TOPOGRAPHICAL VARIATIONS IN THE STRUCTURE OF THE SMOOTH SEPTATE JUNCTION

HELEN LEB. SKAER, J. BARRIE HARRISON AND WILLIAM M. LEE
A.R.C. Unit of Invertebrate Chemistry and Physiology, Department of Zoology, Downing Street, Cambridge CB2 3EF, England

SUMMARY

Smooth septate junctions in the midgut of Musca domestica and in Malpighian tubules of both Musca and Rhodnius prolixus are described. Details of the structures revealed after standard fixation, fixation in the presence of the stain, lanthanum hydroxide, and after freeze-fracture are discussed in the light of models previously put forward to explain the interrelations of the images obtained by these different methods. The organization of the junction between cells of the midgut varies in the apical-to-basal axis. At the apical border the septa (or ridges in freeze-fracture replicas) are packed tightly and follow an undulating but strictly parallel course. This packing loosens towards the middle of the junction until, at its basal extremity, the septa (ridges in replicas) are widely separated and follow independent meandering courses. That these features are found both in lanthanum-infiltrated specimens and freeze-fracture replicas allows a correlation to be made between the septa and the freeze-fracture ridges. The functional significance of these smooth septate junctions is discussed.

INTRODUCTION

Invertebrate septate junctions have become so much subclassified that the term now describes a whole class of junctional types rather than a single structure. The septate junction is recognizable by the size and regularity of the intercellular space (11–15 nm) (Satir & Gilula, 1973; Staehelin, 1974) and frequently by the regular cross banding exhibited in this space. The junction was first described in Hydra by Wood (1959) and has subsequently been found in many invertebrate epithelia (reviewed by Satir & Gilula, 1973; Gilula, 1978). Danilova, Rohlenko & Bodryagina (1969) subdivided the invertebrate septate junction into 2 types, the septate (found in Hydra) and the comb (found in insects), basing the separation on the configuration of the septa as revealed by tangential sections of preparations infiltrated with lanthanum salts.

At about this time a new type of junction, the zonula continua, was described by Noirot & Noirot-Timothée (1967) in the midgut of termites. This structure, like septate junctions, exhibited a highly regular intercellular space, but, unlike them, displayed no septa in cross-sections. However, tangential sections and en face views of preparations negatively stained with lanthanum revealed septa with a morphology...
analogous to but distinct from that of previously described septate junctions (Noirot-Timothee & Noirot, 1974). Flower & Filshie (1975) suggested that these continuous junctions should be considered as a type of septate junction and that septate junctions should be subdivided into the true pleated type and the ‘smooth’ type. These smooth septate or continuous junctions have now been recognized in the midgut of many insects (Noirot & Noirot-Timothee, 1967, 1972; Dallai, 1970, 1975; Oeschman & Wall, 1972; Satir & Fong, 1972; Reinhardt & Hecker, 1973; Noirot-Timothee & Noirot, 1974; Flower & Filshie, 1975; Ito, Vinson & McGuire, 1975; Burgos & Gutierrez, 1976; Lacombe, 1976; Houk, 1977), the hepatic caecum of Daphnia (Hudspeth & Revel, 1971), the hepatopancreas of crayfish (Gilula, 1972; Satir & Gilula, 1973) and the midgut and hepatopancreas of Limulus (Lane & Harrison, 1978). Their presence in these tissues, in which continuous cell replacement is a common feature, appeared to substantiate the original suggestion that they represent a specialization of regenerative tissues (Noirot & Noirot-Timothee, 1967). However, continuous junctions have also been described in insect Malpighian tubule, a tissue in which there is no cell turnover (Lacombe, 1976; Dallai, 1976).

Recently Green (1978) has carried out a survey of invertebrate phyla and has described no less than 8 different types of septate junction. These include the 3 types previously described. The detailed structure and possible functional significance of these new types remains to be investigated further.

In this paper continuous junctions are described from one insect tissue in which cell replacement occurs and from another in which the cells are not replaced. The overall morphology, as well as the detailed structure of the junctional components, is considered in relation to the topography of the tissue.

