FINE-STRUCTURAL CHANGES IN RELATION TO ION AND WATER TRANSPORT IN THE RECTAL PAPILLAE OF THE BLOWFLY, CALLIPHORA

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SUMMARY

The fine structure of the epithelial cells of the rectal papillae in the blowfly, Calliphora erythrocephala Meig., has been investigated to elucidate the possible mechanism of reabsorption of water and ions from the rectal lumen. To observe the variations in the structure of the epithelium in response to the absorptive processes the material was taken (a) from flies at the various stages of their first oviposition cycle, and (b) from freshly emerged imagoes starved for 2 days and injected into the rectum with solutions of various tonicities. It has been found that the complex system of intercellular spaces, formed by a prolific infolding of the lateral plasma membrane of the cells, shows a direct response to the conditions of supposedly maximal and minimal transport of fluid. These spaces are (a) grossly distended in the flies injected with hypotonic media, (b) highly dilated under normal conditions, and (c) completely collapsed in fasting and starved flies. These observations have been discussed in the light of the available theories to explain the mechanism of water transport in biological tissues. It is proposed that the structural design of the rectal papillae favours the application of double-membrane theory to explain the reabsorption of water against osmotic gradients as a consequence of an active transport of solutes into enclosed spaces.

INTRODUCTION

It is becoming increasingly apparent that an understanding of the active transport of ions and water in biological systems will remain incomplete unless the physiological data are integrated with the fine structure of the tissues involved. In recent years a few such integrated studies of vertebrate tissues have already provided promising results (Farquhar & Palade, 1964; Kaye, Cole & Donn, 1965; Kaye & Donn, 1965; Kaye & Pappas, 1965; Curran, 1965; Kaye, Lane, Cole & Donn, 1966; Diamond & Tormey, 1966). A feature of these investigations is the revelation that, besides the cells, extra- and intercellular spaces in the epithelia are believed to play a vital role in creating favourable conditions for the movement of water in isotonic proportions, even against an osmotic gradient, thus obviating the need to provide for an active transport of the solvent. Evidence is now available to show that in many vertebrate systems the transport of water is a consequence of an active transport of ions into narrow intercellular spaces, resulting in localized fluids of very high concentration, which draw water osmotically from the lumen of the organ concerned (Whitlock & Wheeler, 1964;

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A number of physiological studies on the insect rectum (Ramsay, 1950, 1955, 1964; Sutcliffe, 1960; Phillips, 1961, 1964a, b, c) have confirmed the earlier suggestion by Wigglesworth (1932) that this organ is responsible for the resorption of water. Further, it has been found by these workers that, in addition to water, important ions secreted by the Malpighian tubules are also resorbed and that both these transport processes often occur against steep osmotic gradients. In his elegant experiments, Phillips injected measured quantities of solutions of various ionic compositions and strength as well as completely ion-free sugar solutions into the canulated, ligatured rectum of Calliphora erythrocephala (1961) and of Schistocerca gregaria (1964a, b, c) in known physiological states. After suitable periods aliquots of the injected fluids were withdrawn from the rectum and analysed for the amount of ions and/or water absorbed by the rectum. He concluded that in both the insects there is an active absorption of ions and water against an electrochemical and osmotic gradient with respect to the haemolymph. Since the water was absorbed even from isosmotic and hyperosmotic (to the haemolymph) xylose and trehalose solutions, Phillips (1964a) suggested the existence of a pure water pump or a carrier mechanism to enable the rectal epithelium (including the rectal appendages like papillae and pads) to reabsorb water ‘actively’, presumably without any concomitant movement of the solute. Although Phillips does not rule out the existence of some other mechanism responsible for water resorption, his conclusions suffer from a strong general belief that the epithelium lining the rectal pouch and the rectal papillae and pads is a simple sheet of cells in a single layer (Wigglesworth, 1965), and that all the transport is directed into the haemolymph.

Recently, Gupta & Berridge (1966a, b) have described the detailed fine structure of the rectum in Calliphora erythrocephala. It has been demonstrated that the rectal papillae enclose an extensive system of intercellular spaces and sinuses, described earlier by Graham-Smith (1934), which is in only one-way communication with the haemocoel. These intercellular spaces ramify extensively between the lateral boundaries of the cells; the latter are infolded in a complex and prolific manner to form innumerable stacks of membranes closely associated with large mitochondria in the cytoplasm. A magnesium-dependent and pH-specific adenosine triphosphatase (ATPase) has been localized histochemically on these stacked membranes (Berridge & Gupta, 1966). By analogy with vertebrate systems, we have proposed that the stacks of lateral plasma membrane are the sites of a mechanism which actively pumps ions (perhaps KCl) into the intercellular spaces. The presence of these ions in narrow ‘lumina’ of the sacs of uniform width (about 100–200 Å) will create high ionic concentrations to provide for the removal of water from the rectal lumen (compare Whitlock, Wheeler, Kaye & Lane, 1965; Diamond & Tormey, 1966).

In the present paper the results of experiments designed to study the fine structural changes in the rectal papillae during high and low absorptive activity are described. It has also been found that the transported fluid seems to move through the cells into the intercellular spaces, before it is discharged into the haemolymph. The new evidence
has been compared with the published results of similar studies on vertebrate tissues in an attempt to examine the applicability of current theories of water transport in other tissues to the function of the insect rectum.

MATERIAL AND METHODS

Adult females of the blowfly, Calliphora erythrocephala Meig., have been used throughout the present work. Flies were reared in the laboratory and adults that emerged from pupae over a given period of 12 h were kept in separate cages in order to obtain flies of different ages. They were fed on pig's liver and had access to sucrose and tap water. All flies were kept at 25 °C under a constant light cycle of 12 h. Under these conditions flies deposited eggs between 5 and 6 days after emergence from the pupae.

