *Rec*, rectal sac.

ileum by way of a well-developed rectal valve, which is a posteriorly directed funnel formed by a double layer of small ovoid basophil cells that secrete a rather loosely attached chitinous intima. The ileum itself is about 120 μ long and 150 μ wide in a contracted condition. It is lined with a flattened epithelium about 4 μ thick, with nuclei of the same diameter but without distinct cell boundaries. The epithelium is thrown into many small folds in specimens empty of faecal matter. On the dorsal side of the ileum there is an oval glandular pad of cells 30 μ deep, which extends almost the full length of the organ but not down the lateral walls. Where it joins the thin epithelium, the latter is carried up the sides of the gland cells almost to their free border (Plate I, fig. A). The nuclei of the gland cells are 20 μ by 10 μ, and lie close to the free border with their long axes parallel to the border. They are densely granular, and between them and the cell border is a zone 4 μ deep in which the cytoplasm is weakly eosinophil and rather coarsely and irregularly striated. The cytoplasm towards the cell base, below the nuclei, is distinctly eosinophil and very finely striated. Between the nuclei, poorly defined vacuoles or gaps in the cytoplasmic striations are usually present.

The connecting tube is 70 μ wide and 60 μ long, lined with small basophil columnar cells about 20 μ deep. The rectal sac is 350 μ long and 320 μ wide in the contracted condition. Its dorsal and lateral parts are lined with gland-type epithelium, and its ventral wall is composed of thin syncytial epithelium like that in the ileum. This is

deeply folded when the organ is contracted. The glandular epithelium is of two distinct kinds, divided from one another by a junction zone, two or three cells wide, of narrow columnar cells with very dark staining nuclei. The junction is more or less straight and transversely orientated (Plate I, fig. B). The anterior part of the rectal gland occupies about one-third, and the posterior part two-thirds, of the length of the rectal sac. The anterior cells are similar in shape and size, and in nuclear type, to those of the ileum gland, but do not have the striated zone between the nuclei and the cell border. Instead, the nuclei rest in contact with the cell membrane, sometimes being flattened into extensive contact. The cells of the posterior rectal gland are markedly different, having a depth of 65–70 μ, and nuclei which are larger, ovoid in shape with granules clumped against the nuclear membrane, and situated near the base of the cell. The cytoplasm between the nuclei and the free border of the cell is finely striated and definitely eosinophil, whereas that around the nuclei is finely granular and slightly basophil, and extends in strands towards the cell border. The basal part of the cell is composed of weakly eosinophil, faintly vacuolated, cytoplasm.

Hydrometra stagnorum

The hind gut of *H. stagnorum* resembles that of the Hydrocorisae in being formed in two distinct regions, a long narrow tubular "ileum" followed by an expanded, thin walled "rectum". In the Hydrocorisae the ileum is thick walled (except for a narrow ventral strip), the cells of this region being characterised by strongly eosinophil cytoplasm with striations running from base to free border, and nuclei elongated in the same direction. The striations may thin out towards the free border (Goodchild, 1963). This was confirmed by study of *Sigara* sp. (Plate II, fig. B). The Amphibicorisae have been found (Goodchild, 1966) to have simple sac-like hind guts, with an extensive dorsal gland area, the cells of which have an eosinophil striated border about half the depth of the cell and spherical nuclei. The ileum of *H. stagnorum* was found to have a thick wall with a narrow ventral zone of thin rectal cells, the thick part consisting of cells typical of the gland in Amphibicorisae (Plate II, fig. A). In the Hydrocorisae the long narrow ileum might be supposed to enhance the effectiveness of the gland epithelium by increasing the area/volume ratio; it seems however that the similarity of the anatomy of *H. stagnorum* may be a result of its highly elongated body.

Aedes Larvae

The rectal gland cells of larvae of *Aedes aegypti* (Plate II, fig. D) are approximately cuboidal and only slightly thicker than the diameter of the spherical nucleus (25–30 μ). The cytoplasm is mildly eosinophil and densely fibrous in texture throughout the cell, with a narrow border zone along the apical margin. This border is eosinophil, but with a hint of a basophil element in its innermost parts, and is very dense, the striations being only poorly discernible.

In the larvae of *Aedes detritus*, the two types of cell occupy the anterior quarter and posterior three-quarters of the glandular region of the rectum and are separated by a circumferential zone of small unspecialised rectal cells, which extends into the lumen of the rectum as a shallow fold (Plate II, fig. E, bottom). The cells of the anterior part of the gland are cuboidal, with large nuclei extending almost from base to apical margin (Plate II, fig. E). There is a very narrow, sparsely striated, eosinophil border zone along the apical margin, and below that a zone rich in basophil granules. The general cytoplasm is coarsely fibrous, the fibres being orientated from base to apex and more widely separated in the region of the lateral margins of the cell than they are in the vicinity of the nucleus. The overall staining reaction is mildly eosinophil.

In the posterior region of the gland (Plate II, fig. F) the cells are taller than they are wide, the nuclei being similar in size to those of the anterior gland and situated close to the base. The additional cell height is brought about by the very great development of the eosinophil striated apical border. This zone is composed of coarse fibres, which tend to clump together so that clear channels penetrate at irregular intervals towards the nucleus. In the region immediately below the chitinous intima, the striations thin out very markedly, leaving wide open spaces separated by single membranes. The eosinophil striated zone is in distinct contrast to the granular basophil cytoplasm below and at the sides of the nuclei.

Discussion

It is clear that insects subjected to immersion in water of varying salinity, so that on different occasions the excreta must be made either hypotonic or hypertonic to the haemolymph, cannot rely on a single type of rectal gland cell to perform both tasks. The fact that the dipteran *Aedes detritus* and the hemipteran *Halosalda lateralis* belong to very widely separated taxonomic groups, and that their relatives in which the osmotic problem is simple water conservation or water disposal (but not both) have simple glands, must rule out any non-adaptive explanation of the two-fold gland in these species.

Before going on to consider whether this observation contributes significantly to our understanding of cell structure in relation to function, it is necessary to review briefly the most recent work on insect rectal gland structure. Noirot & Noirot-Timothée (1966, 1967) have described the ultrastructure of gland cells of the hind gut of the termite *Cephalotermes rectangularis* Silvestri. In this insect, there is an anterior expansion of the hind gut (referred to as the paunch), connected by way of a narrow colon to a sac-like rectum. Enlarged cells of gland type occur in both the paunch and the rectum. They are different in structure, those of the rectum having a deeply infolded apical membrane with elongated mitochondria abundant among the folds. The cytoplasmic face of this folded cell membrane is evenly coated with a layer of granules. In the gland cells of the paunch, the mitochondria are located towards the base of the cell and are surrounded by invaginations of the basal membrane, the nucleus is central and spherical, and the apical membrane is deeply infolded but lacks the inner granular coating. A light micrograph shows a cell not unlike that of the rectal gland of *Hydrometra stagnorum*.

The authors, using a technique that revealed sodium accumulations in electron micrographs, found that this ion was concentrated in the apical membrane of the rectal gland, in which there is a granular coating, but not in that of the paunch gland, which lacks this coating. They conclude that the rectal gland absorbs salts, and suggest that the paunch gland may be responsible for absorbing fatty acids, the products of bacterial fermentation of cellulose. It seems likely that the cell border of the rectal gland in the termite would appear to light microscopy rather like that of the rectal gland of larvae of *Aedes aegypti*, or the lining of the hind gut of a cicadoid homopteran (Plate II, fig. C), both of which absorb solutes. This cell type may be the site of uptake of organic molecules, as it has been shown (Jackson & Smyth, 1968) that transport of these is sodium-dependent.

Since the structure of the paunch gland cells of *C. rectangularis* is like that of those Hemiptera that absorb water from hypotonic rectal contents only, it is possible that in the termite it is concerned with regulating water content of haemolymph relative to hind gut. The postulated function of absorption of organic molecules faces two objections, firstly the probability that the rectal gland is performing this task, and secondly the likelihood that passive diffusion through unspecialised rectal lining would be significant (Berridge & Gupta (1967) recognise this as a possible pathway). The

large extent of glandular rectal epithelium in those Hemiptera in which water is absorbed with the assistance of an osmotic gradient suggests that it is under active control, perhaps by variation of permeability of the chitinous intima.

The presence of two types of cell in glands of the hind gut of *C. rectangularis* cannot however be strictly comparable to the situation in *Ae. detritus* and *H. lateralis*. In the termite, the deep striated border is on the more anterior of the glands; in both estuarine insects it is posterior. Furthermore, the peculiar physiology of wood-eating termites, with microbial fermentation in the hind gut, would necessitate an additional absorptive area to supplement the normal absorptive function of the mid-gut.

Another type of rectal gland cell, found in the blow-fly *Calliphora* (Gupta & Berridge, 1966*a*, *b*) and in adults of *Aedes aegypti* (Hopkins, 1967), is characterised by only moderate development of the infolded apical membrane, not involving associated mitochondria, and by very considerable development of infoldings of the lateral cell membranes, which open into intercellular spaces and ramify among concentrations of mitochondria. In the blow-fly, the innermost parts of this system are lamelliform spaces, which are intricately assembled into stacks with accompanying mitochondria. According to Gupta & Berridge (1966*a*), the apical cell membrane has a granular inner layer, but the lateral membranes have not. In *Ae. aegypti*, Hopkins could not detect such a layer on any of the cell membranes, but suggested that the lateral membranes bore an extracellular coating, which might be physiologically important. In both species it was observed that, under conditions when the insect was feeding actively, the intercellular spaces and part of the infolded lateral membrane system seemed to be open and engorged with fluid, and fluid-filled spaces developed between the chitinous intima and the apical membrane; when the blow-fly was fasting during oogenesis, and before the mosquito took a blood meal, however, these spaces were closed. This observation accords with the theory of Diamond & Tormey (1966) on the function in fluid transport through epithelia of such long extracellular channels, in that rapid equilibration of water with actively transported ions will enable local osmosis to effectively move an isotonic solution. Although, when these spaces appear open, it is extremely probable that isotonic transport is occurring, this theory does not explain the absorption of water in the later stages when the rectal contents are hypertonic to the haemolymph, and the intercellular spaces are closed.

Although Berridge & Gupta (1967) made out a case for regarding the rectal gland cells of the blow-fly as functioning in the manner of the Curran-McIntosh two-membrane model, the same authors (Gupta & Berridge, 1966*a*) did not rule out the possibility that the granular coating on the apical cell membrane represented the site of a enzyme system capable of water transport, utilising extramitochondrial energy sources. One enzyme mentioned as possibly involved is carbonic anhydrase, which is known to be involved in absorption in the vertebrate renal tubule (Clapp, Watson & Berliner, 1963). It is therefore of interest to record an experiment of mine in which larvae of *Aedes detritus* were kept in weak solutions (20 p.p.m.) of a number of diuretic drugs in either sea or tap water. Those larvae kept overnight in sea water with drugs having anti-carbonic anhydrase activity (acetazolamide, and to some extent chlorothiazide) were shrunken and inactive. Other drugs, and also some local anaesthetics which act by affecting ion transport in nerves, had no effect, even when repeated at twice the concentration. In fresh water, none of the drugs tested, including anti-carbonic anhydrases, had any effect on the appearance or activity of the larvae, nor had the anti-diuretic pitressin, which was also tested.