MATERIALS AND METHODS

The tissues examined were the midgut of the housefly, Musca domestica, and the upper regions of the Malpighian tubules of both Musca domestica and the bug, Rhodnius prolixus. In both cases laboratory-reared adult insects were used. The tissues were exposed by removal of the dorsal body wall and flooded with the fixative. The tissues were then dissected out and placed in fresh fixative solution.

Conventional fixation and embedding

Tissues were fixed at room temperature (about 18 °C) for 1 h in either 2.5 or 3 % glutaraldehyde made up in 0.05 M cacodylate buffer at pH 7.2 with 0.68 % sucrose added. After washing in 0.05 M cacodylate buffer with 9.5 % sucrose, they were postfixed for 1 h in buffered 1 % osmium tetroxide with 8.4 % sucrose added. The tissues were then stained en bloc with 2 % uranyl acetate dissolved in sodium hydrogen maleate buffer, pH 6.0. Dehydration through an ascending series of ethanol, immersion in propylene oxide and embedding in Araldite followed. Thin sections were cut on an LKB Ultratome III, stained with lead citrate and uranyl acetate and examined in a Philips EM300.

Lanthanum staining

Tissues to be stained in the intercellular space with lanthanum were fixed in the presence of lanthanum hydroxide as described by Revel & Karnovsky (1967). Tissues from Musca were fixed at room temperature overnight in 2.5 % glutaraldehyde buffered at pH 7.2 with 0.1 M cacodylate buffer containing 0.2 M sucrose and 1 % lanthanum hydroxide. The
buffer wash also contained 1% lanthanum hydroxide and 0.4 M sucrose. Postfixation followed in 1% osmium tetroxide buffered with collidine at pH 7.2 and containing 0.65 M sucrose and 1% lanthanum hydroxide. Tissues from Rhodnius were fixed for 2 h at room temperature in 3% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.2 containing 1.7% sucrose and 1% lanthanum hydroxide. The wash contained lanthanum hydroxide and 12% sucrose. Postfixation followed for 1 h in 1% osmium tetroxide buffered with 0.05 M cacodylate and containing 10.3% sucrose and 1% lanthanum hydroxide. Tissues from both species were stained en bloc with uranyl acetate and embedded as outlined above. Sections were viewed either unstained or stained as described above.

RESULTS

In both the midgut of Musca and the Malpighian tubules of Musca and Rhodnius continuous junctions are a characteristic feature of the lateral borders between adjacent cells (Figs. 1, 14). Always found towards the apical end of the lateral membranes, they extend basally for about two thirds of the cell height. They are recognizable in conventionally embedded, sectioned and stained material by the constancy of the intercellular space (14–17 nm) which is relatively electron opaque (Fig. 14). After negative staining by infiltrating the intercellular space with lanthanum hydroxide, the details of the junctional structures within the intercellular space may be made out (Figs. 11, 17–22). By means of freeze-fracture the intramembranous structures associated with the junction are revealed (Figs. 1–10) and in addition areas of membrane may be exposed that sometimes encompass almost the entire junctional length (Fig. 1).

Freeze-fracture

Midgut. In freeze-fracture preparations the junctions appear as arrays of more or less parallel ridges on one face of the membrane with corresponding rows of grooves on the other face (Figs. 1–8). The face on which the ridges appear depends on the treatment of the tissue prior to freezing. In fixed tissue (Figs. 1–4) the ridges appear on the PF with complementary grooves on the EF. The ridges are 10 nm in diameter and appear to be made up of short rods (Fig. 2), which in some cases can be seen to be made up of particles 10 nm in diameter (Fig. 3, inset). Adjacent ridges occasionally appear to fuse to give a double structure (large arrows, Fig. 4). Other parts of the PF in the junctional area bear particles 8–12 nm in diameter which are sometimes closely
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incorporated with the ridges. In Fig. 4 some of these particles are lined up between adjacent ridges (between small arrows). Grooves complementing the PF ridges are found on the EF (Figs. 2, 3). They also are approximately parallel in arrangement and follow an undulating course. Numerous 8–10 nm diameter particles lie in these grooves (Figs. 2, 3).

