The structure of the foregut and midgut of nymphs, subimagos and imagos of *Cloeon dipterum* (Ephemeroptera) and the functions of the gut of adult mayflies

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Abstract

The anatomy of the gut and the fine structure of the foregut and midgut of the nymphal, subimaginal and imaginal stages of *C. dipterum* are described. A new description is given of the nymphal foregut–midgut junction and its relationship to peritrophic membrane formation. Although the mouthparts are lost at the end of the nymphal stage, and the structure of the gut undergoes extensive and often degenerative changes both during and after each of the moults to subimago and imago, the structure of the subimaginal and imaginal gut nevertheless suggests that the gut is still performing some active function during both adult stages. This is also suggested by the persistence in subimagos and imagos of rhythmical waves of midgut contraction, an energetically expensive process for an animal which has ceased to feed. Although a very occasional uptake of fluids by imagos has been observed, it has been shown, by the use of a fluorescent stain, that fluid which disappears from the mouth region is most frequently being spread as a very thin film over the body surface rather than entering the gut. Frequency of midgut contraction has been measured in resting subimagos and imagos of *C. dipterum* and *C. simile*, in imagos of *C. dipterum* during and after being held in a dry atmosphere, in ovipositing imagos of *C. dipterum*, in ejaculating male imagos of *C. simile* and *Ecdyonurus venosus*, and after successive bouts of flying by *E. venosus*. Previous claims that the midgut contractions either control or affect oviposition and sperm ejaculation have not been substantiated. The possibility of a key relationship between the midgut contractions and the need for rapid transport of metabolites is discussed.

Key words: Ephemeroptera, mayfly, gut, *Cloeon dipterum*, *Cloeon simile*

INTRODUCTION

The structure and the functions of the alimentary canal of Ephemeroptera during each stage of the life cycle are of particular interest both because the group is extensively used as an indicator of freshwater quality and because Ephemeroptera are the only insects known to moult and change structure during a winged stage (the aquatic nymphal stage gives rise to a first winged aerial stage, the subimago, which later moults to a second winged stage, the imago, although the females of a few species omit the final moult and remain as subimagos). The final nymphal instar of two of the mayflies discussed here, *Cloeon dipterum* (L.) and *C. simile* Eaton, lasts for between 3 and 10 days, the subimaginal stage for only between 7 and 40 h. Although the imagos of both sexes of *C. simile* survive for 5–6 days those of *C. dipterum* show a marked sex-difference in survival time, with the male imago living for 2 to, at the most, 6 days whereas the female imago normally survives for not less than 7 and more often for about 19 days.

Neither subimagos nor imagos have functional mouthparts, and the gut of *Cloeon*, as in other Ephemeroptera, undergoes complex structural changes over a period which begins before the moult from nymph to subimago and ends after the first few hours of imaginal life. As in most Ephemeroptera (Needham, Traver & Hsu, 1935) the subimaginal and imaginal midgut of both sexes of *Cloeon* and of the third mayfly observed here, *Ecdyonurus venosus* (Fabricus), is gas-inflated, and well-defined waves of contraction pass continuously down its length. In both genera the midgut of the male is normally greatly more inflated than is that of the female.

There have been a number of conflicting descriptions of the structure of the mayfly alimentary canal during the different stages of the life cycle, and a variety of functions have been proposed for the inflated adult midgut. Early authors (Swammerdam, 1737; Vayssiere, 1882) described the inner layer of the anterior part of the imaginal midgut as glandular, and claimed that it is only in the posterior region that there is any surrounding musculature. This view was qualified by Pickles (1931) who made a detailed study of the

structural changes occurring in the gut of *C. dipterum* over the course of its lifetime: he described a highly developed muscular layer in the posterior region of the nymphal midgut with some 'weak muscle' in the anterior region, but concluded that a 'dissolution' of all these muscles occurred in the subimago.

Sternfeld (1907) suggested that the inflation of the midgut in the winged stages is brought about, after emergence, by a pumping action of the foregut (stomadeum), but Morgan (1913) and Kimmins (1941) both described nymphs pushing their heads above water and gulping in air in order to inflate the midgut before emerging as subimagos. Palmen (1884) and Needham et al., (1935) ascribed an aerostatic function to the inflated midgut as well as claiming (as also did Sternfeld) that ejaculation is aided by its inflation. Pickles (1931) further concluded that males are able to change their specific gravity by altering the degree of gut dilation and that the rise and fall pattern of the 'dancing flight' is due to these changes. Yet despite having a highly inflated midgut the males of some species fly actively during both the up and down phases of the dance, and some females follow a pattern of undulating flight similar to that of the males despite showing only a very low degree of midgut inflation (Harker, 1992).

The structures of the foregut and midgut of *C. dipterum* during each of the three post-embryonic developmental stages are explored in this paper, and the results of a study of the functions of the subimaginal and imaginal midgut of male and female *C. dipterum* and *C. simile*, and of the imaginal midgut of male *Ecdyonurus venosus*, are discussed.

METHODS

Light microscopy

Horizontal, transverse and longitudinal serial sections of paraffin wax-embedded nymphs, subimagos and imagos of *C. dipterum* were stained with haemotoxylin and eosin.

Electron microscopy

Nymphs, subimagos and imagos of *C. dipterum* were dissected in 2.5% glutaraldhyde + 0.1 M phosphate + 1.6% sucrose, at pH 7.2, post-fixed in 0.5 M osmium tetroxide and embedded in araldite CY212 resin. Sections were contrasted with uranyl acetate and lead citrate and viewed with a Phillips EM300 electron microscope.

Water movement

Drops of water containing acriflavine, indian ink, or both, were placed on the mouths of imagos and the movement of the fluid, both over the outer body surface and into the gut, were observed by fluorescence and transmission microscopy.