In the Hemiptera, rectal gland cells essentially the same as those of the posterior gland of *Ae. detritus* can be found in a large number of species, but particularly among families of Cimicomorpha such as Reduviidae. The habitat and food preferences of these species strongly suggest a need for water conservation. Although the intercellular spaces of the rectal papilla in the blow-fly are visible to light microscopy, the most minute study of sections of the rectal glands of Hemiptera fails to reveal a system

of this kind. In a table summarising published figures for maximum osmotic gradient developed between rectal contents and haemolymph in a number of insect species, Berridge & Gupta (1967) list *Calliphora* as achieving a freezing point depression difference of 0·9° C., the locust *Schistocerca* 2·0° C., and *Ae. detritus* 1·3° C. Much greater differences occur in the larvae of the shore flies *Ephydra riparia* Fallén and *Coelopa frigida* (F.), which achieved differentials of 5·3° C. and 3·7° C., respectively, and the meal-worm *Tenebrio* is quoted as reaching a 9·0° C. difference. Unfortunately no histological information is available for *E. riparia* or *C. frigida*, but the rectal gland of the meal-worm is assisted by the cryptonephric system (Saini, 1964; Ramsay, 1964), in which an osmotic pressure exceeding that of the general bulk of the haemolymph is generated in the narrow perirectal space, thus reducing the osmotic gradient across the rectal gland epithelium. The mechanism by which this is achieved is presumed to be the active secretion of potassium chloride (to a maximum concentration of more than twice molar) into the Malpighian tubules from the haemolymph, for which the tubule cells themselves may be responsible. Water is drawn from the perirectal fluid, but the ionic composition of this fluid remains "normal", the osmotic pressure being made up by non-electrolytes. Ramsay suggested that this ensures that no epithelium is exposed to excessive ionic concentrations on both sides. This point makes it difficult to accept the possibility that in *Calliphora* the rectal contents are made hypertonic to the haemolymph by the secretion into the intercellular spaces (80 to 90 per cent. of the cell surface (Berridge & Gupta, 1967)) of even more concentrated solutions.

Since the larval rectum of *Aedes detritus*, with a simple layer of gland cells, achieves a greater osmotic gradient than does *Calliphora*, it is not unreasonable to conclude that the apical membrane is actively involved in the process. More knowledge of detailed structure is obviously needed before such problems as the high osmotic activity in *E. riparia* and *C. frigida* can be resolved. The detailed structure of the cicadoid Homoptera and hydrocorisid Heteroptera also needs further investigation. In the former, sections show many cells with a cavity below the nucleus (Plate II, fig. C), and it may perhaps be that the cell undergoes a cycle of filling and expulsion of water under pressure. If any of the cytoplasmic elements were contractile under certain ionic conditions, this mechanism would be possible. In the Hydrocorisae, the rectal gland cell seems like the supposed water-absorbing, eosinophil, type with striated border, carried to an extreme, in which the basal granular basophil cytoplasm is eliminated. There is no doubt that the excreta are hypotonic to the haemolymph (Staddon, 1963, 1964), but it is not proven whether the hypotonicity is produced in the Malpighian tubules or conferred upon the excreta during passage along the ileum. Two possible explanations arise from the fact that the excretory product is ammonium bicarbonate, and water is not only absorbed by cuticular osmosis, but is actively ingested to provide a sufficient flow to carry this material at a non-toxic concentration. One is that the Malpighian tubules may be producing an excessively hypotonic fluid, and the ileum may in fact absorb water. The other is that the ion-absorbing aspect of striated border cells, which can be shown to occur (Phillips, 1964) and which must be present in order to restore sodium/potassium balance following the excessive passage of potassium into the Malpighian tubules, may in Hydrocorisae be the main component of their activity. In view of the fact that potassium secretion into the Malpighian tubules is presumed to be concerned with uric acid excretion (Wigglesworth, 1931), the ionic composition of the tubule fluid in Hydrocorisae may be very different from that recorded from terrestrial insects. It is also possible that the ileum cells might actively transport water into the lumen.

It is of interest that in both *Aedes detritus* and *Halosalda lateralis* the presumed solute-absorbing region is anterior to the presumed water absorbing region. This must mean that, at least under one of the alternative conditions of hypo- or hypertonicity of excreta, the solute absorption must precede the water absorption. It has

not yet been shown whether the routine ion balancing that takes place under all osmotic conditions is performed by the posterior gland (thus leaving the anterior gland inactive, except when hypotonic excreta are produced) or by the anterior gland, or by both.

Finally, the lack of resemblance between the histology of the rectal glands of *H. lateralis* and that of the Hydrocorisae supports the current view that the Saldidae are not particularly close to the ancestry of that group. The longitudinal section of the mid-hind gut junction of *Saldula* sp., published by Miyamoto (1961) and repeated, with permission, in my own review (Goodchild, 1966), is misleading (*cf.* fig. 1) in failing to indicate the restricted entry of the Malpighian tubules and the presence of the rectal valve immediately posterior to this point. A restricted pyloric region such as this is compatible with primitive Geocorisid structure, but histological studies on the Dipsocorimorpha would clarify this point.

Summary

1. The hind gut and rectal glands of a salt marsh hemipteran, *Halosalda lateralis* (Fallén), are shown to consist of two cell types comparable to those of the same region in larvae of *Aedes detritus* Edwards. This condition is not found in any other Hemiptera, and is probably related to the estuarine habitat of this species.

2. The rectal gland cells of *Hydrometra stagnorum* (L.) are shown to conform to the structure in other Amphibicorisae, although the hind gut has an overall resemblance to that of the Hydrocorisae.

3. The rectal gland of Hydrocorisae is shown to differ significantly from glands producing hypotonic excreta in other species.

4. It is suggested that for rectal glands of the types described here, some measure of active water transport by eosinophil striated cytoplasmic regions cannot be ruled out. The additional, supposedly solute-absorbing gland cells of estuarine insects may be actively pumping water from the haemolymph.

References

Bahadur, J., 1963, Rectal pads in the Heteroptera. *Proc. R. ent. Soc. Lond.* (A) **38** : 59–69.

Beament, J. W. L., 1965, The active transport of water: evidence, models and mechanism. *Symp. Soc. exp. Biol.* **19** : 273–98.

Berridge, M. J., 1965, The physiology of excretion in the cotton stainer, *Dysdercus fasciatus* Signoret. I. Anatomy, water excretion, and osmoregulation. *J. exp. Biol.* **43** : 511–21.

—— & Gupta, B. L., 1967, Fine-structural changes in relation to ion and water transport in the rectal papillae of the blowfly, *Calliphora*. *J. Cell. Sci.* **2** : 89–112.

Clapp, J. R., Watson, J. F. & Berliner, R. W., 1963, Effect of carbonic anhydrase inhibition on proximal tubular bicarbonate re-absorption. *Am. J. Physiol.* **205** : 693–6.

Curran, P. F. & McIntosh, J. R., 1962, A model system for biological water transport. *Nature, Lond.* **193** : 347–8.

Diamond, J. M., 1965, The mechanism of isotonic water absorption and secretion. *Symp. Soc. exp. Biol.* **19** : 329–47.

—— & Tormey, J. McD., 1966, Role of long extracellular channels in fluid transport across epithelia. *Nature, Lond.* **210** : 817–20.

Goodchild, A. J. P., 1963, Studies on the functional anatomy of the intestines of Heteroptera. *Proc. zool. Soc. Lond.* **141** : 851–910.

—— 1966, Evolution of the alimentary canal in the Hemiptera. *Biol. Rev.* **41** : 97–140.

Graham-Smith, G. S., 1934, The alimentary canal of *Calliphora erythrocephala* L. with special reference to its musculature and to the proventriculus, rectal valve and rectal papillae. *Parasitology* **26** : 176–248.

Gupta, B. L. & Berridge, M. J., 1966*a*, A coat of repeating subunits on the cytoplasmic surface of the plasma membrane in the rectal papillae of the blowfly, *Calliphora erythrocephala* (Meig.), studied *in situ* by electron microscopy. *J. Cell. Biol.* **29** : 376–82.

—— —— 1966*b*, Fine structural organization of the rectum in the blowfly, *Calliphora erythrocephala* (Meig.) with special reference to connective tissue, tracheae and neurosecretory innervation in the rectal papillae. *J. Morph.* **120** : 23–82.

Hopkins, C. R., 1967, The fine-structural changes observed in the rectal papillae of the mosquito *Aedes aegypti* L. and their relation to the epithelial transport of water and inorganic ions. *Jl R. microsc. Soc.* **86** : 235–52.

JACKSON, M. J. & SMYTH, D. H., 1968, Role of sodium in the intestinal transport of organic solutes. *Nature, Lond.* **219** : 388–9.
LICENT, P. E., 1912, Recherches d'anatomie et de physiologie comparées sur le tube digestif des Homoptères supérieurs. *Cellule* **28** : 1–161.
MIYAMOTO, S., 1961, Comparative morphology of alimentary organs of Heteroptera, with the phylogenetic consideration. *Sieboldia* **2** : 197–259.
NOIROT, C. & NOIROT-TIMOTHÉE C., 1966, Revêtement de la membrane cytoplasmique et absorption des ions dans les papilles rectales d'un Termite (Insecta, Isoptera). *C.r. hebd. Séanc. Acad. Sci. Paris* **263** : 1099–102.
—— —— 1967, L'épithélium absorbant de la panse d'un termite supérieur. Ultrastructures et rapport avec la symbiose bactérienne. *Annls Soc. ent. Fr.* (N.S.) **3** : 577–92.
PHILLIPS, J. E., 1964, Rectal absorption in the desert locust, *Schistocerca gregaria* Forskal. I. Water. *J. exp. Biol.* **41** : 15–38.
RAMSAY, J. A., 1950, Osmotic regulation in mosquito larvae. *J. exp. Biol.* **27** : 145–57.
—— 1964, The rectal complex of the mealworm *Tenebrio molitor* L. (Coleoptera, Tenebrionidae). *Phil. Trans. R. Soc.* (B) **248** : 279–314.
SAINI, R. S., 1964, Histology and physiology of the cryptonephridial system of insects. *Trans. R. ent. Soc. Lond.* **116** : 347–92.
STADDON, B. W., 1963, Water balance in the aquatic bugs *Notonecta glauca* L. and *Notonecta marmorea* Fabr. (Hemiptera; Heteroptera). *J. exp. Biol.* **40** : 563–71.
—— 1964, Water balance in *Corixa dentipes* (Thoms.). *J. exp. Biol.* **41** : 609–19.
WIGGLESWORTH, V. B., 1931, The physiology of excretion in a blood-sucking insect, *Rhodnius prolixus* (Hemiptera, Reduviidae). *J. exp. Biol.* **8** : 411–51.
—— 1932, On the function of the so-called "rectal glands" of insects. *Quart. Jl microsc. Sci.* **75** : 131–50.
—— 1933, The function of the anal gills of the mosquito larva. *J. exp. Biol.* **10** : 16–26.