In material that is frozen without prior fixation the fracturing characteristics of the junctions are found to be rather different. Ten-nanometre ridges, sometimes clearly moniliform in substructure (Figs. 5, 6), are found on the EF with corresponding grooves on the PF (Figs. 5, 8) and there are fewer 8–10 nm diameter particles associated with the PF grooves than there are with the EF grooves in fixed material (cf. Figs. 2, 3 and Figs. 5, 8).

In both fixed and unfixed material, terminations of the ridges are frequently found (arrowheads in Figs. 4–7) and in other cases they form blind-ending looped structures (Figs. 6, 8). Loops in the ridges are also found encircling dome-shaped humps in the EF (unfixed tissue, Figs. 5, 7) with corresponding depressions surrounded by grooves in the PF (arrow, Fig. 5). In fixed tissue the humps are also found on the EF but encircled by grooves (Figs. 2, 3) with the depressions on the PF in association with the ridges (Figs. 3, 4).

The apical end of the continuous junction displays a very much more tightly packed system of ridges and grooves (Figs. 1, 2, fixed; 5, unfixed) than does the basal end of the junction (Fig. 3, fixed; Figs 6–8, unfixed). The gradual loosening of the packing can be followed where the plane of fracture reveals extensive areas of the junction-bearing lateral membrane (as in Fig. 1). The highly ordered, closely parallel swirls of ridges and grooves (Fig. 2) become more undulating and less closely packed (Fig. 3) until, at the basal end of the junction, the ridges and grooves peter out in occasional isolated looped or undulating structures (Fig. 8). As the tightness of the

Figs. 1–4. All the figures are of freeze-fracture preparations of the midgut of *Musca domestica* fixed prior to freezing.

Fig. 1. Lateral border between 2 midgut cells. The microvilli (mv) of the luminal border can be seen at the top left and the gradual loosening in the organization of the components of the continuous junction can be followed (arrows) as the lateral membrane extends basally. × 24,000.

Fig. 2. The apical extremity of a continuous junction linking 2 midgut cells. The particle rich membranes of the microvilli (mv) mark the apical limit of the junction. PF ridges, made up of short rods, are in register with grooves in the EF of the adjacent membrane. Both structures are closely packed. × 53,000.

Fig. 3. The middle-to-basal region of a continuous junction between 2 midgut cells. Both the PF ridges and the EF grooves are loosely organized with sizable patches of membrane, decorated on the PF with intramembranous particles, separating the meandering rows of ridges and grooves. × 41,000. Inset: higher power of PF ridges from a similar region of the junction illustrating the particulate nature of the ridge structure. × 65,000.

Fig. 4. PF ridges showing fusion of adjacent ridge structures (thick arrows), ridge termination (arrowheads) and the alignment of intramembranous particles between 2 adjacent ridges (between small arrows). × 54,000.
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<p>| Table 1. Dimensions and separations of the various junctional structures studied |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Midgut, nm</th>
<th>Malpighian tubule, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sections</strong></td>
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<tr>
<td>No lanthanum</td>
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<tr>
<td>Intercellular space</td>
<td>$15.2 \pm 0.12$ (41)</td>
<td>$17.3 \pm 0.12$ (11)</td>
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<td>(range 14-16-5)</td>
<td>(range 16-17-5)</td>
<td></td>
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<tr>
<td>Lanthanum</td>
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<td></td>
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<tr>
<td>Intercellular space</td>
<td>$18.1 \pm 0.33$ (21)</td>
<td>$19.1 \pm 0.31$ (14)</td>
</tr>
<tr>
<td>(range 17-5-19)</td>
<td>(range 17-21)</td>
<td></td>
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<tr>
<td>Interseptal distance (no columns)</td>
<td>$3.2 \pm 0.25$ (23)</td>
<td>$2.6 \pm 0.14$ (12)</td>
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<tr>
<td>(range 2-4)</td>
<td>(range 2-3.5)</td>
<td></td>
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<tr>
<td>Septal width</td>
<td>$8.4 \pm 0.16$ (27)</td>
<td>$7.1 \pm 0.15$ (45)</td>
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<td>(range 7-10)</td>
<td>(range 6-9)</td>
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<tr>
<td>Column diameter</td>
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<td>$4.4 \pm 0.18$ (10)</td>
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<td>(range 4-5)</td>
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<tr>
<td><strong>Freeze-fracture</strong></td>
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<tr>
<td>Fixed</td>
<td></td>
<td></td>
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<tr>
<td>Ridge width</td>
<td>PF $10.1 \pm 0.4$ (21)</td>
<td>EF $11.4 \pm 0.25$ (26)</td>
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<td>(range 9-12-5)</td>
<td>(range 10-14)</td>
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<tr>
<td>Ridge particles</td>
<td>PF $9.8 \pm 0.35$ (20)</td>
<td>EF $10.9 \pm 0.16$ (43)</td>
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<tr>
<td>(range 8-12-5)</td>
<td>(range 9-12)</td>
<td></td>
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<tr>
<td>Particles in grooves</td>
<td>EF $8.3 \pm 0.17$ (24)</td>
<td>PF $10.6 \pm 0.31$ (19)</td>
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<tr>
<td>(range 8-10)</td>
<td>(range 9-12)</td>
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<tr>
<td>Unfixed</td>
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<tr>
<td>Ridge width</td>
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<td>(range 10-12)</td>
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<td>Ridge particles</td>
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<tr>
<td>(range 8-12)</td>
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<td></td>
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<tr>
<td>Particles in grooves</td>
<td>Few 7-5-10</td>
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</table>