Gut contractions

Male and female subimagos of *C. dipterum* and *C. simile*, and the imagos emerging from each, were held individually in small transparent dishes at 19–20 °C and the number of gut contractions per minute in resting individuals recorded at intervals. Similar recordings were made of the gut contractions of male imagos of *E. venosus*. Sperm ejaculation was induced in male imagos of *C. simile* and *E. venosus* either by gently stroking the final abdominal sternite, or by touching the aedeagus with a pair of forceps; the times taken for sequential sets of 10 midgut contractions before, throughout, and for a short period after ejaculation were recorded. The above technique failed to induce ejaculation in *C. dipterum*. Female imagos of *C. dipterum* and *C. simile*, captured in the field from within or near swarms, were kept individually in small cages, without water, for 2–7 days and the midgut contraction frequency recorded at intervals. From time to time during this period selected individuals were placed on water in a Petri dish and the frequency of midgut contraction again recorded: on those occasions when contact with water induced oviposition the midgut contraction frequency was recorded throughout oviposition and for up to 30 min thereafter.

Sustained flight was induced in *E. venosus* male imagos by suspending individuals by a thread attached to the thorax. The frequency of midgut contraction before and immediately following each of a series of 1 min flights, separated by 10 min rest periods, was recorded.

The numbers of gut contractions per minute were recorded from male imagos of *C. dipterum* and *C. simile* while each was held in a small container separated by an open meshwork from a layer of silica gel, and were recorded again after each individual had been moved to a high humidity environment.

RESULTS AND DISCUSSION

Structure

In common with other Ephemeroptera (Needham et al., 1935) there are neither gastric caecae nor salivary glands in nymphs or adults of *C. dipterum*.

Nymphs

Young nymph

The foregut (stomadeum) is very short (Fig. 1a), running from the mouth to the anterior margin of the thorax where it joins the midgut (mesenteron); the
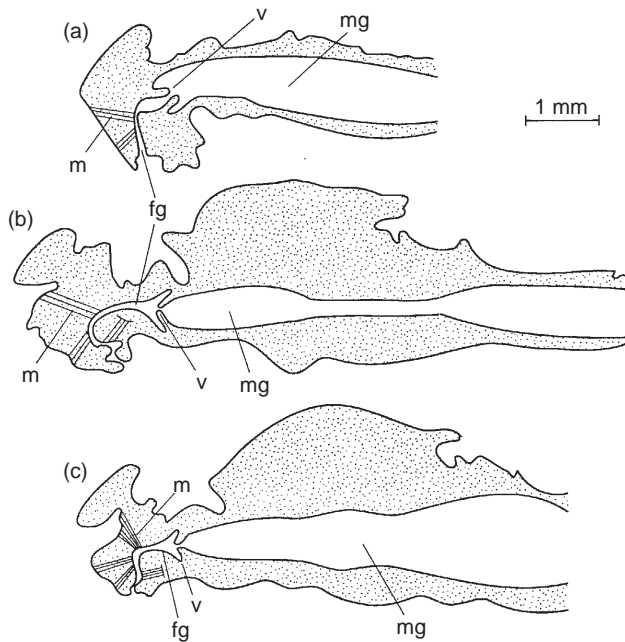
Fig. 1. *Cloeon dipterum*. Midline longitudinal sections of the anterior region of (a) male final instar nymph; (b) male subimago; (c) male imago. fg, foregut; m, muscle; mg, midgut; v, valve at foregut–midgut junction.

likely that in other species studied by Pickles the microvilli also extend right down the midgut although they are perhaps not easily detectable by light microscopy. The apical zone of the epidermal cells, lying immediately under the microvilli, contains many mitochondria.

Saouter *et al.* (1991) drew attention to the presence of regenerative cells in the 'posterior cephalic canal' of *Hexagenia rigida*, a region which they regarded as part of the midgut. This region cannot, however, be part of the midgut since their figure shows it lying anterior to the invagination of the foregut into the midgut as well as having epithelial cells lined by cuticle: both are features of the most posterior part of the foregut. As in many insects the epithelial layer of the mayfly foregut is thrown into longitudinal folds, and in transverse section these give a stellate appearance to the lumen: the 'regenerative nidi' described by Souter *et al.* are almost certainly such folds. In the present study neither regenerative nidi nor any groupings of undifferentiated regenerative cells have been observed in the posterior part of the foregut. Neither have regenerative nidi nor any groups of undifferentiated regenerative cells been observed in the midgut: by contrast sections of the midgut of *Cloeon*, taken at all times during the intermoult period, show a scattering of 'dark' degenerating cells with pycnotic nuclei and the presence of lipid

midgut extends to the posterior margin of the sixth abdominal segment (Fig. 2a), although at times it may be temporarily drawn into the seventh segment. As in other insects the foregut is lined with cuticle and in this species the inner surface of the foregut is thrown into longitudinal folds which are stretched out when food is pushed down the foregut. The epidermal cells, which produce a cuticular lining, are surrounded externally by a thick layer of circular muscle with outer strands of longitudinal muscle. Large radial muscles run between the foregut and the exoskeleton of the head.

The midgut (mesenteron) tapers down its length, narrowing perceptibly in the posterior region of abdominal segment 5 and throughout segment 6, although a temporary increase in diameter occurs in successive regions as food is moved down the gut by peristalsis. In both early and late nymphal instars the epithelial cells of the posterior midgut are smaller than those of the anterior midgut, but in other respects all regions of the midgut appear similar in structure. The midgut epithelial cells are columnar and there are extensive infoldings of the extracellular space into their basal surfaces: the nuclei generally lie in the basal halves of these cells and have not been seen higher than half-way up (Fig. 5a). As in most insects, the apical surfaces (those adjacent to the gut lumen) of the midgut epithelial cells are bordered with microvilli (the 'striated hem' of Pickles (1931) and other early authors) (Fig. 5a, b) and, although Pickles concluded that microvilli are present only in the anterior midgut of *Cloeon* and other mayfly species, microvilli have here been observed at every midgut level, as Saouter *et al.* (1991) also noted in *Hexagenia rigida*. It is
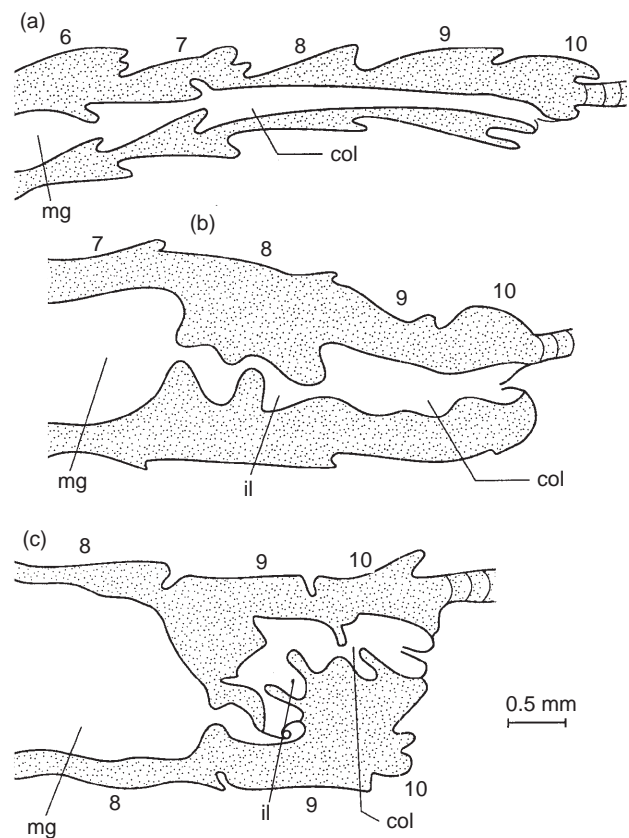