By correlating the weights of the body, crop, gut and ovary of the females from 0 to 6 days of age, Berridge (1966) found that the oviposition cycle of Calliphora may be divided into a period of feeding, digestion and assimilation (0–3 days) and one of egg development in the ovary (4–6 days); the cycle is repeated again after oviposition on the 5th or 6th day. Berridge also found that the Malpighian tubules from flies on the 4th and 5th day were least active in terms of rate of urine flow; this is the period of the cycle when there is no feeding or drinking by the flies and the ovarioles are growing. It would be reasonable to assume that the amount of ions and water to be reabsorbed by rectal papillae is maximal during the period 0–3 days and that it will gradually decrease during the following period until restart of the cycle after oviposition. However, in the second period of the cycle (3–5 days) the work of reabsorbing ions and water would take place against maximal gradients. Furthermore, the developing oocytes would withdraw solutes from the blood, causing dilution of the latter and thus the need for reabsorption of ions in the absence of feeding would perhaps also be maximal. For the purpose of describing fine structure in relation to the physiological state of the fly, rectal papillae have been grouped into 3 categories:

1) Normal—from 0- to 2-day-old actively feeding flies, in which the gut is full, Malpighian tubules are very active, and the rectum is distended; the rectal papillae are swollen, the ovary small, and presumably large quantities of ions (particularly K, being very low in blood and high in urine) and water are being reabsorbed.

2) Fasting—from 3- to 5-day-old flies, which are not feeding; the gut and the crop are generally empty and Malpighian tubules relatively inactive; ovaries gain enormously in weight, the rectum often contains solid material with very little fluid; rectal papillae are thin and elongated; presumably there is little reabsorptive activity.

3) Restored—from 5- to 6-day-old flies which show oviposition and the restart of feeding, normal conditions are restored.

Induced changes

In order to confirm that the structural changes in the rectum were related to the conditions of fasting, newly emerged flies were denied food or water for 2 days. Such flies show anatomical conditions similar to 'fasting' flies except that ovaries do not
develop. Under these conditions the need to conserve ions and water would be maximal; this condition is referred to as starvation. Wigglesworth (1932) had already noted that 2 days are about the maximum period which Calliphora (newly emerged) can survive without food and water under laboratory conditions.

Following Phillips (1961), 0.75 µl of the following solutions were injected through the anus into the rectum of starved flies: (1) isosmotic (with haemolymph) xylose (5.5 g/100 ml); (2) isotonic salt solution (KCl, 1.12 g/100 ml; NaCl, 0.29 g/100 ml); (3) hypertonic salt solution (NaCl, 5.0 g/100 ml); (4) half-isosmotic xylose (2.75 g/100 ml); (5) distilled water. All solutions contained 0.001 M amaranth as an indicator. Flies were anaesthetized with CO₂, and the required fluid injected into the rectum. Material from each lot was fixed after 15 and 30 min. Only the animals which showed a high concentration of dye in the rectum, without any leakage into either the anterior portion of the gut or rectal wall, were used. In all cases experiments were repeated 3 times; in each instance the rectum from uninjected flies, both anaesthetized and unanaesthetized, from the same batch of starved insects, was used as control.

The material was fixed for 2 h in cold 2.5% glutaraldehyde in 0.05 M Na-cacodylate buffer with 0.15 M sucrose (pH 7.2–7.4), washed overnight in cold buffer with 0.3 M sucrose, postfixed in cold buffered 1% OsO₄ for 1 h, dehydrated in ethanol and embedded in Araldite through propylene oxide. Silver or light-gold sections were routinely ‘stained’ in ethanolic uranyl acetate followed by lead citrate, and examined with a Philips EM 200 electron microscope operated at 60 or 80 kV. Further details of the procedure have been given in a previous paper (Gupta & Berridge, 19666).

The electron-microscopic evidence from a study of experimental materials may be questioned on the basis of insufficient sampling. The present results are based on 76 rectal papillae each taken from a different fly, in turn selected at random from a particular batch of fixation. A total of more than 1000 electron micrographs was examined.

RESULTS

Structure in the normal phase

The general organization of the rectum in Calliphora at the electron-microscopic level has been described elsewhere (Gupta & Berridge, 19666), where a brief description of the cells of the rectal papillae was also included. The rectal papillae are conical structures with an epithelial cortex and a connective tissue medulla. The cortex is made up of a single layer of large epithelial cells with their basal surface facing either the haemolymph or an ‘infundibular space’ enclosed between the cortex and the medulla, and covered with a thick basement lamella containing collagen-like fibres. The apical surface of the epithelial cells is covered with a cuticular intima which is continuous with the intima lining the rest of the rectal pouch, but differs from it in fine structure (Figs. 3, 25 and 26). The apical plasma membrane is thrown into continuous infoldings which in sections resemble the profiles of microvilli (Fig. 3), but are, in fact, comparable to the ‘leaflet’s described by Copeland (1964) in the anal papillae of mosquito larvae. Their sheet-like nature becomes evident both in serial
sections and in sections cut normal to the long axes of the leaflets. The cytoplasmic surface of this membrane is covered by uniformly spaced particulate subunits (Figs. 23, 24), described in detail elsewhere (Gupta & Berridge, 1966a). The width of the leaflets, as well as the width of the subcuticular space enclosed by them, varies according to the physiological state of the rectum (Figs. 19–22; see below). ‘Coated’ or pinocytic vesicles (pv), believed to be responsible for the incorporation of large molecules into cells in other tissues (Roth & Porter, 1964; Bowers, 1964; Stay, 1965; Fawcett, 1965) are often associated with the plasma membrane of the leaflets (Figs. 3, 20, 22). The apical region of the cytoplasm contains scattered profiles of endoplasmic reticulum, both with and without granules (ribosomes) on their surface, small mitochondria, free ribosomes, glycogen granules and abundant microtubules (Figs. 3 and 19–24). Dense, lysosome-like bodies (de Duve, 1963; Novikoff, 1963) are also occasionally present (Figs. 20, 21).

In sections observed at low magnifications (Fig. 3) the lateral boundaries of the cells in the apical region appear as extremely dense, sinuous profiles, extending from the base of a leaflet to a large intercellular channel. At this magnification the lateral boundary (lpbm) appears to be composed of thick (sd) and thin (zo) areas of alternating lengths. The thick areas can be resolved into typical septate desmosomes (Wiener, Spiro & Loewenstein, 1964; Locke, 1965a; Gupta & Berridge, 1966b), while the thin portions are sites of membrane fusion, forming occluded zones (Figs. 4, 5). It has been shown in other tissues that both these types of cell junction provide sites of low resistance to the cell-to-cell movement of diffusible substances like ions and water (Loewenstein & Kanno, 1964; Loewenstein et al. 1965; Penn, 1966), but they almost completely prevent any such diffusion of extracellular materials into the intercellular channels without passing through the cells (Farquhar & Palade, 1963, 1964, 1965; Loewenstein & Kanno, 1964).