Values are given ± standard error and with the size of the sample in parentheses.

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Figs. 5-8. All the figures are of freeze-fracture preparations of the midgut of *Musca domestica* frozen without prior fixation.

Fig. 5. EF ridges and PF grooves from an area close to the apical extremity of the lateral border between 2 midgut cells. The particulate ridges and grooves interrupted by very few particles (cf. Fig. 2) are tightly organized, interrupted only by dome-like structures in the EF corresponding with occasional craters in the PF (arrow). $\times 54000$.

Fig. 6. EF ridges from the apical-to-middle region of the lateral border. In this region blocks of ridges run close to each other but individual ridge terminations are frequent (arrowheads) and large areas of clear membrane separate the groups of ridges. On the right-hand side the particulate substructures of the ridges can be seen. $\times 69000$.

Fig. 7. EF of a region showing the basal limit of the junction. The ridges, in places clearly moniliform and frequently ending blindly (arrowheads), are more loosely organized and the prominent EF domes are common. In this case the ridges end abruptly leaving particle-rich non-junctional membranes (at top). $\times 66000$.

Fig. 8. The basal extremity of a junction showing both EF and PF. The EF ridges and PF grooves peter out in loosely organized loops and blind-ending rows. The PF in this region bears unusual arrays of fine, parallel ridges (arrows). $\times 59000$. 
packing of the ridges and furrows decreases, so the frequency of the 8–12 nm particles on the PF increases and at the basal end of the junction the non-junctional membrane is rich in intramembranous particles (Fig. 8). The PF of the basal end of the junction is occasionally characterized in unfixed material by fine hair-like ridges smaller in diameter (4–6 nm) than the EF ridges of the junction and much shorter (arrowed in Fig. 8.) They are arranged in parallel in stacks of 10 or more ridges. No complementary structure has been found in the EF nor have similar structures been seen in fixed tissues.

**Malpighian tubules.** In general the features of the junctions found in Malpighian tubules are similar to those described for the midgut. Table 1 summarizes the dimensions and separations of the various intramembranous structures found and comparison with the figures for the midgut show that the junction exhibits the same characteristics. The intercellular space seems to be slightly larger in the Malpighian tubules (17 nm, cf. 15 nm) and this is also reflected in the greater size of the ridges and ridge-associated particles found in freeze-fracture preparations of fixed material. The moniliform nature of the ridges is clear in both fixed (Fig. 9) and unfixed (Fig. 10) tissues in both species examined (cf. Figs. 9, 10 with Fig. 9 inset). However, one notable difference between the 2 tissues is shown by the fracturing pattern of the junction following fixation. Unlike the midgut, where the ridges adhere to the PF (Figs. 1–4), in the Malpighian tubules of both *Musca* and *Rhodnius* the fracturing characteristics of the unfixed tissue, namely EF ridges with PF grooves, remain unaltered by fixation (Fig. 9).