Fig. 2. *Cloeon dipterum*. Midline longitudinal section of the posterior region of (a) last instar male nymph (b) male subimago (c) male imago. col, colon; il, ileum; mg, midgut. Abdominal segments are numbered.
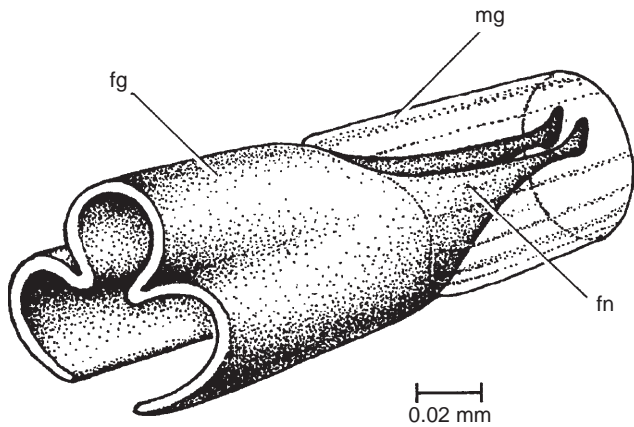
Fig. 3. *Cloeon dipterum*. Reconstruction of the nymphal foregut–midgut junction. fg, foregut; fn, funnel; mg, midgut.

spheres in many cells. Such features suggest that there is a continuous turnover of epithelial cells, and this for *Cloeon*, both because of the brevity of the instars and the very large number of moults, is likely to be more appropriate than the restriction of cell replacement to a specific time in the instar.

Pickles (1931) was unable to find any peritrophic membrane (now often termed the peritrophic matrix (Lehane, 1997)) although Dehn (1933) had earlier claimed both that a peritrophic membrane is secreted by cells lying down the entire length of the midgut and that the posterior cells of the foregut may also produce a membrane. Needham *et al.* (1935) also noted a peritrophic membrane although claiming that it originates entirely from the oesophageal valve. Peters (1992) however has clearly demonstrated that in *Cloeon* the microvilli at the apical surface of at least some of the midgut epidermal cells act as a template for a peritrophic membrane or membranes, that the polymerization of the chitin-containing microfibrils of these membranes takes place in the interstices between the bases of the microvilli, and furthermore, that successive
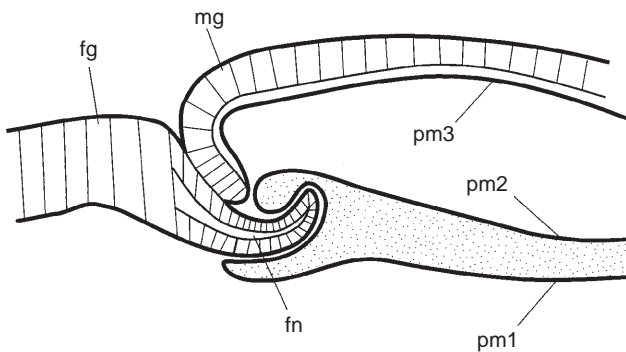
membranes are secreted in this way down at least much of the length of the midgut.

In the present study peritrophic matrices have been seen in sections taken at all levels of the midgut, whether viewed by light or electron microscopy, and both Type I and II membranes (Wigglesworth, 1930) have been recognized. The structure of the foregut–midgut junction is more complex than previously described; at this junction a distinctively shaped incurving funnel protrudes into the midgut (Fig. 3). The cells of this funnel produce a membrane, which, as it detaches from the funnel cells, is pushed down the gut as a continuous sheet; owing to the configuration of the funnel this sheet is thrown into a double fold (Fig. 4). This double fold lies internally to the membrane(s) produced down the length of the midgut so that at any time there is a minimum of three discrete membranes down the length of the midgut, with extra layers being added as new membranes are secreted (Fig. 5b). Although in all the preparations viewed these membranes appear to lie at some distance from the microvilli, such a spacing may be an artefact since in the living insect it seems likely that the pressure of the gut contents, particularly during peristalsis, would force the membranes against the microvilli.

Although in many insects the peritrophic membrane breaks down as it passes into the hindgut (Peters, 1992), such a breakdown does not appear to occur in *Cloeon* and the excretory pellets, which in *C. dipterum* may be as long as 4 mm, are enclosed in membranes. On average the length of the evacuated bolus in mature, actively feeding, female nymphs is 2.8 mm and in male nymphs 2.3 mm: a series of as many as three boluses may be ejected consecutively, followed by a pause of as little as 3 min before the next series is ejected, although there may also be long pauses between bouts of evacuation. One female evacuated a total of 1.7 cm of peritrophic membrane over a 30 min period, an amount which is very high compared with, for example, that of 1.6 mm h^{-1} evacuated by earwigs (Waterhouse, 1954), although less than the record 10 mm min^{-1} produced by a coleopteran (*Rhynchaenus fagi*) just before pupation (Streng, 1973) (however, this is perhaps a special case since the membrane is used for cocoon formation). Since the throughput time for food in *C. dipterum* is sometimes as short as 30 min (Harker, 1989), the amount of substrate lost by the evacuation of the peritrophic membranes appears to be significantly high.