The apical region is only about 5–10 μ in width in cells which measure about 80–100 μ between the basal and apical membranes. Through the rest of the cells the lateral plasma membranes surround a highly arborized system of interconnected intercellular spaces containing tracheal twigs and tracheoles. The larger of these spaces is lined by a very delicate basement lamina which, however, does not extend into the finer (up to 0.2 μ wide) cylindrical diverticula (see Gupta & Berridge (1966b) for further details on tracheal system and basement lamina). At frequent intervals around their devious course, the lateral plasma membranes show areas of close apposition (Fig. 6, asterisks), with tiny desmosome-like densities (pdm) distinguishing such profiles from stacked membranes (see below). Such areas of close apposition alternate with localized sites of membrane fusion, forming maculae or fasciae occludens (Figs. 6 and 9, zo: compare Farquhar & Palade, 1963). Near the basal surface of the cells the lateral plasma membranes again form a complete collar of septate desmosomes accompanied by a zonula occludens (Fig. 9, sd and zo). A similar collar of septate desmosomes closes the intercellular space at the sites of tracheal entry (Gupta & Berridge, 1966b). In a narrow (up to 5 μ wide) basal region of the cells the intercellular spaces extend to form a network of blind diverticulae (Figs. 9 and 10, bd). In the rectal papillae of freshly emerged flies the intercellular spaces are filled
with long loosely packed microvillus-like cytoplasmic processes (Figs. 3, 8) generally directed towards the open ends of the spaces. Thus within the epithelial layer of the papillae the intercellular spaces are completely shut-off from the extracellular environments, namely, the haemolymph and the contents of the lumen.

Fig. 1. A diagrammatic representation of a 3-dimensional image of infolded lateral plasma membrane forming stacks of sacs opening into larger intercellular spaces containing tracheal twigs and tracheoles. Large mitochondria are closely associated with such stacked membranes. The cytoplasm surrounding the infolded membranes has not been shown in this diagram. The stippled surfaces face the cytoplasm, while the luminal spaces are shown blank to simulate their transparent contents in the electron micrographs. The larger intercellular spaces are lined with a thin basement lamina.

In addition to lining the major intercellular spaces, the lateral plasma membranes of the cells are also extensively infolded to form sacs of various dimensions, enclosing a lumen of uniform width (about 150 Å) in 'normal' flies, and occurring in piles of variable number. In sections such piles of sacs appear as stacked profiles of paired membranes, joined at either one or both ends (depending upon the plane of sectioning for an individual sac). The membranes of the stacks are uniformly spaced (about 200 Å). Each membrane can be resolved into a typical unit membrane with about 25–25–25 Å spacing and bears a thin felt-like covering or glyocalyx (Bennett, 1963; Fawcett, 1965) on the outer surface (Fig. 15). Prominent mitochondria with closely packed plate-like cristae and dense matrix are intimately associated with the membrane stacks, and not infrequently form series with them (Figs. 3, 13).
The lumina of the sacs can be shown to open into the diverticulae and branches of the major intercellular spaces (Figs. 3, 6 and 13, arrows). It must be stressed that unlike the basal infoldings of the plasma membrane in many other absorbing and excreting systems (for examples see Rhodin, 1963; Fawcett, 1966) the stacks of paired membranes are not formed by continuous infoldings of the plasma membrane in only one plane. In 3 dimensions each sac in any one pile is generally formed from an intercellular channel lying in a different plane (Fig. 1). Such an arrangement is likely to prevent the distension of the sacs in the event of a hydrostatic pressure developing in the spaces (see Discussion). Myriads of such stacks form the bulk of the apparent cell volume (Fig. 10) in the cortex of the papillae. Only the narrow apical and basal regions and a circum-nuclear zone (Gupta & Berridge, 1966b) are not invaded. All intercellular spaces in the cortex ultimately drain into a large intercellular sinus in the conical tip of the papillae (Fig. 2D); and this sinus, in turn, communicates freely with the funnel-shaped infundibular space opening into the haemocoel at the base of the papillae. In *Calliphora* this opening is guarded by a flap of connective tissue, the medullary valve (Gupta & Berridge, 1966b). In other insects, however, such a valve seems to be absent (Baccetti, Mazzi & Massimello, 1963; C. P. Hopkins, personal communication), and the medulla may be represented only by the tracheal tissue (e.g. in the mosquito (Hopkins, personal communication)).

The cytoplasm of the papillary cells contains scattered profiles of Golgi dictyosomes and rough endoplasmic reticulum, free ribosomes frequently forming polysomic clusters, and a variable population of glycogen granules (identified by criteria used by Revel, Napolitano & Fawcett, 1960; Revel, 1964; Fawcett, 1966). Profiles of microtubules and cisternae of smooth endoplasmic reticulum are also present. The special nature of the basal region of the cytoplasm and its significance in cellular nutrition has already been discussed elsewhere (Gupta & Berridge, 1966b).

**Cyclic changes**

*Normal phase (0–2 days).* In freshly emerged imaginal flies, the subcuticular space is narrow and the apical leaflets are more or less uniformly spaced (Fig. 19). The cytoplasm contains large aggregates of glycogen. Both the small apical mitochondria and the large mitochondria associated with the stacks are regular in shape and uniform in the density of their matrix. All intercellular spaces are fairly distended and larger ones are loosely filled with long and thin cytoplasmic processes directed towards the opening of the spaces (Fig. 8). The lumen of the sacs in the stacks is about 100–150 Å wide.

In 2-day-old flies the subcuticular space is greatly enlarged and in places forms balloon-shaped profiles containing loose flocculent material of moderate density (Fig. 20) and pushing the leaflets farther apart. In many instances mitochondria are seen lying close to the cytoplasmic surface of such distensions (Fig. 20). Pinocytotic vesicles ('coated vesicles' of Roth & Porter, 1964) associated with the leaflets are frequently present. It is not certain whether such profiles in this material indicate an uptake of macromolecules by the cells as in oocytes (Roth & Porter, 1964; Stay, 1965) and pericardial cells (Bowers, 1964) or secretion by the cells. It is to be noted, however,
that the cuticle lining the rectum is believed to act as a molecular sieve permitting only molecules less than 6.5 Å in diameter to infiltrate into the subcuticular space (Phillips, 1965). The intercellular spaces are also greatly distended and by the techniques employed show completely transparent contents (Figs. 6, 10). The glycogen content of the cytoplasm seems to have diminished. The lumina of the sacs vary from 100 to 200 Å in width. The impression gained is that a large volume of some soluble material (perhaps a KCl solution) is now present in the intercellular spaces. The mitochondria are still fairly normal in appearance, although a few enclose 'vacuoles' in the matrix (Figs. 9, 13). Whether these are an accident of fixation is difficult to determine, although their consistently similar appearance in otherwise well-preserved material from older flies would seem to rule out the possibility of such gross artifacts.