Lateral borders are encountered in freeze-fracture replicas of Malpighian tubules

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Fig. 9. Continuous junction from a *Musca* Malpighian tubule that had been fixed before freezing. The moniliform EF ridges and PF grooves, obscured only very occasionally by particles, can be seen. Domes are found in the EF, corresponding craters occurring in the PF. × 86,000. Inset: PF grooves and EF ridges from a continuous junction linking two cells from a fixed *Rhodnius* Malpighian tubule. The fracturing characteristics and other features of the junction closely resemble those of *Musca* Malpighian tubules. × 49,000.

Fig. 10. Continuous junction from a Malpighian tubule taken unfixed from *Musca*. The moniliform ridges are still found on the EF. × 67,000.

Fig. 11. Continuous junction from a Malpighian tubule of *Rhodnius*, infiltrated with lanthanum during fixation. The clear septa are separated by lakes of stain which, except where the septa run very closely parallel (arrows), are punctuated by electron-lucent spots. Arrowheads indicate where the septa end abruptly. × 190,000.

Fig. 12. PF from the apical end of the lateral border between 2 *Musca* Malpighian tubule cells fixed before freezing. Undulating rows of 10-nm particles indicate the presence of a pleated septate junction. × 49,000.

Fig. 13. Area from the basal region of *Musca* Malpighian tubule cells, fixed in the presence of colloidal lanthanum. The lanthanum has stained the intercellular space, infiltrating the gap junction which can be seen both in transverse section (large arrow) and in en face view. The stain has penetrated around the particles comprising the gap junction and in some cases (small arrows) appears to have filled the channel down the centre of the particles. × 90,000. Inset: freeze-fracture image of the EF of the basal region of the lateral border between 2 *Musca* midgut cells showing the characteristic clustering of 13-nm particles of the insect gap junction. × 77,000.
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far less frequently than in the midgut and it is not yet possible to analyse the apical-to-basal characteristics of the junctions. Preliminary results indicate that there is a fair degree of order in the packing of the ridges all the way down the junctional area; extensive areas of extremely tight packing (as found in the apical region of the midgut (Fig. 2)) and loosely meandering ridge systems (as in the basal regions of the midgut junction (Fig. 8)) have not been found. However, a feature never seen in the midgut has occasionally been found in the Musca Malpighian tubule. At the extreme apical end of the lateral border there is sometimes the suggestion of a pleated septate junction (Fig. 12). These areas display undulating rows of 10-nm particles on the PF, that meander across the membrane face approximately in parallel. They are less readily recognizable than the smooth septate junctions in this tissue (cf. Figs. 9, 10 with Fig. 12) and indeed are less striking than the pleated septate junctions found in many other insect epithelia (see for example Satir & Gilula, 1973; Noirot-Timothee, Smith, Cayer & Noirot, 1978).

Fig. 14. Conventionally fixed and sectioned preparation of the apical region of a junction between 2 midgut cells of Musca. The regularity of the intercellular space and its uniform electron density characterize the continuous junction in this region. ×106000.

Figs. 15, 16. Conventionally fixed and sectioned preparations from the middle region of the lateral border between 2 Musca midgut cells. The ladderlike arrangement of septa in the intercellular space can be seen clearly. The convoluted nature of the lateral border occasionally gives rise to circular profiles in fortuitous sections as in Fig. 16. Fig. 15, ×169000; Fig. 16, ×142000.

Fig. 17. Apical region of the lateral border between 2 Musca midgut cells, fixed in the presence of lanthanum. The section passes transversely through the intercellular space which appears regular and uniformly electron dense. mV, microvilli. ×110000.

Figs. 18, 19. Lanthanum-infiltrated preparations of Musca midgut from the apical-to-middle region of the junction. In this case slightly tangential sections reveal the septa of the junction and also the electron-lucent spots between them (Fig. 19) which in some cases appear as a continuous band, thinner than the septa (arrowed Fig. 18). Fig. 18, ×154000; Fig. 19, ×201000.