Wigglesworth (1972), Martoja & Ballan-Dufrancais (1984) and Billingsley & Lehane (1996) have all drawn attention to the presence of goblet cells in the midgut of mayfly nymphs. None of these authors cites the source of this observation although internal evidence in Wigglesworth's description suggests it to have been Shinoda (1927). Shinoda, in giving the first detailed description of goblet cells in a number of insect groups, referred to 'goblet-like cells' in Ephemeroptera and cited his own figure of a transverse section of the midgut of *Cloeon dipterum*: this figure shows a group of columnar
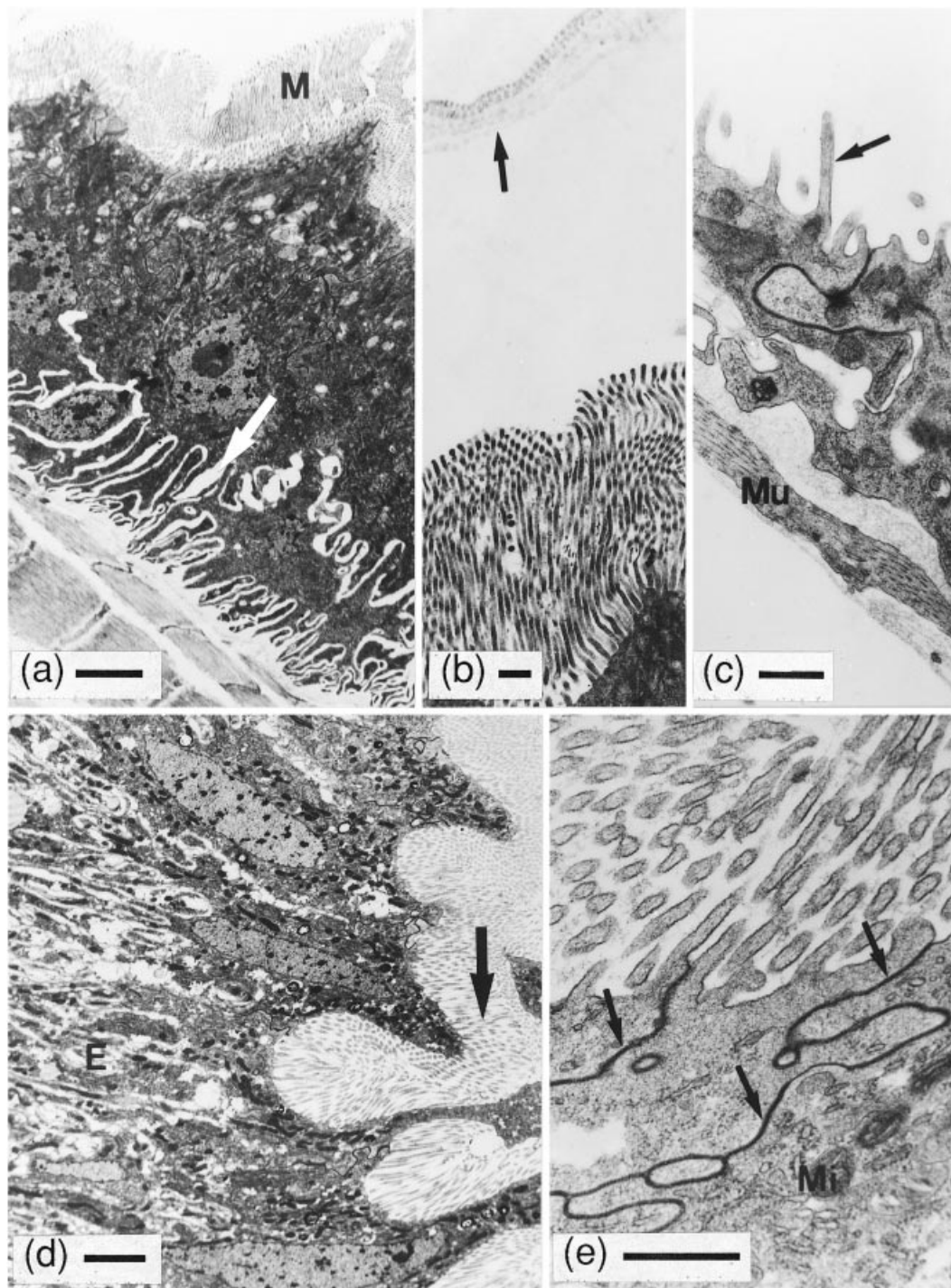

Fig. 4. *Cloeon dipterum*. Midline longitudinal section of foregut–midgut junction, showing the two sources of peritrophic membranes. fg, foregut; fn, funnel; mg, midgut; pm1, pm2, pm3, peritrophic membranes 1 to 3.

Fig. 5. *Cloeon dipterum* midgut. (a) Final instar nymph. Arrow, infoldings of extracellular space, Scale bar 0.5 μm. (b) Final instar nymph. Arrow, multilayered peritrophic membrane. Scale bar 2 μm. (c) Imago. Arrow, reduced microvilli. Scale bar 2 μm. (d) Subimago. E, infoldings of the extracellular space. Arrow, folds in the distal margin. Scale bar 0.5 μm. (e) Subimago. Arrows: cell boundries. Scale bar 2 μm. M, microvilli; Mi, mitochondria; Mu, muscle.

epithelial cells one of which lacks microvilli and has only a thin layer of cytoplasm above the level of the nucleus (although the latter is at the same level as those of adjacent cells). Martoja & Ballan-Dufrancais (1984) define the characteristic feature of goblet cells as 'the invagination of the apical border of the cell to form a deep cavity which confines the nucleus to the basal region': in the present study goblet cells of this form have never been observed in nymphs, although cells bearing a superficial resemblance to goblet cells occur in the subimago, as is described below.