(Manuscript received 28th February, 1968)

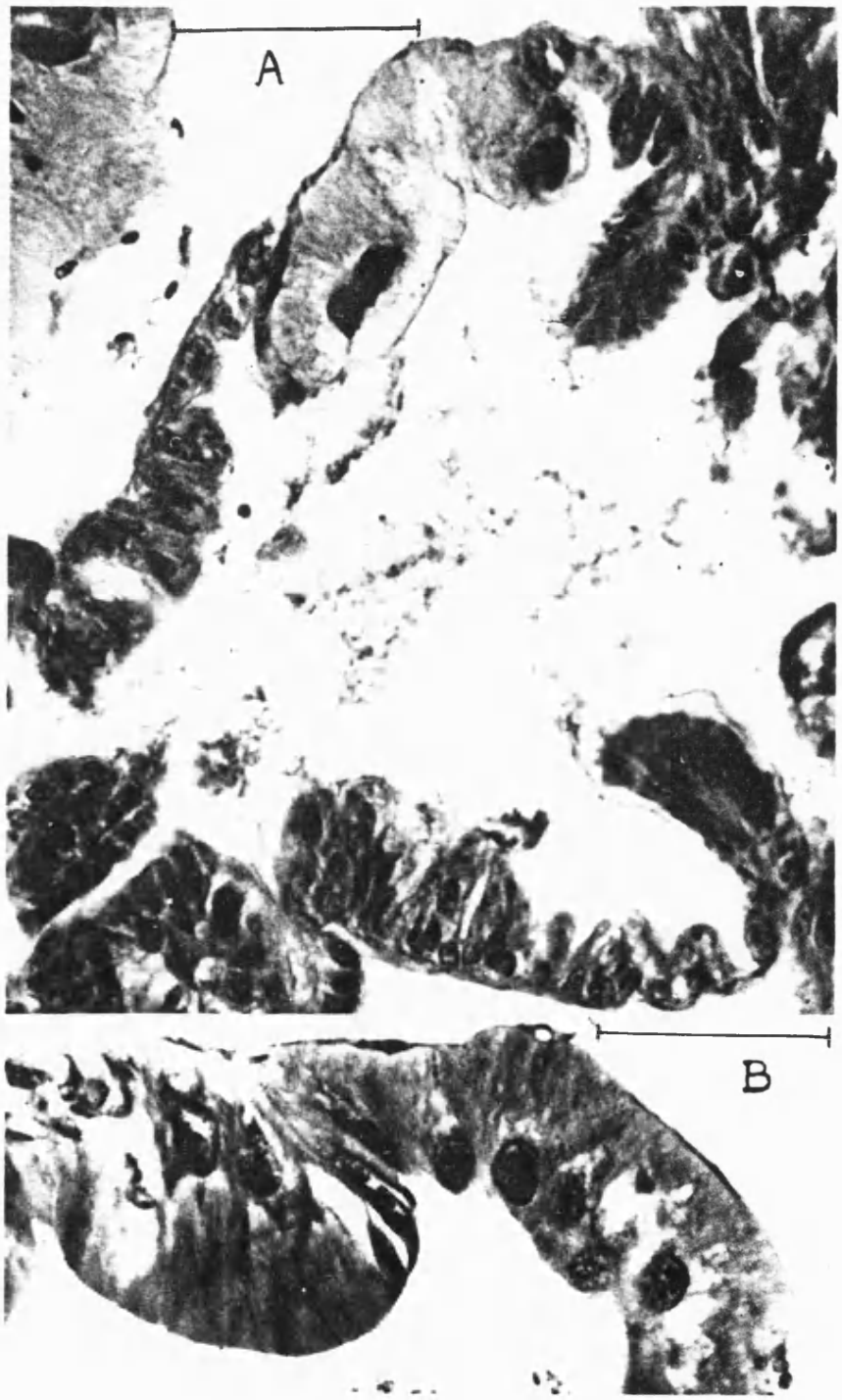


FIG. A.—Oblique sagittal section through contracted ileum of *Halosalda lateralis*, showing gland cells, rectal valve and other structures.

FIG. B.—Part of sagittal section through rectal gland of *H. lateralis*, showing anterior (on right) and posterior cell types, and junction zone.

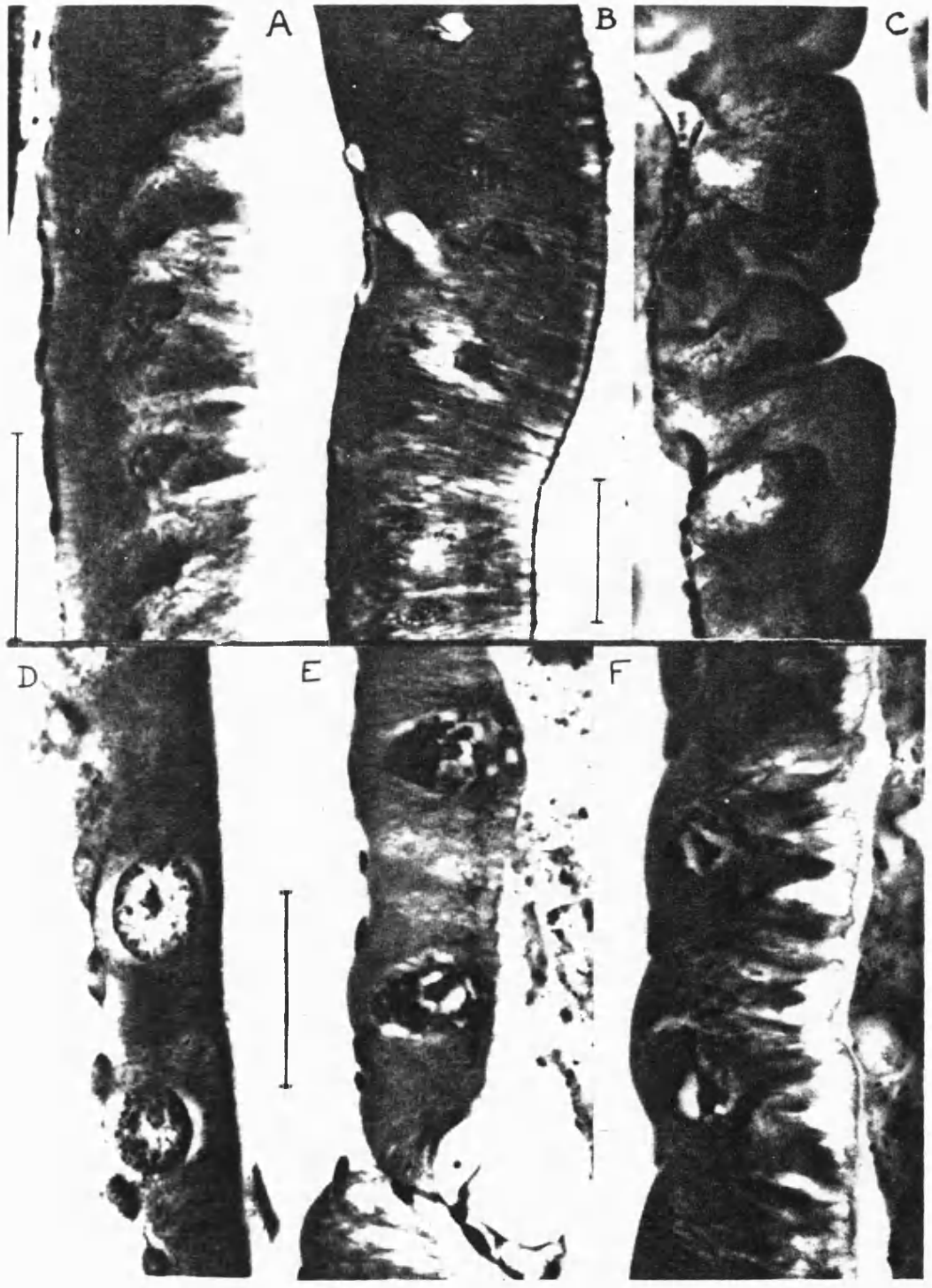


FIG. A.—Rectal gland (ileum) cells of *Hydrometra stagnorum*.
FIG. B.—Rectal gland (ileum) cells of *Sigara* sp.
FIG. C.—Hind-gut cells of *Ptyelus flavescens* F. (Homoptera: Cercopidae).
FIG. D.—Rectal gland cells of *Aedes aegypti*.
FIG. E.—Anterior rectal gland cells of *Ae. detritus*.
FIG. F.—Posterior rectal gland cells of *Ae. detritus*.
Scale, 50 μ (B and C; D, E and F, to same scale)

APPENDIX 8

(*Rev. Zool. afr., 91, n° 4*) (*A paru le 30 décembre 1977*).

Bionomics, Aggregated Feeding Behaviour, and Colour Variations in the Sap-sucking Bug *Mygdonia tuberculosa* Sign.

(*Hemiptera: Coreidae*)

By A.J.P. GOODCHILD

(School of Biological Sciences, Bath University, England)

SYNOPSIS

Mygdonia tuberculosa is a sap-sucker which feeds on shoots of the tree *Markhamia platycalyx* in East Africa. Feeding occurs in an aggregated manner on suitable leaves, and several distinct colour varieties are found in the immature stages. Aggregated feeding seems to be necessary for efficient exploitation of the food, and therefore the colour variations may render the clusters of nymphs less conspicuous to predators.

INTRODUCTION

The medium to large sized bugs of the sub-family Coreinae (Division Mictaria Stål 1873) are fairly distinctive insects, widely distributed through the wet tropics. Typically of uniform dull brown, grey, or black colour, in many genera with foliaceous expansions of pronotum or abdomen, and large hind legs bearing single massive femoral or tibial spines, they are of regular occurrence, though never very abundant. The hind legs, especially in the males, may be strongly curved, and they are sometimes referred to as « bow-legged bugs ».

A few species rank as minor pests of a wide range of tropical crops. *Mygdonia tuberculosa* is of no economic importance, but was sufficiently abundant on the campus of Makerere University, Kampala, Uganda, for the observations recorded here to be made, in July-August 1966.

BIOLOGY OF *MYGDONIA TUBERCULOSA*

The adults of this species are dull black, except for a variable sized patch of deep red on the dorsal surface of the abdomen. The pronotum is rough, and profusely covered with small tubercles. The only host plant in East Africa is the tree *Markhamia platycalyx* Sprague (Bignoniaceae), a widely distributed medium sized (c. 40-50 ft.) tree with vigorous power of regeneration when coppiced, and normally producing abundant vegetative suckers from its base. Pairs of adults may be found feeding near the tip of a sucker or coppice shoot (Fig. 1), and remain in this situation for a week or more, before copulation occurs. The duration of mating is unknown, but in all cases studied, the pair were no longer present on the shoot the day after mating had been observed. More than one pair to a shoot tip have not been found, though sometimes two of one sex and one of the other are present. It would seem that adults alighting on the shoot move towards the tip (where soft plant tissues suitable as food may be found), but the presence of a pair already in possession has a repellent effect, so that later arrivals wander until they find a vacant shoot. Since the feeding of a single pair does not usually prevent the shoot tip continuing to grow out, it is likely that it could support several adults, though multiple feeding might cause the tip to shrivel. The adult behaviour pattern in relation to food is therefore dispersive, and ensures the continuation of a food supply for the larval stages.

The eggs are laid in a continuous string along the mid-rib, on the underside of a fully hardened leaf near the base of the shoot, there being up to 36 in a batch. It is not known how many such batches the female can produce during her life. The eggs are more or less prism shaped, cemented to the leaf by one flat side, the others rising to a blunt ridge aligned in the direction of the leaf mid-rib, with a ring of minute micropylar processes at one end. They hatch in three weeks, and the first instar nymphs remain near the egg shells for a further week, and then moult to the second instar without having fed.