Fig. 20. Lanthanum-infiltrated preparation of the apical region of Musca midgut cells. The section provides en face as well as transverse views of the junction, revealing the straight septa, in this region often lying very close together (large arrow and inset). In other regions the septa are separated by a single row of electron-lucent spots running parallel to the septa (arrowed). mV, microvilli. ×75000. Inset: higher power of a region shown in Fig. 20. The subunit nature of the septa can be made out, especially where arrowed. ×170000.

Fig. 21. Lanthanum-infiltrated preparation of the middle region of the junction between 2 Musca midgut cells. The en face view displays a looser organization of the septa than in the apical region, the adjacent septa being separated by one (at arrows) or more rows of electron-lucent spots. ×75000.

Fig. 22. Lanthanum-infiltrated preparation of the basal region of the junction between 2 Musca midgut cells. The septa meander in an irregular fashion and are often widely separated by intercellular spaces containing many electron-lucent spots. However, in some places these spots appear to line up along the septa (at arrows). ×124000.
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**Lanthanum infiltration**

*Midgut.* Although details of the intercellular components of the smooth septate junctions can be seen most clearly after infiltration with lanthanum (Figs. 17-22), some indication of intercellular septa can be found in conventionally prepared and stained preparations (Figs. 15, 16). However, images revealing the intercellular septa are not found at the apical end of the junction, where a uniformly electron-opaque intercellular space is encountered (Fig. 14). Septa can be found in non-infiltrated preparations only in the middle and basal regions of the junction (Figs. 15, 16). After infiltration with lanthanum, electron-lucent septa can be made out, separated by areas filled with the electron-dense stain, which is punctuated by electron-lucent spots 4 nm in diameter (Figs. 18, 19). These spots may become aligned in rows running parallel to the septa (arrows, Figs. 20-22). The septa are approximately 7–8 nm in width and may run closely parallel to each other. The separation between them is very variable, from 2.5–3 nm (in which case no translucent spots punctuate the lanthanum-filled interseptal space (arrowhead Fig. 20, and inset)) to 9 nm (with one row of spots as in Figs. 19–21), 17 nm (with 2 rows of spots, Fig. 18) and increasing distances with large numbers of spots in the interseptal space, not necessarily aligned in parallel rows (Fig. 22). The degree of separation of the septa is correlated with their position on the lateral border. Towards the apical end of the junction (Fig. 20) septa separated by only 2–3 nm are frequently encountered but further down the lateral border the septa are more loosely packed, with more rows of spots interspersed between them (Fig. 21). At the basal end of the junction (Fig. 22) the separation between the septa can be large, with many electron-lucent spots between them. These, however, may still become aligned parallel to the septa, in which case they lie adjacent to them (arrows, Fig. 22).

Details of the structure of the septa can be made out at higher resolution (inset, Fig. 20). Electron-lucent subunits with an electron-dense core are arranged in a linear fashion and these particles could themselves be composed of subunits.

*Malpighian tubule.* Reference to Table 1 shows that the dimensions of junctional features revealed by negatively staining the intercellular space are very similar to both the midgut and Malpighian tubule. The intercellular space is again slightly larger than in the midgut but the other dimensions are in close agreement. The septa are separated by a variable distance and may run closely parallel, with no electron-lucent spots interspersed between them (Fig. 11, arrowed). As was found with freeze-fracture ridges, the septa may also end abruptly (arrowheads, Fig. 11).

Lanthanum-infiltrated images of apically positioned pleated septate junctions have not yet been found, nor have striking differences in the septal organization of the smooth septate junctions been seen corresponding to the apical-basal axis of the lateral border. However, in both freeze-fractured and lanthanum preparations of midgut and Malpighian tubule, gap junctions are encountered towards the basal end of the lateral border (Fig. 13, and inset). These junctions display the characteristic reduced intercellular space in transverse section (large arrow, Fig. 13), the lanthanum-outlined particles with an electron-dense core in more tangential section (small
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arrows, Fig. 13) and the clusters of 13-nm particles in freeze-fracture (Fig. 13, inset).