**Fasting and starved flies.** The general image of fine structure of the rectum in fasting flies (3–4 days old) is similar to that of newly emerged females kept without food and water for 2 days (controls for injection experiments). The fact that a fasting condition can be induced by starving the flies will, therefore, tend to support the 'factual' nature of the recorded fine-structural changes.

In fasting flies the subcuticular space is almost completely obliterated (Fig. 21). In several places the particle-bearing plasma membranes of the leaflets (Gupta & Berridge, 1966a) are separated by only a narrow space 20–30 Å in width, mostly filled with material of the glycocalyx (Figs. 23, 24). Cisternae of smooth endoplasmic reticulum frequently lie close to the cytoplasmic surface of the leaflets (Figs. 21, 23). There is no noticeable change in the small mitochondria of the apical region (Fig. 21).

The intercellular spaces in the bulk of the cortex are completely collapsed. The lateral plasma membranes of adjacent cells are now separated by a narrow space of 100–200 Å, and tightly surround the tracheal and tracheolar tubes where the latter are present in the micrographs (Fig. 7; compare Fig. 6). This effect is quite pronounced even in the narrow basal region of the cell containing the blind diverticula (Fig. 11 from a starved fly; compare Fig. 10). The membranes in the stacks show a similar dramatic change. The lumina of the sacs are for the most part reduced to less than 100 Å in width. At several points, curiously coinciding in all the sacs in one stack, the membrane-pairs become tightly apposed and are separated by a space of only about 20 Å (Figs. 12, 14). At such sites of close apposition, both in the stacks and in the leaflets, the unit-membrane structure of the component membranes is unaltered (Figs. 14, 23 and 24).

The mitochondria associated with stacks frequently have a vacuolated appearance. In numerous instances subspherical myelin-like aggregates of concentric membranes were found attached to the bounding membrane of the mitochondria at one point. Such aggregates are, in such stages, also found free in the cytoplasm. The whole process is somewhat reminiscent of the reorganization of mitochondria during spermatelosis of some animals described by André (1962) and Gupta (1964). André believes that such myelin-like structures represent a portion of mitochondrial membranes together with some transparent material of the matrix ('pseudomatrix') discarded by the mitochondria in response to osmotic changes in the cytoplasm. As
will be discussed later, similar changes in the osmotic concentration of the cortical cells may be expected in response to the high concentration of the rectal contents in a desiccated condition of the flies.

The cells in starved flies show similar changes except that the paired membranes in the stacks as well as in the leaflets remain separated by a space of about 100 Å along their whole lengths (Figs. 7, 22).

Restoration phase (5–6 days). In flies which had started feeding after oviposition, the rectal papillae showed a fine structure very similar to that of 1- to 2-day-old flies.

Injected flies

Since all the injection experiments were carried out on fresh imaginal flies starved for 2 days, the structural changes are to be compared with the fine structure depicted in Figs. 7, 11 and 22.

Isotonic solutions (xylose or KCl + NaCl). The subcuticular spaces appear dilated and similar in appearance to those in Fig. 20. Intercellular spaces were restored to a degree of expansion found in normal young flies, although the change was not consistent throughout the cortex of the papillae. The contents of the intercellular spaces were completely transparent. No other change in the cells was noticed.

Hypertonic salt solution. Phillips (1964a) noticed that, after an injection of 1 M NaCl into the rectum of the locust, water moved from the haemolymph into the rectum. In the present study subcuticular spaces were completely obliterated both in the rectal epithelium and papillae (compare fasting flies, Fig. 21). Intercellular spaces remained collapsed and unchanged. The fine structure of the cells showed no other signs of distortion or shrinkage, except that mitochondria in the papillae seemed to have more myelin-like attachments than in the controls.

Hypotonic xylose solution and distilled water. Injections of hypotonic media produced the most dramatic changes in fine structure of rectal papillae. When papillae were fixed 15 min after injection, the intercellular spaces appeared maximally dilated throughout the cells (Fig. 16). The stacked membranes maintain their structural arrangement and the lumina of the sacs are rarely wider than 300 Å. The mitochondria appear swollen and transparent but the orderly arrangement of cristae is undisturbed. At higher magnifications, granular inclusions like ribosomes and glycogen show a high degree of clumping. The plasma membrane in the stacks and elsewhere continues to have an uninterrupted unit-membrane configuration. All junctional complexes, apical leaflets and subcuticular spaces remain unchanged.

When rectal papillae from the same batch of flies injected with hypotonic solutions were fixed after 25–30 min, their fine structure presented an entirely different picture. The cytoplasmic structures now appear quite normal (compare Figs. 10 and 18). The mitochondrial matrix is restored to its normal density. The only noticeable difference between the cytoplasm of this material and that of the controls is that the cisternae of rough endoplasmic reticulum are swollen and filled with some material of very high electron density. The intercellular spaces continue to be dilated but their contents are no longer transparent. As compared to flies fixed after 15 min (Fig. 16) they are now filled with a uniformly dispersed flocculent material of moderate density.
The material is confined to the larger intercellular spaces and does not penetrate the finer diverticulae or lumina of the sacs; as already mentioned, a fine basement lamina covers the openings of the finer spaces into the larger ones. In the basal region of the cells (Figs. 17, 18) extremely dense granules, measuring up to 2 μ in diameter, are found in the laminated part of the basal lamella. Similar granules are also present in small pockets of the basal plasma membrane (arrows '4') and within the blind diverticula of the intercellular spaces. It seems likely that the material of the dense granules is being phagocytosed and dumped into the intercellular spaces by fusion of the phagocytotic vacuoles with the blind diverticula. Within the intercellular spaces the material of the dense spheres appears to disperse in the form of a flocculent material (Fig. 17). Although a detectable content has never been found in the intercellular spaces of the rectal papillae of blowflies in any other condition, from the present evidence it would seem that the blind diverticula are the channels which provide the nearest access to the haemolymph for any interchange of materials. However, such an interchange must occur through the cytoplasm of the cells.