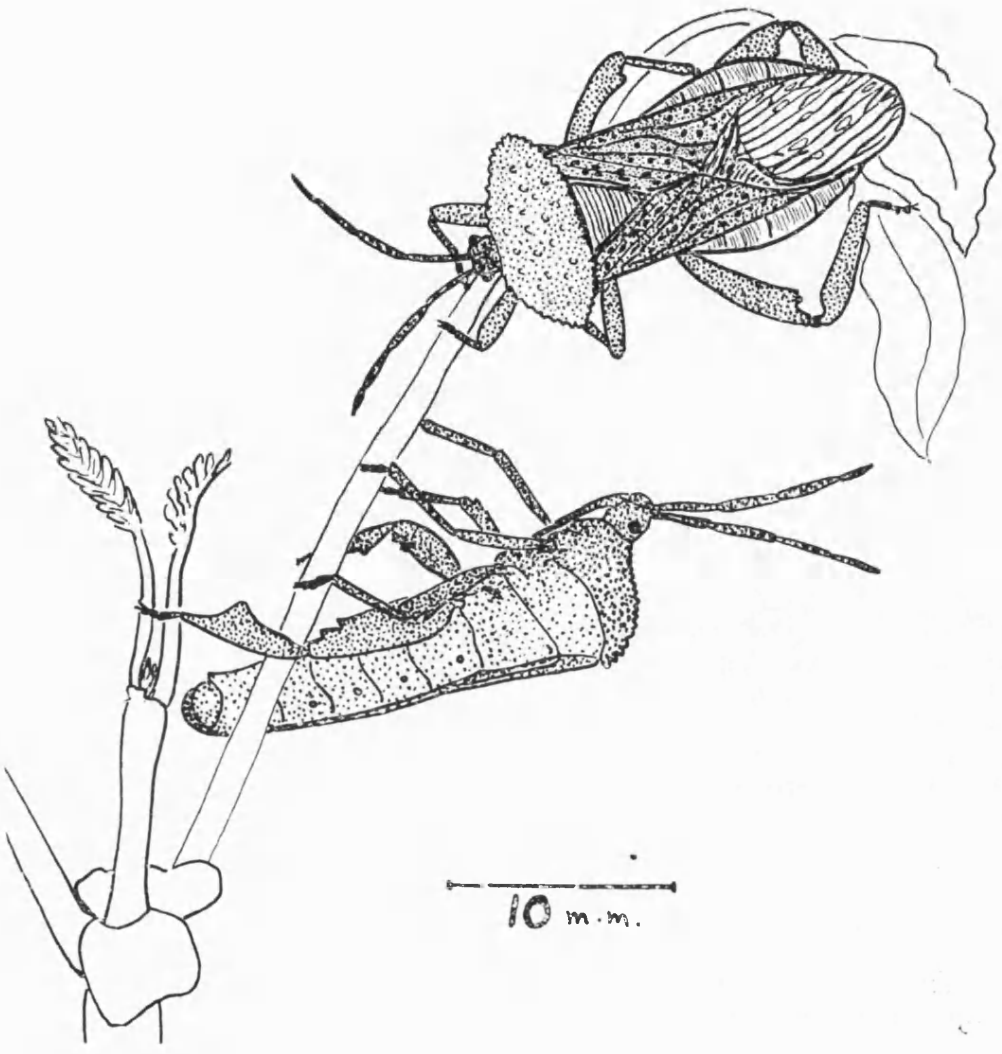


Fig. 1. — Pair of adult *M. tuberculosa* (female above) on shoot tip previous to mating (drawn from a photograph)

The second instar nymphs migrate to the upper part of the shoot and congregate on a partly expanded leaf. The growth of the shoot tips of *Markhamia platycalyx* produces opposite pairs of pinnate leaves of 7 to 11 leaflets, with overall length of 8 to 18 inches, and when growing under favourable conditions there will be a pair of leaves just unfolding, a pair half expanded, and a pair fully expanded

but not hardened, on the top three nodes of the shoot. *M. tuberculosa* does not feed on hardened leaves, and only in the final instar on the terminal part of the shoot axis. The unexpanded leaf primordia do not provide enough space for a full batch of nymphs, but they may be forced to feed on this site if the shoot is growing slowly, when the leaves at lower nodes have hardened. Under average conditions, the leaves at a node take four to five weeks to expand fully and harden. All the nymphs in a batch cluster on a single suitable leaf, with few if any on the opposite member of the pair. They occupy both upper and lower sides, with their mouth parts inserted into the mid-rib or petiole within a very restricted area, and their bodies directed radially away from that region. Feeding continues at the same point throughout an instar, even though the distal part of the leaf may wilt and shrivel. If few nymphs are present (due to predation or egg parasitism) the effect on the leaf is slight, though it has been observed that when the leaf hardens while nymphs are feeding (which happens on infrequent occasions) a black scar forms along the mid-rib, the outer hardened lamina remaining green.

In nearly every case observed, some half dozen or so second instar nymphs colonised the leaf a few days before the remainder of the batch, presumably as a result of earlier hatching, and remained an instar in advance of the others throughout the nymphal period. Moulting takes place on the leaf, and the instar length is 7 to 8 days. There is a pause in feeding a day or two before the moult, during which the nymphs rest in the centre of a leaflet, randomly orientated with a tendency for those on the periphery of the group to face outwards. There is also a delay of a day or so before feeding is resumed after the moult. If, in the meantime, the leaf has hardened, several days may elapse before the batch of nymphs migrates to a suitable leaf higher up the stem, or on an adjacent stem if it is in contact with the first. The third and fourth instars also feed in this aggregated manner, but the fifth (and last) nymphal instar may be found scattered over several small or unexpanded leaves at the shoot tip, or may feed on the apical stem axis. After feeding for about a week, they they cluster again on a hardened leaf lower down the stem, and moult to the adult eight days later. Some feeding on the original shoot may occur in the adult stage, but after a week all the newly fledged adults will have dispersed. Growth in length and weight during the nymphal instars is rapid, as indicated in Table I.

TABLE I.

Length and weight of unfed nymphs of *M. tuberculosa*

Instar	Body length (mm.)	Mean weight (mg.)
II	7.0	8.0
III	8.5	17.0
IV	12.0	60.6
V	16.5	173.5

AGGREGATION BEHAVIOUR

The feeding aggregations of nymphs are maintained by a form of « homing » behaviour which is all the more noticeable because their reactions to disturbance are stongly dispersive. There are two stages in their alarm reaction. The first one is initiated when rain or wind shake the plant vigorously, or by the sudden casting of a shadow on the leaf. The nymphs disperse over the whole surface of the leaf, and remain motionless with quivering antennae. In this condition they cling with considerable tenacity to the smooth surface of hardened leaves, and even to a clean glass surface, if they have been collected in a glass vessel. This must ensure that wind or rain do not dislodge them from the plant. The tarsi bear the usual pair of claws and pseudarolia, the latter being rounded and longitudinally ridged above, but membranous below and perhaps capable of a sucker-like action.

If nymphs in this first state of arousal are approached with a collecting tube, which might simulate a predator, their behaviour changes again, and they release their foothold, and, with legs drawn up to the body, tumble off the leaf. Although they readily cling to any leaf or stem which they touch in their fall, many drop to ground level. However, after a few hours, or the following day, the cluster will be found to have reformed, and in a high proportion of those observed, on the original leaf. If several suitable shoots are close together more than one feeding group may form after artificial disturbance, but less commonly after natural alarms. This behaviour makes it very easy to transfer colonies of nymphs from one area to another, for convenience of observation, as it is only necessary to shake them out of the container over a suitable shoot.

The disturbed nymphs show a strong negative geotaxis, but it is not known whether they have any ability to discriminate between the species of plant at ground level, or whether it is simply the high probability that the nearest stems belong to the plant on which they were feeding which ensures that most individuals return to a suitable shoot. Occasionally nymphs may be seen on the upper leaves of other species of plant in the same hedgerow. The insects may employ tactile, gustatory or visual senses to detect unhardened leaves, but the tendency to return to the original leaf even when adjacent ones are equally suitable, suggests a reaction to traces of the insects own scent, as has been found with cotton stainers (Youdeowei & Calam, 1969).

Solitary specimens, or groups of two or three, are commonly seen, the result of predation and parasitism on a batch of eggs. They do not remain on one shoot for long, and in fact the life cycle of such individuals has proved impossible to follow because of this. They do not seem to feed with any persistence, and may be more vulnerable to predators than nymphs in large groups. When a wandering nymph meets one which is feeding, it touches it with its antennae and immediately probes the substram with its proboscis. If it encounters a cluster of nymphs it is more strongly stimulated, and not only probes, but thrusts itself in among the others in an excited manner. A curious feature of the nymphal aggregations is the presence among many of them of a few nymphs of the related coreid *Phyllogonia biloba* Sign., particularly during a period in 1966 when many adults of this species were found on *Markhamia* shoots. No clusters of *P. biloba* nymphs alone were found, though nymphs feeding in their normal solitary fashion on creepers (*Cissus* sp.) were seen.

Aggregation implies toleration by insects of the close presence of other individuals, and in this case extended even to individuals of a different species. It must therefore be recorded that the larger nymphs of *M. tuberculosa* showed clear signs of discomfort when a smaller nymph walked over them. If the intruder paused on the dorsal surface of the larger insect, a distinct behaviour pattern was elicited from the latter, a rhythmical sideways rocking about once a second until the smaller insect moved on.

COLOUR VARIATION

A conspicuous feature of the immature stages of *M. tuberculosa* is the existence of a range of colour variations which are apparent in the third to fifth instars. There is not any such variability in the adults, except in the extent of red colouring on the abdominal dorsum (the correlation of which with nymphal colour is unknown). Second instar nymphs are all alike, with black thorax, head, and antennae, and abdomen which in the unfed state is iridescent greenish black, but which soon becomes shiny dark brown when the insects feed, presumably due to elimination of fine surface wrinkles which gave diffraction colour effects. The legs may be black with a red band around the middle of the femur, or all red except for the tarsi.

In the third instar colour bands of variable extent, and either red or yellow, are present on both femora and tibiae; the thorax may be all black, or have orange-yellow wing pads and a broad border of the same colour round the pronotum; and the abdomen may be uniform dark brown, or have small creamy-white spots on the lateral margins of each segment. Correlated with this last variation, the leg bands and pale areas of thorax are also creamy-white.

The variations observed in the fourth and fifth instars can be grouped into four types, in some of which there is a wide range of variability of expression.

Type 1 (« speckled »). This is the commonest type, and ranges from very dark to light coloured spotted forms. Head, legs, and antennae black; underside dark sepia-brown. pronotum, wing pads, and lateral margins of abdominal segments orange-brown. Variable sized patches of creamy-orange on sides of head, coxae, femuro-tibial joints, posterior margins of thoracic pleura, and as irregular longitudinal stripes on either side of midline of abdominal dorsum and scutellum. In lighter coloured specimens creamy stripes occur also along abdominal midline and adjacent to the orange-brown border, and the dark brown ground colour of the abdomen is isolated as irregular lateral and submedian spots in each segment. Included in Type 1 are the darkest forms in which there is no trace of creamy-orange patches.

Type 2 («white »). Second commonest form, resemble the lighter variants of Type 1 in pattern, but with the range of variation within

the type extending further in the encroachment of pale areas on the dark ground colour. Legs with broad bands or almost all pale, sides and ventral part of thorax with pale patches, abdominal venter with pale segmental spots ranging to extensive pale areas with dark residual spots. General dilution of colour so that areas orange-brown or creamy-orange in Type 1 are grey-white, dark areas grey-brown.

Type 3 (« red leg »). Similar to dark form of Type 1, but legs all red or red-brown, instead of black.