DISCUSSION

The appearance of the smooth septate junction described here, after preparation by 3 different methods, serves in general to confirm the structural model put forward by Flower & Filshie (1975). They suggested that the septa, revealed by lanthanum staining as clear, stain-excluding bands, abutted onto the 2 junctional plasma membranes and that at these points freeze-fracture preparations revealed rows of particles which in fixed material fused to form rods. The intercellular columns, revealed as clear, electron-lucent spots after lanthanum infiltration, had no counterpart in the freeze-fracture image and therefore were held not to insert into the interior of the junctional membranes. The absence of clear septa in conventionally sectioned and stained preparations was explained by the presence in the intercellular space of both septa and columns. As a result, only those sections cut in a plane perpendicular to the longitudinal axis of the septa would be likely to show differences in electron opacity between the septal regions and the intersertal regions containing columns (see Flower & Filshie, 1975; fig. 1 b). These features are found in the present study with both midgut and Malpighian tubule and the various dimensions given in Table 1 agree with those measured by other authors (Dallai, 1975, 1976; Flower & Filshie, 1975).

The exact correlation of structures seen in the intercellular space by lanthanum infiltration with the structure revealed in the interior of the membrane by freeze-fracture has been called in question recently (Noirot-Timothee et al. 1978). Working with pleated septate junctions they found evidence conflicting with the assumption made by some authors (Gilula, Branton & Satir, 1970; Satir & Fong, 1973; Satir & Gilula, 1973; Flower & Filshie, 1975) that the particles seen in freeze-fracture correspond with the intercellular septa at a precise and repeated position with respect to the phase of the septal undulations. Noirot-Timothee et al. (1978) found no precise correspondence between the separation of the freeze-fracture particles and the periodicity of the undulations of the septal ribbons. From this evidence they concluded that there could be no 'structural continuity throughout the entire thickness of the septate junction, contrary to the evidence available on the organisation of gap junctions'. Their evidence could equally well be interpreted to show that the insertion of the septa into the membrane was irregular with respect to the undulations of the septa, not that it was non-existent. This latter interpretation would leave unexplained the presence of the rows of particles in freeze-fracture replicas. However, the topographical correlation between the rows of particles and septal bands was good. This is also found with smooth septate junctions (c.g. Lane & Harrison, 1978). In the present study, correlation between the ridges found in freeze-fracture and the septa found in lanthanum-infiltrated preparations extends to variations in the separation between them, to their organization in the apical-to-basal axis in midgut and to the occurrence of blindly-ending septa (and ridges) in both midgut and Malpighian tubules. It seems likely that the intercellular and intramembranous structures are both related to the septa in some way but that, as with
pleated septate junctions, the precise mode of insertion of the intercellular structures into the cell membranes must await further elucidation.

Fixation of tissue before subjecting it to freeze-fracture has a marked effect on the fracturing characteristics of the ridges. Without prior fixation, the rows of rods or particles fracture almost exclusively onto the EF, whereas in tissue that has been fixed the particles tend to fuse to form ridges which fracture both onto the PF and EF but with a greater preponderance on the PF (Flower & Filshie, 1975; Dallai, 1976). This effect of fixation has been confirmed in the midgut of Musca but in Malpighian tubule preparations, fixation has little effect on the fracturing characteristics. Particles are found in the EF in both fixed and unfixed preparations. This contrasts with the findings of Dallai (1976) using the Malpighian tubules of Periplaneta americana where the fracturing characteristics alter in the way described also by Flower & Filshie (1975). This discrepancy presumably reflects a slight variation in the bonding between the junctional components and the membrane, so that alterations produced by aldehyde fixation result in different fracturing patterns. The degree of fusion of the junctional particles is greater in fixed than in unfixed preparations. The natural in vivo structure is not known, since alterations might be produced by either fixation or glycerination. Filshie & Flower (1977) found that the particle rows of septate junctions of Hydra were apparently lost if glycerination of unfixed material continued for longer than 5 min and it is possible that the structure of the smooth septate junctions could be altered by similar treatment. On the other hand, fixation could also promote fusion of normally separate junctional particles. Whether the ridge structure in vivo is moniliform or consists of short bars or continuous ridges, cannot be elucidated until a method is devised that circumvents both fixation and glycerination (see for example, Skaer, Franks & Echlin, 1978).