Changes in the rectal epithelium

A general description of the fine structure of the flattened cells lining the rectal pouch has been provided elsewhere (Gupta & Berridge, 1966). It remains here only to comment briefly on the structural changes in these cells during the feeding cycle and in injected flies. In normal flies the rectal pouch is distended and the cells are stretched into a thin sheet. The subcuticular space is prominent. In fasting and starved flies, the wall of the rectal pouch is thrown into villus-like folds separated by narrow channels of extracellular material, presumably haemolymph. The apical plasma membrane of the cells is closely apposed to the cuticular intima, thus completely obliterating the subcuticular space (Fig. 25). The general cytoplasmic organization of the cells remains unchanged. There is no marked structural response in the cells of the rectal wall to injections of the various solutions. Thus unlike papillae cells, rectal cells do not show any osmotic shock with hypotonic injections. However, the subcuticular space is enlarged under such conditions (Fig. 26).

The relative surface areas of the epithelial cells in the rectum

If all other parameters determining the movement of solutes and solvents through a cell membrane were the same, the rate of passive diffusion through a plasma membrane (unit membrane) will then be largely determined by the ratio of the surface area to the volume of the cells concerned (Davson, 1964). In other words, if the volume of the cells were to remain more or less constant, then in the event of an osmotic change the cells will tend to come to steady-state equilibrium with that environment which faces their maximum surface area. It will therefore be of interest to make an estimate of the proportions of the total area of the plasma membranes of the epithelial cells in the blowfly rectum which face each of their 3 environments, namely, the haemolymph, rectal lumen and intercellular spaces. Since, in sections, both types of epithelial cells are roughly rectangular in outline, the areas of the plasma membranes were calculated by determining the lengths of their profiles in electron micrographs of unit areas of the
cells (by running a commercial map-measure path-finder on the profiles). Final relative areas were estimated for the total volume of the rectal papillae and rectal pouch determined from light-microscope sections.

In flattened rectal epithelium, the ratio of the surface area of the cells facing the haemolymph is approximately equal to the area of the surface facing the lumen (subcuticular space). The lateral boundaries of these cells, as also the basal and apical membranes, show few infoldings. No particular specialization of structure indicating a large-scale 'active transport' by these cells has been observed, either in the blowfly (Gupta & Berridge, 1966b) or in other insects (Baccetti et al., 1963; C. P. Hopkins, personal communication). This highly attenuated layer of cells is therefore likely to act as a simple physiological membrane for the purpose of passive diffusion between the haemolymph and rectal lumen.

The extensive infoldings of the plasma membranes of the epithelial cells in rectal papillae causes an estimated 100- to 1000-fold increase in their total surface areas. The basal plasma membrane facing the haemolymph (or infundibulum) is more or less straight, and constitutes only 0.5-2% of the total surface area in these cells. The apical membrane, infolded to form leaflets, accounts for about 10-20%; the remaining 80-90% of the cellular surface constitutes the lateral boundaries and faces the intercellular spaces. Out of the total surface at the lateral boundaries about 80-90% forms the surface of the sacs in the stacks; only 10-20% lines the larger intercellular channels and is covered by a thin basement lamina. Thus the area facing the larger intercellular spaces is approximately equal to the luminal (apical) surface area. If rates of diffusion across each of the 3 surfaces are related to surface area, then the lateral boundaries would be highly permeable, the apical surface less so, while the basal surface would be relatively impermeable.

It may also be of interest to note that the total surface area exposed to the intercellular spaces in the rectal papillae is at least twice the entire surface area of the rest of the plasma membranes of both types of epithelial cells in the rectum.

**DISCUSSION**

Insects are not alone in their ability to transport fluids either in the absence of an apparent osmotic gradient or even against substantial gradients. Several vertebrate epithelia are also capable of similar mechanisms, and Table 1 summarizes the situation in a few such known systems. However, in vertebrates it is now believed that the transport of water is not itself an 'active' process but is a consequence of 'active' secretion of ions in the tissues concerned (Robinson, 1965; Durbin & Moody, 1965; Smyth, 1965; Curran, 1965; Diamond, 1965). A major reason for the hesitation on the part of the workers on vertebrate tissues to accept an 'active' transport of water is the fact that, whenever investigated extensively in vitro, these systems did not show any transport of water in the complete absence of a concomitant transport of ions (Curran, 1965; Diamond, 1965).

Insects, however, do seem to be capable of absorbing water through their body surface from an atmosphere of subsaturated air, some of them in relative humidities
as low as 50% (see Beament (1964) and Treherne (1965) for references). In his
experiments Beament (1964) found that droplets of 1% sodium chloride or of
saturated solutions of sodium cyanide and sodium fluoride applied to the surface of a
cockroach disappeared at a rate similar to that of distilled water, leaving solid crystals
of salt outside. Theoretical treatment indicated that water was being absorbed against
osmotic forces of 300 atmospheres. Beament (1964, 1965) has proposed an ingenious
model in which the superficial (waxy) layer of lipid molecules (Locke, 1965b) is
believed to act as a ‘valve mechanism’ regulating the water movements between the
outside medium and underlying protein layers. Cyclic changes in the isoelectric