Type 4 (« black »). In fifth instar only (as observed so far), entirely black or deep sepia brown. In two instances, fourth instar nymphs of Type 3 were found to moult into this Type.

Although these variations pose an interesting genetic problem, there is insufficient data at present to do more than guess at the mechanisms involved. There are probably multiple genes governing the extent of pale area in Types 1 and 2, and maybe a single gene for colour dilution accounting for the difference between these types. The situation with regard to Types 3 and 4 is obscure.

The maintenance of a polymorphic population implies that the variability is of survival value, and since the effect of these variations is to give the feeding cluster a variegated appearance, they may have the beneficial result of disguising it from some forms of predator. Adult *M. tuberculosa* are probably not taken by predators to any great extent, as they produce abundant pungent secretions from their metathoracic glands (Remold, 1963, discussed the functions of this secretion), and also have been found to use the spines of the hind tibia in a defensive manner. If an adult is picked up by its abdomen, the hind legs are raised above the abdomen and the spines forced firmly inwards in such a way that they would encounter the face of a lizard, bird or mammal predator attempting to capture the insect.

The nymphal stages have no such defence mechanisms. Although they have presumed scent glands in the dorsal abdomen, they do not produce the pungent odour typical of the adults. Predation upon nymphs by spiders, mantids, and ants has been observed, and strong suspicion falls on lizards and reduviid bugs found in the neighbourhood of nymphal colonies which had suffered losses.

DISCUSSION

Feeding in dense aggregations is characteristic of sap-sucking Hemiptera, since the plant vascular system supplies a continuous source of food, in contrast to the local destruction of tissue by Miridae or Tingidae or mesophyll-feeding Homoptera such as Jassidae. Also characteristic of sap-suckers is their ability to feed on mature stems, even woody tree branches. Among the small Homoptera the Coccidae have this habit, and it has been observed by the present writer (1963a, 1963b, 1967) in Tettigometridae (Fulgoroidea), Cercopidae (Cicadoidea), Tessaratomidae and Plataspidae (Heteroptera). The area of the plant suitable as a feeding site for these forms is much in excess of that occupied by the insects, but they tend to remain clustered near to the egg mass from which they emerged. Reduced powers of movement and lack of a positive dispersal behaviour lead to these aggregations. The same can be said of aphid colonies on soft plant tissues, Aleyrodidae, and Psyllidae. So far no species of large sap-sucking Coreidae has been found to feed on woody stems of its host plant. The American Squash Bug, *Anasa tristis* De G., is said to be gregarious in its early stages, but later in its infestation of cultivated Curcubits it is gregarious only in the sense of competing for restricted feeding or resting sites. African species observed by the present writer usually feed on small and widely scattered points of soft growing tissue, which can only support one or two of the mature nymphs. These species are *Phyllogonia biloba* (creepers, *Cissus* spp.), *Anoplocnemis curvipes* (on a wide range of herbs and shrubs), *Anoplocnemis signata* (on a herb, *Aspilia africana,* Compositae), and *Cossutia stali* (on a tree, *Bridelia micrantha,* Euphorbiaceae). Their behaviour pattern must be dispersive, like that of the adult *Mygdonia,* and it may be significant that no colour varieties have been observed in the nymphal stages of these species.

As has been described, the plant host of *Mygdonia tuberculosa* provides large areas of suitable food in its slow maturing leaves, but the insects aggregate very strongly in only a small part of this. The insertion of the mouth parts of all members of the colony into a very short length of the leaf axis must maximise sap availability. It cannot be an adaptation to avoid predation, because the reaction to danger is immediate dispersal. Fuseini & Kumar (1975) found that the clusters of bright red nymphs of cotton stainers resembled flowers, and the insects were not easily alarmed. The absence of preda-

tion on the clusters could not be explained by aposematic colouring as the nymphs were readily eaten by lizards and mongoose.

The evidence suggests that the variegated colouring of the *Mygdonia* nymphs must be beneficial, in order for the polymorphism to persist in the population, but it is not clear how it functions. It may be that the cluster is in some way made less conspicuous, or the pattern presented in the first phase of the alarm reaction confuses a predator, but the answer may lie in an entirely different direction, in each colour gene carrying with its compensating advantages and disadvantages in physiological efficiency or resistance to parasitoids.

SUMMARY

1. The coreid bug *Mygdonia tuberculosa* and its host plant *Markhamia platycalyx* are briefly described.
2. The instar length and behaviour associated with moulting are shown to be related to the duration of availability of the immature leaf as a source of food. The insects cannot feed on hardened leaves or woody stems.
3. The formation, and re-formation after disturbance, of aggregations of nymphs into feeding clusters is described.
4. Various distinct colour patterns of the older nymphs are described. Nymphs of related species which feed in a solitary manner have not been found to be variable in colour, and it is suggested that the variability of *M. tuberculosa* may render the clusters less conspicuous to predators.

REFERENCES

FUSEINI, B.A. & KHUMAR, R. (1975). — Ecology of cotton stainers (Heteroptera : Pyrrhocoridae) in Southern Ghana. — *Biol. J. Linn. Soc.*, 7, 113 - 146.

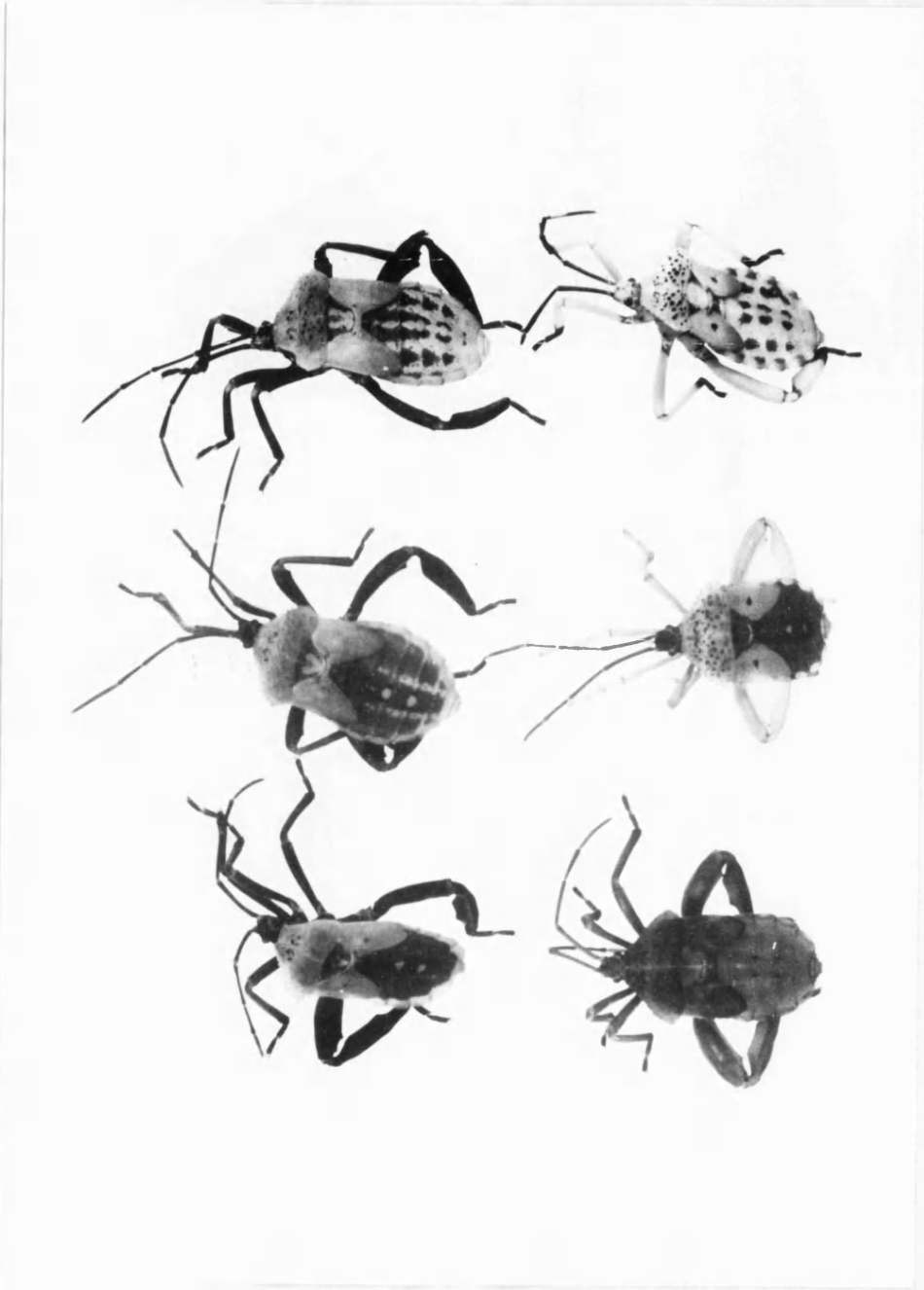
GOODCHILD, A.J.P. (1963a). — Some new observation on the internal structures concerned with water disposal in sap-sucking Hemiptera. — *Trans. R. ent. Soc. Lond.*, 115, 217 - 237.

GOODCHILD A.J.P. (1963b). Studies on the functional anatomy of the intestine in the Heteroptera. — *Proc. zool. Soc. Lond.*, 141, 851 - 910.

GOODCHILD, A.J.P. (1967). — Shield bug (*Piezosternum calidum* Fab.) infestation of Oyster Nut. — *East Afr. Ag. For. J.*, 33, 192 - 196.

REMOLD, H. (1963). — Scent-glands of land-bugs, their physiology and biological function. — *Nature, Lond.*, 198, 764 - 768.

YOUDEOWEI, A., & CALAM, D.H. (1969). — The morphology of the scent glands of *Dysdercus intermedius* Distant (Hemiptera : Pyrrhocoridae) and a preliminary analysis of the scent gland secretions of the fifth instar larvae. — *Proc. R. ent. Soc. Lond.* (*A*), 44, 38 - 44.



Supplement to Appendix 8 (illustration not accepted for original publication), The colour variants of fifth-instar larvae of Mygdonia tuberculosa.

APPENDIX 9

Ent. exp. & appl. **23** (1978) 177—188. *Ned. Entomol. Ver. Amsterdam*

THE NATURE AND ORIGIN OF THE MID-GUT CONTENTS IN A SAP-SUCKING HETEROPTERAN, *PIEZOSTERNUM CALIDUM* FAB.(TESSARATOMINAE), AND THE ROLE OF SYMBIOTIC BACTERIA IN ITS NUTRITION

BY

A. J. P. GOODCHILD

School of Biological Sciences, Bath University, England

Evidence is presented in support of the hypothesis that in *Piezosternum calidum* the mid-gut contents consist of symbiotic bacteria from the gastric caeca, and are not derived from the plant sap ingested. A similar process has been observed in other large sap-sucking Heteroptera, of which *Mygdonia tuberculosa* (Coreidae) has been studied as an example, the site of bacterial digestion is usually posterior to an interruption in the continuity of the mid-gut, but in *P. calidum* the gut is continuous and the contents fill it completely. It is suggested that the mid-gut contents of some other sap-sucking Heteroptera with continuous guts may also prove to be derived from their bacterial symbionts.