Externally the midgut epithelial cells are surrounded

by several thick layers of circular muscle, and, in contrast to Pickles' (1931) claim that longitudinal muscles occur only at the anterior and posterior ends of the midgut, well-defined strands of longitudinal muscles lying outside the circular muscles have been seen at all midgut levels, as in most insects.

Last nymphal instar

A series of events, which appears unique for an insect, occurs during this instar: *two* layers of new cuticle are laid down successively without an intervening ecdysis. This can be particularly well seen in developing wings in which both the subimaginal cuticle and the imaginal cuticle are laid down during the last 2 days of nymphal life (pers. obs.). The changes in gut structure which occur over this period may therefore reflect changes induced by *each* of these episodes of moulting.

Nymphs feed during most of the last nymphal instar, but some hours before the emergence of the subimago the mouthparts cease to move and the associated muscles degenerate. Murphy (1922) recorded a similar degeneration of the mouthpart musculature in *Hexagenia*, although in that species it occurs as many as 5 days before eclosion.

Pickles (1931) believed that in the course of the last nymphal instar the cells of the mesenteron lose their well-defined boundaries, the cell contents begin to break down, and vesicles containing cell material are extruded into the lumen; Needham *et al.* (1935) likewise described the disappearance of all well-defined epithelial cell boundaries at this time. By contrast in the present study, although empty vesicles have been seen in some epithelial cells of last instar nymphs with darkened wing buds (20–48 h before emergence of the subimago), the majority of the mesenteron epithelial cells still contain large numbers of mitochondria, have well-defined cell junctions similar in structure to those of earlier stages, and show no signs of breakdown (Fig. 5a). Pickles also described a breakdown of the muscle fibres during this period, but in the present study these fibres appear both structurally unchanged (Fig. 5a) and fully functional: indeed, in view of the fact that the midgut of the subimago contracts rhythmically immediately after (and sometimes throughout) eclosion, and the gut of the imago continues to show strong waves of peristalsis throughout its lifetime, muscle breakdown is very unlikely.

Subimago

Foregut

The lumen diameter (Fig. 1b) is considerably less than in the nymph and by contrast with the nymph the epidermal cells of the region lying immediately anterior to the midgut are no longer thrown into folds.

Mid and hindgut

At the moult to the subimago there is a change in the orientation of the cells lying at the junction of the foregut and midgut (those cells which secrete the double fold of nymphal peritrophic membrane): instead of forming an invagination into the midgut these cells now form a circular valve in which the cells are at right angles to the foregut lumen (Fig. 1b). Peritrophic membranes have not been observed either adjacent to these cells or to those of the rest of the midgut, although some of the debris left in the subimaginal midgut is surrounded by a membrane; this latter membrane has almost certainly been carried over from the nymph since not all of the midgut contents with their surrounding membranes are evacuated at the moult to the subimago.

Pickles (1931) described a narrowing of the lumen of the midgut during the final nymphal instar and at the beginning of the subimaginal instar, followed by a redilation of the gut in the late subimago. In living subimagos it can, however, be seen that, although the lumen of that part of the midgut which lies in the mesa- and metathorax does often appear narrower than in the nymph, the lumen of that part lying in the abdomen is, from the moment of emergence, at least partly (but usually entirely) dilated by gas (Fig. 1b). Throughout the subimaginal instar, waves of muscular contraction pass down the entire length of the midgut, and as the final remnants of the gut contents are moved into the hindgut so the gas-filled region is pushed down until it reaches the middle of abdominal segment 8 (Fig. 2b). At this time a firm plug of food debris is formed at the junction with the ileum. The hindgut, which initially lay in abdominal segments 7–10, continues to receive material from the Malphigian tubules and remains fluid-filled for some hours after the subimago emerges. In the course of the subimaginal instar some of this fluid is lost and the air-filled midgut continues to inflate even further until its anterior end occupies a large part of the thorax and its posterior end, in pushing against the hindgut, compresses the latter into the last two abdominal segments. As the midgut pushes into segment 8 the ileum is thrown into a curve, and the colon, although still retaining a wide lumen, is pushed posteriorly (Fig. 2b). At 18 °C the time taken for the compression of the hindgut, and the expansion of the midgut into the 8th abdominal segment, is 5–6 h in females but 4 h or less in males.

As the midgut inflates, so the surface area of the epithelial cells increases, with a corresponding decrease in the 'depth' of the cells: at this time the infolding of the extracellular space into the epithelial cells becomes even more marked than in the nymph (Fig. 5d). Although Needham *et al.* (1935) described a complete disintegration of the epithelial nuclei at this time, the majority of nuclei in the present preparations appear to be unchanged. Although there is a general decrease in the 'depth' of the epithelial cells the apical margins of some of these cells are thrown into folds, bringing the

nuclei closer to the distal cell surface; this, in places, gives an appearance bearing a superficial resemblance to the goblet cells of some other insects (Fig. 5d). There are still many mitochondria present below the microvilli and the epithelial cells retain their well-defined boundaries.

Contrary to the description by Pickles of the phago-cytosis of the muscular wall of the midgut, a well-defined muscular wall has been observed at all levels, and regular waves of contraction pass down the length of the midgut throughout the subimaginal instar.

Imago

Foregut

Longitudinal sections show the foregut remaining open to the exterior (Fig. 1c), as it must do since the foregut subimaginal cuticle is drawn out through this opening at the moult to the imago. Throughout the length of the foregut there is a thick cuticular lining, which anteriorly bears setae curving outwards towards the mouth. The oesophagus is surrounded by a thick band of circular muscle and large muscles run between the anterior head cuticle and the dorsal side of the oesophagus, and between the large apodeme in the posterior region of the head and the ventral side of the oesophagus (Fig. 1c).

Twenty-one hours after the emergence of the imago the epidermal cells of the foregut still contain many mitochondria and the basal extracellular space is wide and considerably infolded into the epidermal cells.

Midgut

The midgut inflates further, and is no longer narrowed in any region of the thorax. The posterior end of the midgut contains a small concentrated mass of food debris, which remains throughout imaginal life. As the gut continues to inflate so the posterior end of the midgut is pushed towards the end of segment 8, com-pressing the ileum and causing the hindgut to be thrown into further folds as the colon is pushed posteriorly into segment 10 (Fig. 2c). Despite the compression of the hindgut few, if any, of its contents are evacuated so that the diameter of the colon is increased. This is of importance in males because as a result each vesicula seminalis comes under an increasing pressure from both the inflated midgut and the compressed hindgut: in females, by contrast, it is only the midgut which can bring pressure on the reproductive system since the oviducts open to the exterior between segments 7 and 8.