The topography of the smooth septate junction in Musca midgut reveals a distinctive pattern following the apical-to-basal axis of the cells. Apically the junctional ridges of freeze-fracture replicas are very closely associated in a regular and parallel fashion. This high degree of order is also seen in the arrangement of the intercellular septa seen after lanthanum infiltration. Both the ridges and septa become less highly ordered further down the junction and more intercellular columns are found interspersed between the septa, until at the basal extremity, both freeze-fracture and intercellular lanthanum reveal a very loosely organized junction with few septa meandering across the junctional area. This pattern is also reflected in the images obtained by conventional sectioning and staining. In the apical part of the junction where the septa and columns are closely packed, sections reveal the familiar, uniformly electron-opaque intercellular space with no clear septa visible (Fig. 14). Only sections perpendicular to the long axis of the septa would have any likelihood of revealing differences in electron opacity (see fig. 1b Flower & Filshie, 1975) and even if such a section were cut, the tendency of the septa to curve (albeit in parallel) within the thickness of the section would obscure the contrast in electron density between the septa and the spaces between them. However, as the order loosens, the chance of a section revealing septa increases and in the middle region of the junction, sections do occasionally show septa spanning the intercellular space (Figs. 15, 16).
Variations in smooth septate junction

When smooth septate junctions were first described it was suggested that they represented structures analogous to pleated septate junctions and that smooth septate junctions might be associated with regenerating tissues or those of endodermal origin, whereas pleated septate junctions were associated with non-regenerating tissues or those of ectodermal origin (Noirot & Noirot-Timothee, 1967). The results described here and by Dallai (1976) and Lacombe (1976) conflict with this interpretation since smooth septate junctions occur in Malpighian tubules which are ectodermal in origin and in which constant cell turnover does not occur. Moreover, the occurrence of smooth and pleated septate junctions side by side in Malpighian tubules (see also Dallai, 1976) argues against the 2 types of junctions being analogous structures.

The physiological significance of septate junctions is still not clear. While it is generally agreed that they have an adhesive function (see, for example, Wood, 1959, 1977; Noirot-Timothee & Noirot, 1974; Baskin, 1976; Noirot-Timothee et al. 1978), suggestions have been made that they facilitate the movement of fluids through the intercellular space (Dallai, 1976) or that they act as a trans-epithelial permeability barrier (Wood, 1959, 1977; Dan, 1960; Berridge & Oschman, 1969; Newell & Skelding, 1973; Lord & di Bona, 1976; Filshie & Flower, 1977; Noirot-Timothee et al. 1978). Physiologically both the tissues studied here are involved in fluid transport (for reviews see Treherne, 1967; Maddrell, 1972, 1977) and in both cases there is evidence of selective resistance to free diffusion. The upper Malpighian tubule, for example, shows passive permeability to organic solutes, the ease of passage depending on molecular size, whereas the permeability to charged moieties is more complex (Maddrell & Gardiner, 1974). The midgut may also support ionic gradients (Shaw, 1955; Harvey & Nedergaard, 1964; O'Riordan, 1969). It may be that smooth septate junctions are responsible for limiting the backflow of ions, while allowing the passive uptake of uncharged organic molecules. The presence of complex arrangements of septa will retard passive flow through the junction (Filshie & Flower, 1977) and the chemical nature and charge characteristics of the electron-dense material trapped between the septa (Noirot & Noirot-Timothee, 1967; Dallai, 1970) would confer specificity on this otherwise unselective retardation (for a similar suggestion see Staehelin, 1974). Some evidence to support this comes from a study by Gupta, Berridge & Prince (unpublished data, 1972) in which the septate junctions of Calliphora Malpighian tubule were shown to be permeable to the anion, sulphate, but not to a heavy metal cation, barium. The exact permeability properties of a septate junction would depend on the chemical characteristics of the matrix held between the septa and the geometry of the septa themselves. Thus in the midgut, the highly organized nature of the junction at the apical end of the lateral border may represent the area of junction with the highest reflexion coefficient; towards the basal end of the lateral membranes where the junctional structure is looser, the reflexion coefficient may be lower.

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