Phytophagous Heteroptera obtain their food from many different sites on the plant. They may feed on the endosperm of ripe seeds, destroy areas of plant tissue with their toxic saliva, suck out the contents of mesophyll cells or ovules, or imbibe sap from the vascular bundles. Though many species can feed in more than one of these ways, or show changes in feeding site through the life cycle, one mode or another usually predominates. It has been observed (Goodchild, 1963b) that the anterior sac of the mid-gut is filled with thick paste-like contents in those species which feed on plant tissues, but is empty, except for a little fluid and air bubbles, in the sap-suckers. Where observational evidence of feeding habits is lacking, the nature of the gut contents might be regarded as proof of the source of food. Even in sap-suckers, however, solid contents accumulate in a bulbous region lower down the mid-gut ("mid-gut bulb"), and these may include substances precipitated from the ingesta in the process of detoxifying plant defence chemicals.

In a study of some large tropical sap-sucking Heteroptera (Goodchild, 1963b), it was found that the mid-gut lumen was interrupted posterior to the mid-gut bulb, and that an additional bulbous expansion, absent from the mid-guts of tissue feeders, was developed behind the point of interruption. This bulb ("pre-caecal bulb") contained paste-like material, though it was in communication only with the posterior mid-gut, on which are borne the numerous gastric caeca filled with symbiotic bacteria. In these species also, the gastric caecal openings into the gut tube were not restricted as they are in tissue feeders, and recognisable caecal bacteria could be observed in the mid-gut lumen, extending anteriorward into the

pre-caecal bulb. The staining characteristics of the bacteria merged gradually into those of the bulb contents, suggesting that a process of digestion of the symbiotic bacteria was taking place. The occurrence of mid-guts with essentially the same pattern in a number of different families seemed to indicate that it was a functional adaptation to sap sucking (Goodchild, 1966). As far as the Tessaratominae are concerned, early observations by the present author (unpubl.) on occasional specimens captured in West Africa suggested an unspecialized continuous gut. However, in order to include the group in the study mentioned above (1963b), the gut of unfed specimens of *P.calidum* was described, and compared with the typical sap-sucking pattern. Later, an opportunity to study a large colony of maturing adults (Goodchild, 1967) made it clear from their feeding behaviour that they were true sap-suckers, while examination of their intestines showed that there was no interruption of the lumen, and that they became filled, anterior sac included, with a paste-like contents. The histology of the mid-gut and its contents suggested the possibility that they did not originate from the ingested sap, but were the equivalent of those found in the pre-caecal bulb of interrupted guts, i.e., they were derived from digestion of the caecal bacteria. This unusual situation was felt to be worthy of as rigorous a proof as the available material would permit, and therefore the intestinal contents of a sample of both *P.calidum* and a Coreid with a typical interrupted gut, *Mygdonia tuberculosa*, were examined by physiological, biochemical, and microbiological methods. The sap-feeding habit of *M.tuberculosa* has been verified by field study (Goodchild, 1977).

MATERIAL AND METHODS

Piezosternum calidum is a moderately large shield bug, about 20 mm long, dark green above and orange under. Widely distributed through forest regions of tropical Africa, its wild hosts are probably *Momordica* spp. (Cucurbitaceae).

Superficially similar to the common *Nezara viridula*, it differs from the latter in its short proboscis and conspicuous sternal keel. *Mygdonia tuberculosa* is a dull black coreid bug, up to 25 mm long, with a similar distribution, found only on shoots of the tree *Markhamia platycalyx* (Bignoniaceae) in Uganda, but on *Clerodendron* sp. (Verbenaceae) in West Africa.

Material of both was collected in Kampala, Uganda, where the observations on freshly dissected insects were made. Histological studies were made on guts from insects fixed whole (after removal of wings and some abdominal tergites) in Brasil's alcoholic Bouin fluid, and on dissected guts fixed in formol saline. Wax-embedded tissues were sectioned at 10 μm and stained by Masson's Trichrome method (iron haematoxylin/acid fuchsin/light green).

Chemical tests and microbiological studies were made on a consignment of live insects received by air from Uganda, also air-dried host-plant material from the same source. Thin film cellulose powder chromatography was used for amino acid analysis, using one way runs with either butanol/acetic acid/water (12:3:5) or butanol/pyridine/water (equal vols.) as solvents.

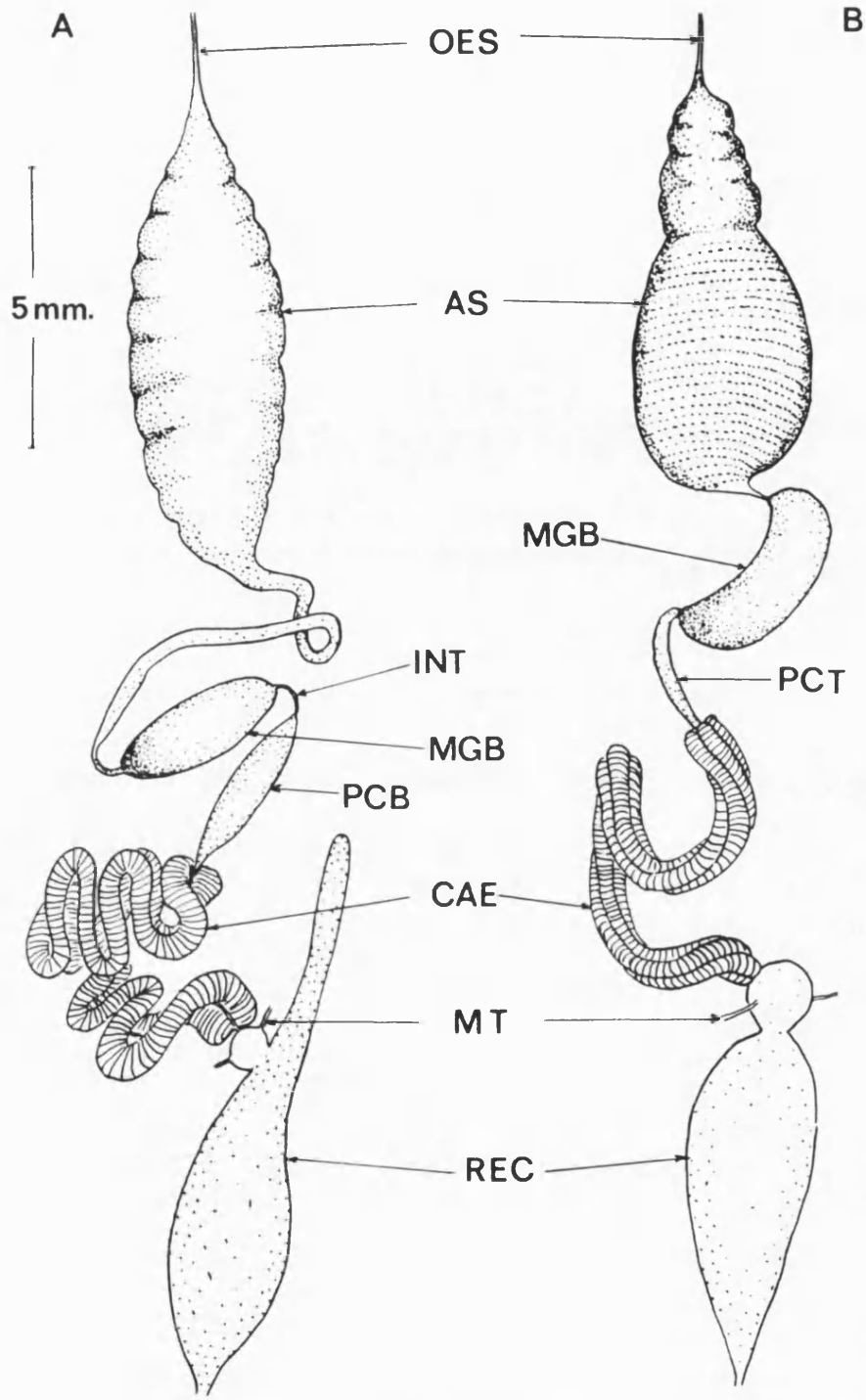


Fig. 1. A. Alimentary canal of *Mygdonia tuberculosa* (Coreidae). B. Alimentary canal of *P. calidum*. Key to abbreviations: AS - anterior sac of mid-gut; CAE - gastric caeca (two rows in A, four rows in B); INT - site of interruption of gut lumen; MGB - mid-gut bulb; MT - bases of Malpighian tubules joining excretory vesicle; OES - oesophagus; PCB - pre-caecal bulb; PCT - pre-caecal tube; REC - rectum.

RESULTS

Anatomy and physiology of the mid-gut

The gross anatomy of the alimentary canals of the two species is shown in Fig. 1.

In the case of *M.tuberculosa*, there is a mid-gut bulb, the contents of which are dark brown and disperse easily in water, and a pre-caecal bulb with creamy-green contents of mucoid consistency which do not mix readily with water. The contents are also quite distinct in histological sections, the former having an intrinsic golden-brown colour and staining only with neutral red, nile blue, toluidine blue or cresyl fast violet, while the latter is very faintly eosinophil. Between the two is the interrupted region of the mid-gut.

In *P.calidum*, the single mid-gut bulb arises from a short extension of the anterior sac. The point of entry into the bulb is extremely narrow (lumen about 30 μm wide), but the presence of histologically identical contents on either side showed the junction to be effectively continuous. From the mid-gut bulb a short pre-caecal tube leads to the caecal region. This tube, about 2 mm long and 0.5 mm wide, is lined with columnar cells 40 μm × 15 μm, with lobed tips and a narrow striated border. The gut in the caecal region is narrow, 60—80 μm wide, lined with columnar cells 20 × 8 μm, with basal nuclei and an apical zone containing secretory vacuoles. Globules of secretion, which take up light green stain, can also be seen between the lobes of the cell tips in the pre-caecal tube. The caeca are disclike, 0.4 mm across and 27—33 μm deep (in the axial direction), closely packed, with adjacent walls fused together. This region is about 8 mm long, and there are four rows of caeca, so that there must be about 1000 caeca altogether. The caecal openings are wide in nymphs, but restricted in adults, except when a bolus of caecal contents is being extruded. The cells of the caeca are pavement-like, though there are a few domed cells filled with granules staining with fuchsin.

In fresh material, the caeca, pre-caecal tube, and mid-gut bulb are dense white in colour, and the latter moderately well filled in all insects examined. In sections, the caeca and the gut tube in that region, contain basophil masses of bacteria with a scattering of acidophil granules and secretion globules. Unlike *M.tuberculosa*, where the pre-caecal bulb is the site of a change in staining reaction, in *P.calidum* unchanged caecal contents can be traced far forward into the pre-caecal tube. This tube has not, however, been found to be completely filled in any of the specimens examined. The contents of the mid-gut bulb are a mixture of small ovoid or rod-like granules which stain with fuchsin, and larger ovoid globules 6—12 μm in diam., together with coalescent masses of such globules up to 0.5 mm across. The globules take up light green stain more and more strongly as they increase in size, and within many of them, clusters of granules staining intensely with fuchsin may be detected. The small fuchsinophil granules predominate in the outer layers and in the opening to the pre-caecal tube, while the large coalescent masses are mainly in the centre of the bulb.