By 4–6 h after eclosion the midgut epidermal cells are reduced to thin, flattened cells with many inclusions and membrane-lined vacuoles: the microvilli are greatly reduced both in length and number. By 21 h after eclosion the remaining microvilli are still further reduced in length and the infoldings of the extracellular space (basal labyrinth) into the epidermal cells have virtually disappeared (Fig. 5c). Peritrophic membranes have not been seen in the imago except that the small pellet at the posterior end of the midgut, which has been carried over from the nymph, may still be invested in remnants of the nymphal peritrophic membrane. The epithelial cells are still surrounded by a thick layer of circular muscle (Fig. 5c).

There does not appear to be any fundamental sex-difference in gut structure. However at the beginning of the male subimaginal stage the sperm move posteriorly into the vesicula seminales (lying in the last two abdo-nimal segments), and as the fat body is also largely concentrated into these posterior segments considerable room is left for the expansion of the male gut within segments 1–8. In the female, by contrast, there is little room for gut expansion since the ovarioles, containing large numbers of eggs, extend from the first abdominal segment to the opening of the oviducts at the posterior margin of segment 7, and the female fat body is more widely dispersed throughout the abdomen than is that of the male. The marked sex-difference in gut volume therefore appears to be related to spatial rather than to structural factors, a view strengthened by the fact that when females contain very few eggs (as occurs, for instance, after nymphs have been reared on a sparse diet) then the degree of midgut inflation becomes com-parable to that of males.

FUNCTIONS OF THE IMAGINAL GUT

The loss of the mouthparts and the extensive changes in midgut structure which occur at the moult to the imago and over the following 2–3 h, particularly the great decrease in the number of epithelial microvilli, a decrease in, or even complete loss of, the epithelial extra-cellular space, and the loss of the peritrophic membranes, are all in keeping with a loss of digestive function. Nevertheless, despite these changes, the fine structure of the majority of epithelial cells is such as to suggest that they still have some function and this, together with the fact that frequent and regular waves of contraction pass down the length of the midgut (an energy-consuming process), suggests that the midgut plays some active role in the life of the imago. Further-more, the structure of the foregut and the presence of well-developed muscles attached to the oesophagus and oesophageal valve suggest that the imago has a degree of control over the gut contents.

Entry of fluids and air into the gut

There is some evidence that very small amounts of fluid can be taken in through the mouth and into the gut: drops of fluid placed on the mouths of *Cloeon dipterum* do sometimes, although infrequently, enter the mouth. The interpretation of the disappearance of a drop from the outside of the mouth requires extreme care, since

some or all of the drop may spread over the surface of the extremely waxy outer cuticle, where it forms a film so thin that it is almost impossible to detect unless a fluorescent dye is added to the fluid, as has been done here. In nine cases, however, at least some of the drops of water-based indian ink placed on the mouths of a male *C. dipterum* imago were seen entering the midgut (which is clearly visible through the transparent cuticle). In four of these cases, by 70–84 min after the drop had been placed on the mouth, dark particles appeared in the haemocoelic fat body. This latter observation is of particular interest because phagocytosis of particles by insect midgut cells is extremely uncommon (Wigglesworth, 1972) and phagocytosis almost certainly does not occur in the nymphal stage when in any case the peritrophic membrane would prevent particles of this size from reaching the epidermal cells. Despite these observations it does not, however, seem likely that there is active or frequent uptake of liquid by imagos of *Cloeon*, both because the surface of the mouth area is so water-repellent and because the stance of the imago, as in other mayfly species, makes it very difficult for the mouth to be brought into contact with the substratum or even with adjacent foliage. Takemon (1993) has, however, described imagos of *Epeorus ikanonis* alighting momentarily on the surface of a stream and picking up water droplets: but his conclusion, that because droplets disappear from the head region they must enter the gut, fails to take into account the alternative possibility that the droplets disperse as a thin film over the outer surface of the imago, the process so frequently observed in the present study.

By contrast to fluid, air may enter the gut very rapidly: this can be seen, for example, during oviposition, when, as the eggs (which are laid in a continuous stream) move away from the area surrounding the midgut, the midgut simultaneously expands into the vacated area. It is possible that, as in mosquitoes (Gillett, 1983), some of the gas which expands the midgut at this time is drawn indirectly from the tracheal system rather than through the mouth. However, it is unlikely that the entire volume could come from the tracheal system both because of the speed of the inflation and the pressure involved, and because such an exchange would need to be supported by a complex spiracular valve system, whereas only very primitive valveless spiracular slits are present in Ephemeroptera. The more likely route for air entry is therefore through the mouth particularly since even fluid droplets may enter the gut by this route. The mouth itself, on the other hand, does not appear to have any closing mechanism to prevent gas being expelled by the contractions of the inflated gut: it appears, rather, that it is the valve at the foregut–midgut junction (Fig. 1c) which controls the midgut contents, acting as a one-way valve which allows air to enter but prevents it from escaping back into the mouth. Although the posterior end of the midgut lacks a valve the debris block in this area provides a stopper so efficient that, in a freshly killed insect, it is not even possible to force air past the block. The efficacy of these two seals can be demonstrated by temporarily blocking the abdominal spiracles with wax: under these circumstances the gut swells as the rising level of carbon dioxide in the tissues brings a transfer of gas into the gut, but even when the gut volume has nearly doubled no gas escape occurs.

The frequency of midgut contraction

(1) The frequency of midgut contraction was measured for male imagos of *C. simile*, *C. dipterum* and *E. venosus* and in *C. simile* and *C. dipterum* comparisons were also made of the contraction frequency in males and females of both imagos and subimagos. For these species Fig. 6 shows the mean values from measurements on many specimens and Fig. 7 shows the counts made for each of four individuals which were measured during both the subimaginal and imaginal stages. Variability within any given category (species, stage and sex) was small, but there were clear differences between categories. In resting imagos of the three species the frequency is not directly related to sex (ratio of male imago wing to body lengths *C. dipterum*: *C. simile*: *Ecdyonurus venosus* 1:1.2:2 and females in all three species are larger than males).