Table 1. A summary of some vertebrate and insect tissues which are capable of
transporting water against an osmotic gradient. Where available, the approximate
osmotic gradient which develops between lumen and body fluid is included.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>The maximum osmotic gradient (Δ°C) (O.P._lumen – O.P._body fluid)</th>
<th>Reference</th>
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<tr>
<td>Rat jejunum and ileum</td>
<td></td>
<td>Parsons &amp; Wingate (1961)</td>
</tr>
<tr>
<td>Dog ileum</td>
<td></td>
<td>Annegger &amp; Wakefield (1962)</td>
</tr>
<tr>
<td>Fish gall bladder</td>
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<td>Rabbit gall bladder</td>
<td></td>
<td>Diamond (1964a, b)</td>
</tr>
<tr>
<td>Human kidney tubule</td>
<td>2.1</td>
<td>Smith (1951)</td>
</tr>
<tr>
<td>Rat kidney tubule</td>
<td>10.0</td>
<td>Smith (1951)</td>
</tr>
<tr>
<td>Aedes detritus rectum</td>
<td>1.3</td>
<td>Ramsay (1950)</td>
</tr>
<tr>
<td>Dilappus morosus rectum</td>
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<td>Ramsay (1955)</td>
</tr>
<tr>
<td>Coelopa frigida rectum</td>
<td>3.7</td>
<td>Sutcliffe (1960)</td>
</tr>
<tr>
<td>Ephydra riparia rectum</td>
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<td>Sutcliffe (1960)</td>
</tr>
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<tr>
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<tr>
<td>Tenebrio rectum</td>
<td>9.0</td>
<td>Ramsay (1964)</td>
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* These experiments did not determine whether an osmotic gradient occurred *in vivo*, but
they did show that these tissues could reabsorb water against an artificially induced osmotic
gradient.

points (perhaps induced by the epithelial cells) of the cuticular proteins continuously
alters their degree of hydration and provides the driving force for the system. It has
been suggested (Beament, 1965) that other insect systems which are lined with a
reflected cuticle (intima in the rectum) may also be able to transport water similarly.
In the rectum of the mealworm, *Tenebrio*, water can be resorbed from rectal contents
in equilibrium with an atmosphere of as low as 75% relative humidity (Ramsay, 1964).
Similarly, Phillips (1964a) found that the rectum of *Schistocerca gregaria* absorbed
water from an isotonic trehalose solution and thus in the absence of any apparent ion
movement. The rate of water absorption was found to be dependent on the osmotic
gradient between the haemolymph and rectal lumen. Water was resorbed at a rate of
17 μl/h/rectum in the absence of an osmotic gradient but fell to below zero against a
gradient exceeding 0.6 osmoles. It must be pointed out, however, that influx of ions
from the haemolymph or the epithelium down their concentration gradient, and their
subsequent reabsorption was not totally excluded. In fact Phillips observed a concomitant absorption of 1 molecule of K, Na or Cl for every 500 molecules of water, even when pure sugar solutions were injected into the rectal lumen. Taking the possible errors of estimation into account, the actual ratio of ion/water molecules could be much less. It is also significant that the water absorption occurred against a much greater osmotic gradient when ions were freely available in the lumen. Similar results were obtained for *Calliphora* (Phillips, 1961).

In contrast to the surface cuticle, the ability to reabsorb ions against steep electrochemical gradients is a distinct feature of the insect rectum (Phillips, 1964). Ion reabsorption takes place simultaneously with that of water. The rectal lumen *in vivo* receives urine which is almost continuously produced by the Malpighian tubules and which has a high content of inorganic ions (mainly KCl). It is not, therefore, immediately apparent how Beamant's model could be applicable to insect rectum, as the former completely excludes ions. Moreover, Phillips (1965) has recently shown that the cuticular intima of the rectum in *Schistocerca gregaria* acts as a molecular sieve with estimated water-filled pores of about 6.5 Å in diameter (compare Kümmel, 1956). It is freely permeable to both ions and water, but severely restricts the penetration of larger molecules, irrespective of their surface charges. Phillips therefore concludes that control of the observed absorptive processes in the rectum must lie in the underlying epithelium.

The basic problem of ion and water transport in the rectum of *Calliphora* and *Schistocerca* is not unlike that in vertebrate systems such as the gall bladder (Diamond, 1964, 1965; Dietschy, 1966), i.e. how to explain the absorption of several hundred molecules of water for every molecule of salt transported 'actively' across the epithelium. It now becomes of interest to examine two theories to explain what is called 'isotonic transport' in vertebrate systems and to compare the structural basis of these mechanisms with observations on the rectum of the blowfly.

**Double-membrane model.** This model was proposed by Curran (1960) and Durbin (1960) to explain fluid transport across the gastric mucosa of vertebrates. The model prescribes, in series, two 'membranes' or diffusion barriers with different reflexion coefficients. Thus the first membrane, 'α', has a restricted permeability to solutes but is more permeable to water, while the second membrane, 'β', is non-selective and freely permeable to both. If solute is actively transported across the first membrane into a confined space between the two membranes (Fig. 2A, '2') then the solute will exert a greater effective osmotic pressure across the fine-pored membrane 'α' than across the leaky membrane 'β'. Water thus crosses the first membrane by osmosis, builds up a high hydrostatic pressure in the confined space and is forced out of the coarse membrane under this pressure head. The hypothesis has been successfully tested in a completely artificial model by Curran & McIntosh (1962) and Ogilvie, McIntosh & Curran (1963). Extensive discussion of this model has been provided by Curran (1965), Whitlock & Wheeler (1964), Diamond (1964 a, b, 1965) and Durbin & Moody (1965). Detailed mathematical treatment of the analogue is given by Patlak, Goldstein & Hoffman (1963).

In seeking a structural analogue of the double-membrane model, Curran (1965)
Fig. 2. A, schematic representation of the double-membrane model after Curran (1960, 1965) to explain the movement of water coupled to the active transport of solute. Compartment '1' is the lumen of the organ, '2' intercellular spaces and '3' lymphatic and blood vessels. The selective membrane 'α' is represented either by the entire epithelial cell (Curran, 1960) or by only the lateral plasma membranes (Curran, 1965), while the non-selective 'leaky' membrane 'β' may be formed by any combination of diffusion barriers at the serosal aspect. In an artificial model made from a lucite cylinder (Curran & McIntosh, 1962) 'α' was a cellophane membrane and 'β' a sintered glass disk. At the beginning of the experiment compartment '2' contained 0·5 M NaCl solution to simulate 'active' transport. Solutions in compartment '1' varied from 0·1-0·5 M NaCl and in '3' from 0·02-0·5 M NaCl, and were stirred. Net volume flow occurred from compartment '1' to compartment '3'.

B, diagrammatic representation of the transposition of Curran's model on the rabbit gall bladder as described by Whitlock & Wheeler (1964) and Whitlock et al. (1965). 1, lumen; 2, intercellular spaces between the lateral boundaries; 3, lymphatic and blood vessels. 'Pumps' (p) for the active transport of NaCl are presumed to be localized on the lateral plasma membranes of the cells (membrane 'α') while the connective tissue, etc., on the serosal aspect constitute the barrier 'β'.