The anterior mid-gut sac of *P.calidum* is, in the unfed adult, about 2.5 mm wide and 7 mm long. The anterior part (about 3 mm) is pale orange, and the remainder translucent buff colour. The wall is thrown into many transverse folds. During the long (10—11 weeks; Goodchild, 1967) pre-oviposition feeding period, the mid-gut bulb becomes more distended, and dense white material gradually fills the anterior sac. In sections the contents of the sac are similar to those of the mid-gut bulb,

though with very few of the small fuchsinophil granules, and with larger coalescent masses of globules. The anterior sac is also well filled in the nymphal stages, but the contents are digested and absorbed before moulting takes place. The scanty contents remaining in newly fledged adults appear in sections as a homogeneous mass staining deeply with light green. The histological evidence is consistent with the view that caecal bacteria, mixed with digestive secretion, pass forward to the mid-gut bulb, where a rapid change in character is due to digestion proceeding in the presence of the low pH which has been found in that region. The formation of the typical globules might then be due to dissolution of bacterial cell walls and the running together of their protein contents. It is probable also that nucleic acids are broken down at this stage, thus destroying the basophil staining reaction of the bacteria.

Nature of intestinal contents

Attempts were made to produce the characteristic globules by the action of salivary gland extract on water extract of dried host plants, and by the action of pre-caecal tube extract on a suspension of caecal bacteria. The mixtures were allowed to react overnight, and dried, heat-fixed smears were stained by Masson's Trichrome method. The plant extract/salivary gland material did not resemble globules at all. The pre-caecal tube/bacterial suspension mixture, at pH 7 and pH 3, produced clumping of the organisms, with staining reaction similar to that of gut contents, but not typical globules. Boiled controls had the same effect. The result, therefore, is suggestive but not conclusive.

Pooled gut contents from 44 insects, anterior sacs and mid-gut bulbs separately, were analysed by suspending in excess of distilled water and centrifuging. The supernatant was freeze-dried in a weighed container, and the precipitate re-suspended in N/20 HCl and the process repeated. The second precipitate was suspended in N/20 NaOH and centrifuged. This supernatant was neutralised by drop-wise addition of HCl. A cloudy precipitate appeared at about pH 5.8, dissolving at lower pH. The precipitate was centrifuged down, dried, and weighed. The dry weights of the different fractions from the two gut regions are given in Table I. The item under mid-gut bulb "precipitated by high speed centrifuge" refers to the fact that the first supernatant was cloudy, and was cleared by centrifuging at 15.000 r.p.m. for one hour. Stained smears were prepared from each precipitate and supernatant. These showed that suspension in water broke up the large coalescent masses, and that acid destroyed the globular structure altogether. The ultracentrifuge precipitate consisted entirely of what looked like bacterial fragments, and the final insoluble residue was a very weakly staining mass of strands and small globules. None of the smears reacted positively with the periodic acid/Schiff's reagent test for polysaccharides.

The dried fractions all had a white or creamy, fluffy crystalline appearance. The water-soluble material contained sugars, amino acids, and protein. Some of this protein was a digestive enzyme, as this fraction showed strong enzyme activity.

TABLE I

Dry weight of fractions of intestine contents in P. calidum

Region of Mid-Gut	Fraction	Weight (mg)	Proportion of whole, %
	Water soluble	22.1	66.8
	Acid soluble	8.4	25.4
Anterior Sac	Alkali soluble	1.5	4.5
	Insoluble	1.1	3.3
	Total	33.1	100.0
	Water soluble	18.5	50.8
	Acid soluble	11.0	30.2
Mid-gut Bulb	Alkali soluble	1.9	5.2
	Insoluble	1.7	4.7
	Precipitated hy high speed centrifuge	3.3	9.1
	Total	36.4	100.0

The acid-soluble fraction seemed to be entirely protein, yielding amino acids only after hydrolysis, but showed no enzyme activity. The amino acid spectrum of the acid and alkali-soluble material, after hydrolysis, was the same as that of the amino acids in the water-soluble fraction.

A comparison was made with the amino acids in a water extract of host-plant material. Dried plant material was soaked in distilled water, homogenised, and centrifuged, all at 0°. The extract contained amino acids, non-reducing sugars, and traces of phenolic compounds, but was negative to tests for reducing sugars, peptides, and heat-coagulable protein. A positive result was given to a test for nitrate. The amino acid spectrum was not affected by incubation with salivary gland extract. The typical chromatograms obtained, and those of mixtures of known amino acids, are shown in Fig 2. The contents of the two gut regions gave similar results, but differed from the plant material, most notably in the following respects.

1). Proline was present in gut contents but not in the plant extract. This amino acid was recognised by the characteristic yellow spot when developed with ninhydrin, which was confirmed by development with isatin. A spot at about the same level in the chromatogram of plant extract was blue with ninhydrin, and could have been aminolaevulinic acid, common in plants as a chlorophyll precursor.

2). A spot at about Rf 15 with butanol/acetic solvent was observed in the gut content but not the plant extract chromatograms. Lack of pure samples to act as standards prevented identification, but the position suggests a sulphur-containing amino acid or a diamino acid.

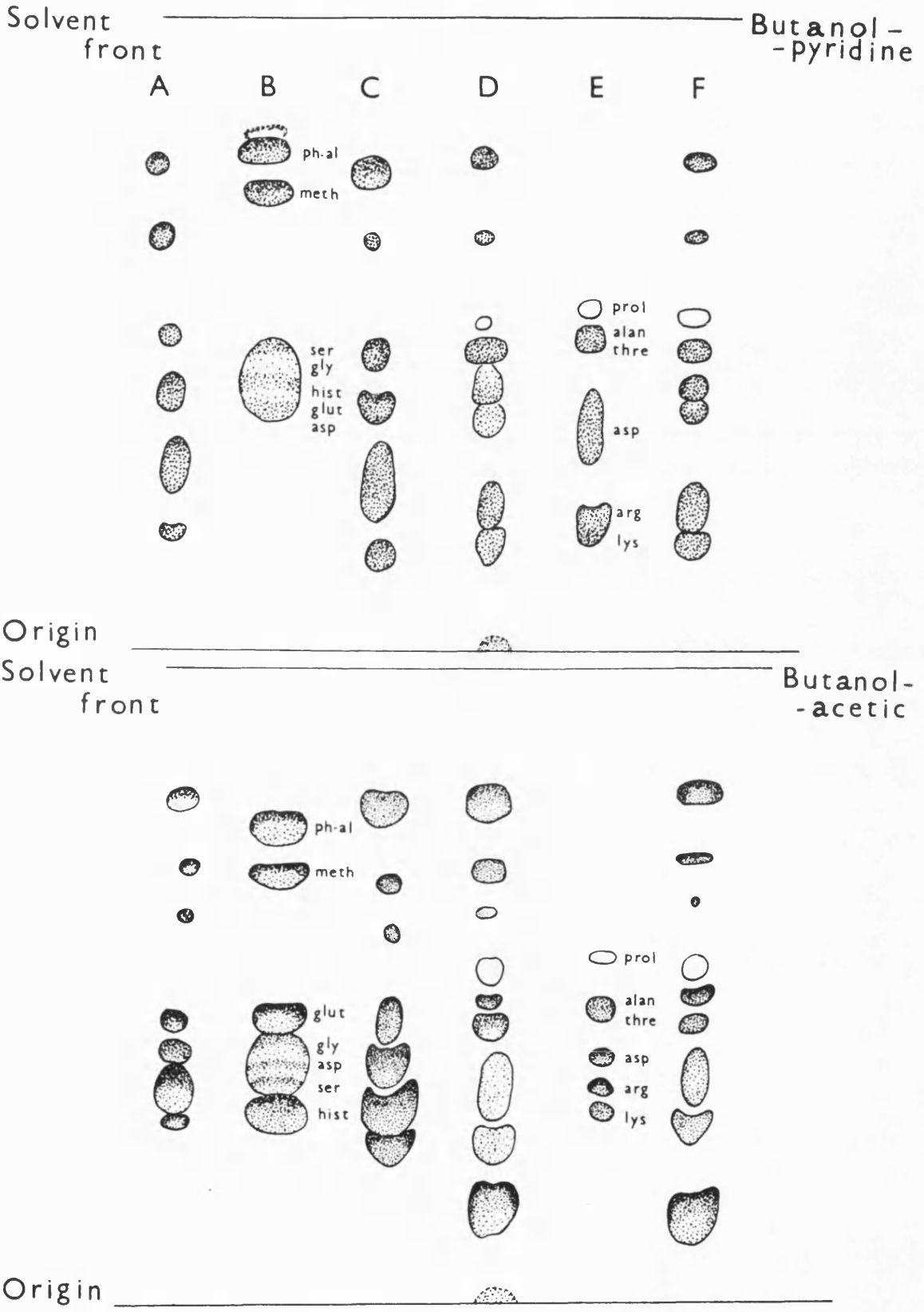


Fig. 2. Typical chromatograms of amino acids in host-plant extract and intestinal contents of *P. calidum* (water-soluble fraction). A - alcoholic host-plant extract; B - reference amino acid mixture (asp. - aspartic acid; glut. - glutamic acid; gly. - glycine; hist. histidine; meth. - methionine; ph-al. - phenyl alanine; ser. - serine.); C - aqueous host-plant extract; D - intestinal contents (anterior sac); E - reference amino acid mixture (alan.-alanine; arg. - arginine; asp. - aspartic acid; lys. - lysine; prol. - proline; thre. - threonine); F - intestinal contents (mid-gut bulb).

3). The general ninhydrin colour reaction differed in that all the plant extract spots were blue-purple, but the gut contents, in addition to the yellow of proline, gave a large brownish spot near the glycine position.

The amino acid spectrum of gut contents was also compared with that of an acid hydrolysate of caecal bacteria grown on a synthetic medium with an inorganic nitrogen source, and with an amino acid chromatogram of the contents of the pre-caecal bulb of *M. tuberculosa*. All three were strikingly similar, whereas that of the mid-gut bulb in the latter species was quite different, with a single large ninhydrin-positive spot at very low Rf and only faint traces of any other components.

The water extract of plant material will undoubtedly contain substances not present in the sap as ingested by the insect, and therefore the absence in the gut contents of compounds found in this extract cannot be taken as evidence against the origin of those contents from the ingesta. The probable source of the ingesta was xylem sap, because the host plant did not bear any green leaves during the period when the maturing adults were feeding (Goodchild, 1967), and phloem transport must therefore have been minimal. Xylem is recorded as the food source of many Homoptera (Cheung & Marshall, 1973; Marshall & Cheung, 1975). These authors observed solid residues in the guts of the insects, but they were mineral in nature. In other sap-sucking Homoptera, residues in the gut have been identified as carbohydrate (Edwards, 1965). Xylem sap would contain amino acids and nitrate (Bollard, 1960; Pate, 1973), but little if any, protein.

The protein nature of the gut contents of *P. calidum*, and the resemblance of its amino acid spectrum to that of caecal bacteria, and to that of the contents of the pre-caecal bulb of *M. tuberculosa*, are therefore in favour of the hypothesis of their origin from the caecal bacteria. On this view, the contents of the anterior sac would have undergone more prolonged digestion than those of the mid-gut bulb, which would explain the presence in the former of a higher proportion of water-soluble amino acids and a lower proportion of the acid-soluble protein.

Physiology of digestion and of the caecal bacteria

The digestive enzymes of *P. calidum* were studied to see whether they showed the capacity to deal with caecal bacteria on the large scale suggested. Salivary glands and anterior sac tissue were tested. The anterior sacs were divided into the orange anterior portion and the buff hind portion, but mid-gut bulb tissues were not tested owing to the small amount available. The only salivary enzyme detected was a weak invertase, but both parts of the anterior sac showed strong invertase, weak lipase and peptidase, and faint acid protease activity. It was realised too late that tests for lysozyme and ribonuclease would have been valuable. (Barnard (1969) suggested that the high level of pancreatic ribonuclease in ruminants may be due to the importance of bacteria in their nutrition). The weakness of the protease may be related to the long period of adult maturation, during which the protein gut contents accumulate. It is possible that at sexual maturity a more active protein digestion occurs.