The frequency in *C. simile*, in all stages and in both sexes, but particularly in males, is higher than that in *C. dipterum*. In both species the frequency in subimagos is higher than that in the imagos. The two species differ in their sex-dependence. In *C. simile*, for both imagos and subimagos, the frequency in the male is much higher than that in the corresponding female; by contrast, in *C. dipterum* the frequency differs between stages but not between sexes. It is particularly noticeable that no statistical difference has been found in the gut contraction frequency of male and female imagos of *C. dipterum*
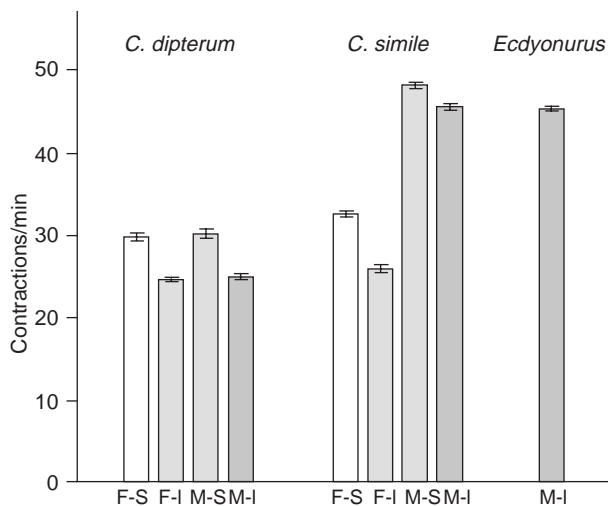

Fig. 6. Mean frequency of midgut contractions in *C. dipterum*, *C. simile* and *E. venosus*. F-S, female subimago; F-I, female imago; M-S, male subimago; M-I, male imago. (*C. dipterum* F-S and F-I 128 sets of counts for each; *C. simile* F-S and F-I 192 sets of counts for each; *E. venosus* M-I, $n = 168$). Error bars: 95% confidence limits for the means.
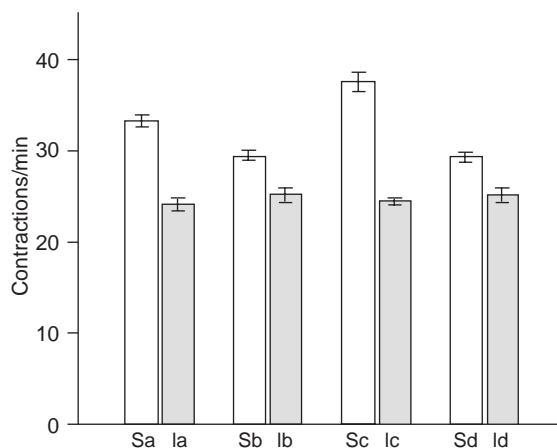
Fig. 7. Mean frequency of midgut contractions of four individual *C. dipterum* (a–d) during the subimaginal and imaginal stages. S, subimago; I, imago. Number of counts for each stage = 8. Error bars: 95% confidence limits for the means.
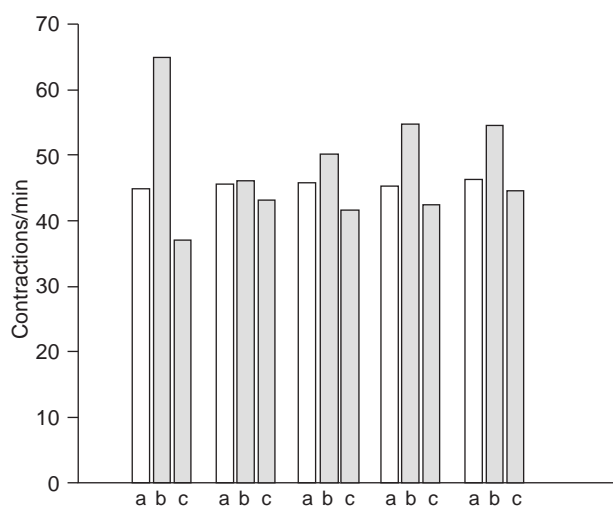

Fig. 8. Mean frequency of midgut contractions of five individual *C. simile* male imagos (a) before ejaculation, (b) during ejaculation and (c) immediately after ejaculation.

despite the very high compression of the female midgut by the eggs: this observation confirms the conclusion that frequency is not related to midgut volume.

(2) No relationship has been found between the frequency of midgut contraction and the humidity of the surrounding atmosphere; the frequency of contraction in imagos confined individually to small chambers with a dry atmosphere (in the presence of silica gel) showed no significant change when the humidity was increased, or when it was again decreased.

(3) During ejaculation the midgut contraction frequency of *C. simile* (Fig. 8) is sometimes, but not always higher than that of resting imagos; immediately post-ejaculation it may fall very slightly below the previous resting frequency. By contrast, however, ejaculating *E. venosus* either show no significant change in gut contraction frequency or the contractions cease altogether and

Table 1. The mean ($\bar{x}$) number of gut contractions per minute in female *C. dipterum* imagos immediately before and during oviposition. *n*, number of consecutive 1 min counts

Animal	Pre-oviposition			Ovipositing		
	$\bar{x}$	SE	*n*	$\bar{x}$	SE	*n*
A	26.2	0.2	24	25.3	0.2	8
B	26.9	0.4	33	17.1	0.5	8
C	24.7	0.1	32	24.6	0.4	8
D	25.2	0.1	32	24.0	0.4	8
E	25.6	0.3	31	26.0	0.4	9
F	23.9	0.7	33	24.6	0.7	9
G	26.4	0.6	14	15.3	1.9	7

restart only after up to 10 min post-ejaculation. Ejaculation was also induced in an *Ecdyonurus* imago in which the midgut, as a result of accidental puncture, had completely deflated: therefore in this species, at least, ejaculation cannot be dependent on midgut contraction. Overall these observations show that although there may sometimes be a change in frequency of midgut contraction during ejaculation, ejaculation is not itself driven by the midgut contractions.