C, diagram representing Diamond's theory of local osmosis to explain the 'isotonic' transport of fluid in the rabbit gall bladder. As in Fig. 2B, the solute (NaCl) is believed to be actively pumped (p) into the lateral intercellular spaces, thus making them...
proposed that the selective barrier ('membrane α') will probably be represented by the basal (serosal) and/or lateral plasma membranes of the epithelial cells, while both the central compartment and the non-selective barrier ('membrane β') could be composed of any combination of spaces and diffusion barriers between the cells and the serosal aspects. A further discussion on the application of Curran's model in vertebrate intestine has been provided by Fordtran & Dietschy (1966).

The double-membrane model has, perhaps, been most rigorously tested in studies of rabbit gall bladder, both in vivo and in vitro (Whitlock & Wheeler, 1964; Dietschy, 1966). In this organ, bile in the lumen is concentrated by removal of salts (mainly NaCl) and water in isotonic proportions, frequently against a substantial osmotic gradient. The wall is composed of a single layer of epithelial cells which enclose intercellular spaces (Hayward, 1962; Johnson, McMinn & Birchenough, 1962). In general aspects, therefore, the gall bladder bears close resemblance to the rectum in insects where the excretory material is concentrated by the absorption of useful ions and water.

In the gall bladder of the rabbit, Whitlock & Wheeler (1964) suggested that the intercellular spaces between the loosely interdigitated lateral boundaries of the epithelial cells may act as compartment '2' in Curran's model (Fig. 2B). Subsequently they observed (Whitlock et al. 1965) that the intercellular spaces between the lateral boundaries appeared: (1) grossly distended (5 μm in width) in gall bladders transporting water in vivo, but (2) less dilated in vitro, (3) closed or inconsistently dilated when the lumen contained bile, and (4) closed when water transport was osmotically arrested in vitro (also see Diamond & Tormey, 1966). The changes observed in the rectum of Calliphora, in vivo, recorded in this paper are in close agreement with those in the gall bladder. In the latter material it has been proposed (Whitlock & Wheeler, 1964; Whitlock et al. 1965) that the lateral plasma membrane is the seat of an 'active' transport of solute (in this case NaCl), while the basement lamina and serosal tissue act as non-selective diffusion barriers. By employing a histochemical method at the electron-microscopical level, Kaye et al. (1966) found a high concentration of sodium: (1) in the intercellular space in the gall bladder in actively transporting state, but (2) on the cytoplasmic surface of the lateral membrane when the transport was blocked with ouabain. Heavy localization of adenosine triphosphatase (ATPase) has been demonstrated histochemically at the electron-microscopical level on the lateral boundaries (Kaye, Lane, Wheeler & Whitlock, 1965). Similar observations have been made on a

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D, schematic representation of the rectum in blowfly to explain the resorption from the lumen according to Curran's double-membrane model. Thick broken line traces the pathway of water from the lumen. Membrane 'α' is represented by the stacked infolds of the lateral plasma membranes, presumably the site of an 'active' transport of KCl into the narrow lumina of the sacs in the stacks (2). The non-selective barrier 'β' is constituted by the resistance to diffusion provided by the basement lamina, infundibular bridges, medullary tissue and medullary valve. Compartment '3' is the haemocoel. It is thought that the rectal epithelium is the site of passive diffusion between the lumen and the haemolymph.
number of other vertebrate tissues (Farquhar & Palade, 1964; Kaye & Pappas, 1965; Kaye & Donn, 1965).

Another tissue involved in the concentration of urine by an absorption of ions and water is the toad urinary bladder (Bentley, 1966). Intercellular spaces, which are shut off from the lumen, have also been described in this tissue by a number of workers (Choi, 1963, 1965; Peachey & Rasmussen, 1961), and an ATPase has been localized on the lateral boundaries (Bartoszewicz & Barrnett, 1964). Physiological aspects of water transport in the toad bladder have been discussed by Davson (1964), Leaf & Hays (1962) and Hays & Leaf (1962a, b). The possibility of applying the double-membrane hypothesis to this tissue has been considered by Patlak et al. (1963).

Local osmosis. In his recent studies on the gall bladder, Diamond (1964a, b, 1965) has argued that, while the double-membrane theory could explain the passive uptake of water by an active transport of ions, the final concentration of the absorbate will depend on a critical balance of the two barriers. He therefore proposes that 'isotonic' transport could only be achieved by creating local osmotic gradients within the tissue by actively secreting solutes. Water will then follow down the created gradient in isotonic proportions. Diamond (1965) also predicted that, in such transporting tissues, channels for the movement of solvent in response to the active transport of solutes must be different from those for a passive diffusion of solutes and solvent (see also Patlak et al. 1963). Recently, Diamond & Tormey (1966) have suggested that the active channels are situated on the lateral boundaries (Fig. 2c). It has been pointed out by Whitlock & Wheeler (1964) that local osmosis is only a special condition of Curran's model. At any rate the choice of one or other of the two theories is largely dependent upon critical information on the nature of the absorbate (Diamond, 1965). No such information is available so far on the rectum of insects.