In insects utilising micro-organisms as a food source, it has been found, that

limited regions of the gut have a high degree of acidity (dipteran larvae: Hobson, 1931; termites: Randall & Doody, 1934). This has been interpreted as a means of killing the micro-organisms prior to digestion. The mid-gut of *P. calidum* has also been found to possess such a region. The pH of the contents of the gut was investigated by extending whole guts on dry narrow-range indicator paper, and puncturing each region so that its contents wetted the paper. The mid-gut bulb was in the range pH 3 to 4, while the anterior sac was pH 6.7 to 7.0 and the caecal region and rectum were pH 7.0 to 7.3. Furthermore, the dried water-soluble fraction of the mid-gut bulb showed a pH of about 4 when redissolved. Hobson (1931) was able to detect phosphoric acid in the gut of Diptera, and he assumed that it was secreted by the gut. It is this acid, however, which would be liberated by the action of ribonuclease. Unfortunately, no tests for phosphate were made on the gut contents of *P. calidum*, but those of the pre-caecal bulb of *M. tuberculosa* gave an abundant precipitate with the ammonium molybdate test for phosphate.

The caecal bacteria grew readily on nutrient agar, and some trials were carried out to determine their capacity for growth with inorganic nitrogen sources or metabolic waste nitrogen, and thus to assess their possible value to the insects. Although the caecal forms are thread-like and up to 25 μm long, the forms in culture are Gram-negative short rods 2 μm long, with polar flagellae, and can be assigned to the genus *Pseudomonas* (kindly determined by Dr. R. G. Board). This is in accordance with the identifications of caecal bacteria made by Steinhaus, Batey & Boerke (1956). Culture media were prepared, using as energy sources sucrose, glucose, or di- or tricarboxylic acids (citric, tartaric, etc.), and as nitrogen sources peptone, mixed amino acids, uric acid, nitrate, nitrite, or ammonium salts. Low concentrations were used (0.2% energy source, 0.01% nitrogen source) as it was thought that this might be a better approximation to conditions within the caeca. Trials were also carried out on bacteria isolated from *M. tuberculosa*, which were of similar morphology and taxonomic position. It was found that in many cases growth was better if both carboxylic acid *and* glucose were supplied. A summary of the response of the bacteria to different nitrogen sources is given in Table II. It can be seen that the bacteria are well able to utilise the inorganic nitrogen present in the plant sap, and to recycle metabolic waste nitrogen, but the organisms from *P.*

TABLE II

Response of caecal symbionts of P. calidum *and* M. tuberculosa *to the nitrogen supply in the medium*

N supply	Uric Acid	NO_3	NO_2	NH_4	no N
M. tuberculosa	+ + +	+ + +	—	+ +	±
with citric acid	+ + +	+	+	+ + +	±
P. calidum	+	±	—	+	—
with citric acid	+ + +	+ +	+ +	+ + +	—

Basic medium: 1% agar, 0.2% glucose, nutrient salts
— no growth, ± growth just visible, + weak growth
+ + moderate growth, + + + vigorous growth

calidum did not grow in the absence of a nitrogen source. Those from *M. tuberculosa* showed a slight amount of growth without nitrogen, and might therefore be able to fix nitrogen to some degree (see Discussion in Goodchild, 1963b).

DISCUSSION

The evidence supports the idea that, in the nutritional process of *P. calidum*, there is large-scale exploitation of the caecal bacteria. This brings it into line with the other large tropical sap-suckers in which such a process is clearly taking place. It also provides an explanation for the presence in the anterior sac of the mid-gut, of cell types normally associated with the absorptive process in the intestinal tube (Goodchild, 1963b). What is remarkable, however, is the apparent absence of any residues originating from the ingesta, such as are found in the mid-gut bulb of interrupted intestines. This could be due to the absence from the plant sap of toxic compounds requiring sequestration. A comparison may be made with the gut of the Red Pumpkin Bug (*Coridius janus* Fabr.) which, according to Rastogi (1966) is continuous and with uniform pasty contents; whereas a close relative in the pentatomid subfamily Dinidorinae, *Aspongopus xanthopterus* Fairm., which as far as is known feeds on Passifloraceae or Bignoniaceae, has a typical interrupted intestine (Goodchild, 1963b). The guts of the cucurbit-feeding coreids *Leptoglossus membranaceus* F. (Goodchild, unpubl.) and *Anasa tristis* De G. (Breakey, 1936) are continuous; but the morphology of the gut of *Megymenum* spp. (Dinidorinae), cucurbit-feeders of S. E. Asia and Australia, has not yet been described. In regard to the two coreids named, the nature of the mid-gut bulb contents has not yet been determined, and it would be of great interest in the present context, if they were found to be of caecal origin. Material probably of this nature was found in the short connecting tube between caecal region and mid-gut bulb in several unspecialised coreids (Goodchild, 1963b).

The contents of the mid-gut bulb in the uninterrupted gut of Pentatomidae Phyllocephalinae is of the same histological type as that of the pre-caecal bulb of interrupted guts (Goodchild, 1963a), but the host plants of the species studied were not known. Most members of that sub-family seem to feed on Gramineae. The gut morphology of other species of Tessaratominae than *P. calidum* has not been described, but examination of material of the Bronze Orange Bug (*Musgraveia sulciventris* (Stål)), a citrus pest in Australia, showed it to be of much the same pattern. Other Tessaratominae of agricultural importance are also pests of tree crops (Goodchild, 1967).

Plant families and genera differ in their content of defensive or insect-repellent substances (Swain, 1977) and in the nature of the nitrogen compounds transported in the vascular tissues (Pate, 1973). It would not therefore be surprising to find that the guts of sap-sucking Heteroptera show functional adaptation to these differences. It would be expected that utilisation of the caecal flora would increase with the level of inorganic nitrogen in the sap, or with deficiency of essential

amino acids, while the isolation of the posterior gut by interruption of the lumen would be associated with plant-defence chemicals.

Very little investigation has yet been made into the details of these adaptations, but it must be significant that Cucurbitaceous hosts are so often associated with continuous guts, and that the widest range of hosts is reported from species (Coreidae, Mictinae) with interrupted guts. The Tessaratominae seem to be somewhat restricted in their host range, and this may be associated with the nature of their mid-guts.

I thank Mr. M. Lubega and Mr. E. Roberts, Makerere University, Kampala, Uganda, for supply of insects and plant material; Dr. T. E. Woodward, University of Queensland, Brisbane, for supply of Bronze Orange Bug; and Dr. R. G. Board, Bath University, for determination of the caecal bacteria.

RÉSUMÉ

LA NATURE ET L'ORIGINE DU CONTENU DE L'INTESTIN MOYEN CHEZ UN HÉTÉROPTÈRE SUCEUR DE SÈVE, PIEZOSTERNUM CALIDUM *(TESSARATOMINAE) ET LE RÔLE DES BACTÉRIES SYMBIOTIQUES DANS SA NUTRITION*

Les Hétéroptères suceurs de sève possèdent sur le mesenteron postérieur de nombreux coecums remplis de bactéries symbiotiques. Chez beaucoup d'espèces, par exemple *Mygdonia tuberculosa* (Coreidae) le mesenteron est interrompu dans sa région moyenne et la partie postérieure renferme un contenu digestif qui représente les bactéries coecales digérées. La partie antérieure contient peu de matière solide provenant de la sève de la plante-hôte. Chez *Piezosternum calidum*, suceur de sève sur des cucurbitacées; l'intestin moyen n'est pas interrompu et est complètement rempli par un matériel presque solide. Ce contenu digestif est uniforme d'aspect sur coupes histologiques et sa composition riche en acides aminés, le rapproche davantage des bactéries coecales que de la sève de la plante-hôte. Le mesenteron présente d'ailleurs une région très acide où les bactéries pourraient être digérées. Ces bactéries se cultivent sur un milieu à base d'acide urique, de nitrate ou d'ammoniaque, en ce qui concerne la source d'azote. L'utilisation massive des bactéries symbiotiques par l'insecte lui est peut-être nécessaire car la source d'azote dans la plante n'est pas directement assimilable. La continuité de la lumière intestinale est peut-être liée à l'absence dans la sève de la plante d'une substance qui serait nuisible aux bactéries.

REFERENCES

BARNARD, E. A. (1969) Biological Function of Pancreatic Ribonuclease. *Nature, Lond.* **221** : 340—344.

BOLLARD, E. G. (1960). Transport in the xylem. *Ann. Rev. Pl. Physiol.*, **11** : 141—166.

BREAKEY, E. P. (1936). Histological studies of the digestive system of the Squash Bug *(Anasa tristis* De G., Hemiptera, Coreidae). *Ann. ent. Soc. Amer.* **29** : 561—577.

CHEUNG, W. W. K. & MARSHALL, A. T. (1973). Water and ion regulation in Cicadas in relation to xylem feeding. *J. Insect Physiol.*, **19** : 1801—1816.

EDWARDS, J. S. (1965). Some observations on the solid components of the gut contents of aphids. *Proc. XIIth. Int. Cong. Ento.*, p. 173.

GOODCHILD, A. J. P. (1963a). Some new observations on the intestinal structures concerned with water disposal in sap-sucking Hemiptera. *Trans. R. ent. Soc. Lond.*, **115** : 217—237.

—— (1963b). Studies on the functional anatomy of the intestines of Heteroptera. *Proc. zool. Soc. Lond.*, **141** : 851—910.

—— (1966). Evolution of the alimentary canal in the Hemiptera. *Biol. Rev.* **41** : 97—140.

GOODCHILD, A. J. P. (1967). Shield Bug (*Piezosternum calidum* Fab.) Infestation of Oyster Nut. *E. Afr. Ag. For. J.* **33** : 192—196.

—— (1977). Bionomics, aggregated feeding behaviour, and colour variations in the Coreid bug, *Mygdonia tuberculosa* Sign. *Rev. Zool. Afr.*, **91** : 1032—1041.

HOBSON, R. P. (1931). Studies on the nutrition of blow-fly larvae. I. Structure and function of the alimentary tract. *J. exp. Biol.*, **8** : 109—123.

MARSHALL, A. T. & CHEUNG, W. W. K. (1975). Ionic balance of Homoptera in relation to feeding site and plant sap composition. *Ent. exp. & appl.*, **18** : 117—120.

PATE, J. S. (1973). Uptake, assimilation and transport of nitrogen compounds by plants. *Soil Biol. Biochem.*, **5** : 109—119.

RANDALL, M. & DOODY, T. C. (1934). Hydrogen-ion concentration in the Termite intestine. *In* KOFOID, C. A. *et al., Termites and Termite Control*, pp. 99—104, Univ. of California Press, Berkeley, Calif.

RASTOGI, S. C. (1966). Studies on the digestive organs of the Red Pumpkin Bug, *Coridius janus* Fabr. (Hemiptera: Dinidoridae). *Rev. Zool. Bot. Afr.*, **74** : 63—82.

STEINHAUS, E. A., BATEY, M. M. & BOERKE, C. L. (1956). Bacterial symbiotes from the caeca of certain Heteroptera. *Hilgardia*, **24** : 495—518.

SWAIN, T. (1977). Secondary compounds as protective agents. *Ann. Rev. Pl. Physiol.*, **28** : 479—501.

Received for publication: December 2, 1977.