(4) Oviposition in *C. dipterum* (Table 1) also appears to be largely independent of the midgut contractions for, although during oviposition some imagos may show a decrease of about 25% in gut contraction frequency, the oviduct pulsations have a higher frequency than the gut contractions and the two are asynchronous. Moreover, in three individuals (not included in the calculations) gut contraction ceased completely during the entire period of oviposition.

(5) Regular and sustained flight was successfully induced only in *E. venosus*. In this species the midgut contraction frequency was higher during the immediate post-flight period than before flight, and there was a successive increase in the post-flight frequency after each of the series of one minute flights followed by a 5 min rest period (Fig. 9); these successive increases in the post-flight frequency occurred despite the fact that after each flight the frequency fell to its initial resting level within at least 4 min. These observations suggest a possible distributive role for the midgut, as is discussed below.

The role of the gut in the distribution of air and metabolites

The rate of transport of fuel to the flight muscles is a limiting factor for flight, whether an insect is flying only to the swarming site and back to water to oviposit, or is undertaking long, energetically expensive, swarming flights.

Sartori *et al.* (1992) have shown that in *Siphlonurus* most of the carbohydrate reserves are depleted before the beginning of the imaginal stage, leaving the fat reserve as the main source of energy for flight. In all the species examined here the fat reserves are largely concentrated in the abdomen, and in males particularly
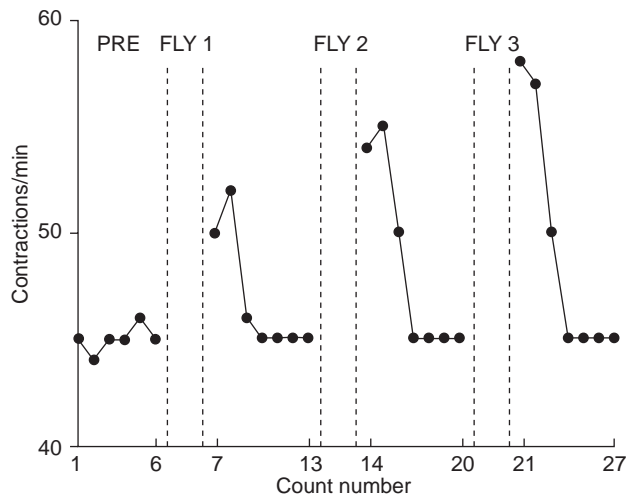
Fig. 9. Contractions per minute of the midgut of a male *E. venosus* imago during 27 counts made before and after each of three successive 1 min flights.

in the posterior abdomen, as they are in a wide range of other species (pers. observ.). As a consequence the rate of transport of fuel from the abdomen to the flight muscles is of primary importance during flight. As the blood system is an open one the inflation of the midgut confines the blood to a thin layer between itself and the body wall; this in itself brings more of the blood into direct contact with the muscle. The waves of contraction of this inflated midgut have a major effect on the overall blood movement and fuel transport, and the frequency of these contractions must therefore have a key role in defining the rate of transport and so the sustainability of flight, particularly during swarming. This conclusion is further supported by the observed levels of the contraction frequency after each successive bout of flying. It is suggested that the successive increases in frequency after each flight reflect successive increases in the level of 'inflight' frequency as each bout brings an increasing need for substrate transport, but that once flight ceases the demand falls so rapidly that the gut contraction rate falls back to the base line over similar time-intervals.

Although in females the bulging oviducts may themselves play some part in confining the blood to a thin peripheral layer, the bulges also tend to trap individual pools of blood, and it can be observed that it is only by the contractions of the partially inflated midgut that these potentially isolated pools are released. Yet there does appear to be somewhat less overall movement of the haemolymph in females than males, and this may be related both to the fact that the fat body is more widely dispersed throughout the abdomen of females and that female flight is generally of much shorter duration than that of males.

In both sexes the gut contractions must also affect the movement of air within the tracheae, for although the tracheae cannot be compressed by the gut waves they are successively stretched and relaxed by the passing waves; since Ephemeroptera, as was noted earlier, have only a very primitive and inefficient control of spiracular

regulation any gut-induced movements are likely to have a considerable effect on air flow.

Corbet (1988) has suggested that it may be possible for insects to regulate their water economy by 'muscular manipulation of the hydrostatic pressure in a gas-filled cavity', and that such manipulations could involve either a loss of, or an increase in, the water content. If metabolic water is produced during very active flight then any transient post-flight water burden might be dispelled by vigorous pumping of a gas-filled cavity. The successive increases in the post-flight gut contraction frequency observed here might suggest such a response, but, as discussed above, since mayfly flight appears to be lipid rather than carbohydrate-fuelled the water balance should not be very sensitive to the effects of vigorous activity so that a relationship between gut contraction frequency and the elimination of water is unlikely. The alternative possibility, that the midgut contractions may be related not to dispelling water but to condensation cycles and active water uptake, also seems unlikely since, as has been shown here, there is an absence of any relationship between gut contraction frequency and ambient humidity.

The arguments outlined above raise another question: if the benefits of the effect of the regular gut contractions on the movement of metabolites, and perhaps of air, are sufficient to outweigh the energetic costs of the contractions themselves, then why should the frequency of midgut contraction in subimagos be higher than that of imagos although the subimago flies both more slowly and more briefly? It is possible that two factors that are unique to the subimago may be involved: (1) a need for rapid distribution of ecdysial hormone, and (2), perhaps more important, the role of the midgut in providing the pressure needed to split the subimaginal cuticle at emergence: it is notable that on those rare occasions when the midgut fails to inflate emergence is never successfully completed.

Overall, therefore, it appears that the inflation of the gut may be primarily of importance in diminishing the volume of the haemocoele and in channelling the haemolymph, and that the primary importance of the contractions of the inflated gut is in moving the haemolymph and metabolites around the body. It is unlikely that the gut plays an important role in direct water uptake, in reproductive processes, or in the mechanism of flight, although it may have a behavioural role during mating flights when the glint of the inflated gut of the male imago may act as a signal both to other males and to females (Harker, 1992).

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