The rectum of the blowfly as a structural analogue of Curran's model

From the observations reported in this communication, it seems, without doubt, that a change in the composition of the rectal contents is reflected in structural changes of the rectal papillae, particularly in the spatial arrangement of the lateral plasma membranes and their associated structures, as well as in the intercellular spaces. It will be reasonable to conclude, therefore, that during reabsorption, water is routed into the intercellular spaces. Fig. 2D is a highly simplified diagram summarizing the main findings on the fine structure of the rectum in Calliphora (Gupta & Berridge, 1966b). By comparison with the vertebrate systems discussed above, it will be seen that the double-membrane model can easily be applied to the structural organization of this organ. Thus, it may be postulated that the stacked infolds of the lateral membrane are the seats of an active transport of ions into the long narrow lumina of the sacs. The high osmotic concentration thus created draws water from the cells, and ultimately from the lumen. Since the stacks are resistant to expansion due to their characteristic geometrical arrangement (Fig. 1), a hydrostatic pressure will develop in the sacs and the absorbate will be forced through the basement membrane into the larger intercellular spaces. The last ultimately drain into the infundibular space which in turn opens into the haemocoel. The flow through the intercellular spaces can be restricted
by: their complex distribution in the tissue; the connective tissue medulla and infundibular bridges in the infundibular cavity; and by a connective tissue flap (medullary valve) at the opening of the infundibular space into the haemolymph (Fig. 2D). An appreciable hydrostatic pressure is therefore likely to be maintained in the larger spaces as well. Some of this pressure is perhaps translated into the volume change and is probably the reason for the dilatation of the larger spaces in the micrographs of the papillae when copious absorption of the fluid is to be expected; i.e. in normal flies and when isotonic and hypotonic solutions are injected into the lumen of starved flies. The sustained hydrostatic pressure will also maintain a flow of absorbate towards the haemolymph and may therefore be largely responsible for preventing a back-flow of haemolymph into the intercellular channels. Thus the lateral plasma membrane of the papillary epithelial cells may be compared with membrane ‘a’ of Curran's model. The non-selective barrier ‘β’ will then be constituted by the thin basement lamina, ‘tissue pressure’ of the cells (i.e. the structural resistance of the larger spaces to expansion), infundibular bridges and the medullary valve. The intermediate compartment, ‘2’, is formed by the narrow lumina of the sacs (Fig. 9). The histochemical localization of a magnesium- and pH-specific ATPase almost exclusively on the membranes in the stacks tends to support the choice of these areas as sites of a solute pump (Berridge & Gupta, 1967).

Since the membranes in the stacks are presumably secreting a solute over their entire surface facing the narrow lumina of the sacs, it is unlikely that the secretion could be diluted to isotonic proportions within the sacs before flowing into the larger spaces. Water will thus continue to flow even into the larger spaces. The final equilibration with the lumen may only occur in the infundibulum where both the hydrostatic pressure and the flow-drag are likely to be very low. Such an arrangement will maintain a range of osmotic concentrations in the intercellular spaces and allow a very large surface for the diffusion of water from the lumen.

As pointed out by Whitlock & Wheeler (1964) for the gall bladder, a basic objection to such a translation of the double-membrane hypothesis is that compartment ‘2’ is separated from compartment ‘1’ (lumen) by an intracelluar compartment. The same applies to rectal papillae (Fig. 2D), where compartment ‘2’ is adjacent to an intracellular compartment whose other boundaries face both the haemolymph and lumen. Consequently, instead of 3 compartments and 2 barriers (Fig. 2A), it would be more correct to include at least 4 compartments and 3 different barriers. However, Whitlock & Wheeler (1964) have suggested that, because of the presence of microvilli, the area of the luminal surface of the gall bladder is much greater than that of the basal surface; and, of course, this is also true for rectal papillae. Thus the intracellular compartment will communicate preferentially with the mucosal (compartment ‘1’) rather than the serosal (compartment ‘3’) environment and thus its osmolarity should be determined mainly by the composition of the mucosal fluid. The whole system can then be reduced to Curran's original model (1960) where membrane ‘a’ constituted the entire epithelial layer (mucosa). It has already been noted that on the basis of the surface areas the cells of the rectal papillae will tend to maintain a steady-state equilibrium with the larger intercellular spaces on the lateral side and with the rectal
contents (subcuticular space) on the luminal side. The surface exposed to the haemocoel is so small (0.5–2 % only) that the relative influence of the haemolymph osmolarity on the papillary cells is likely to be very low. Since the contents of the intercellular spaces will presumably be considerably hypertonic due to an extensive solute secretion, it may not be unreasonable to assume that the osmolarity of papillae cells is, for the most part, intermediate between the intercellular spaces and the rectal lumen and thus always slightly hypertonic to the rectal lumen, at least under conditions in vivo.

If the transport mechanisms in the rectum are to be explained according to the double-membrane theory as postulated above, the peculiar osmolarity of the cells and an extremely small surface area exposed to the haemocoel will greatly restrict the passive diffusion between the haemolymph and rectal contents across the papillae. The arrangement, therefore, will meet the requirements of theoretical predictions whereby the channels for active processes must be separated from those for passive diffusion. In the rectum of insects with papillae and pads, etc., the last may largely occur over the flattened rectal epithelium.

Source and transport of ions. If it is accepted that ions are continuously being pumped into the intercellular space and ultimately drained into the haemolymph to which the cells have a highly restricted access, the papillary epithelium may conceivably run out of solutes. It may, therefore, be appropriate to summarize briefly the parameters of the function of rectal papillae. The rectum of the blowfly (and perhaps of most other insects) may be conceived to have 4 different functional states: (1) in insects with free access to food and water, diuresis is high (Phillips, 1961, 1964c; Maddrell, 1963; Berridge, 1966), absorption of potassium and other ions is obligatory, and transported fluids are probably isotonic; (2) in insects with no access to water but with food available, diuresis is high (Maddrell, 1963; Berridge, 1965), absorption of both ions and water is obligatory, and maximum concentrations may be achieved in the rectal contents; (3) in fasting flies (periods of ovariole development in Calliphora), diuresis is very low, and absorption is in very small quantities only; and (4) in starved flies injected with ion-free solutions into the rectum. As long as the Malpighian tubules are excreting (conditions (1) to (3), i.e. under normal conditions) it is unlikely that the cells of the rectal papillae will face an ion-depleted medium in the lumen. However, when ion-free hypotonic media are injected into the ligated rectum of the flies (condition (4)) water will rush into the epithelium down a high osmotic gradient and ‘dilute’ the entire contents of the cells as well as the intercellular spaces. Such a phenomenon is probably responsible for a highly ‘diluted’ appearance of the papillary epithelium in the electron micrographs of such flies fixed soon after the injection. It is significant, therefore, that such papillae are apparently able to recover from the osmotic shock within a short time by ‘phagocytosing’ some electron-dense material from the haemolymph and releasing it into the intercellular spaces. By analogy with the perirectal space of the cryptonephric system in Tenebrio (Ramsay, 1964), it may be assumed that this material is some non-electrolyte of high onotic pressure (see Davson, 1964) which helps in restoring the osmotic pressure of the fluid in intercellular spaces and thus extracting excessive water from the cells. Moreover, according
Fine structure of rectal papillae in blowfly

to the theoretical predictions by Patlak et al. (1963) it will slow down a further flow of water across the epithelial cells.

The cells of the rectal epithelium on the other hand do not show any ill effects of such gross hypotonic exposures. It is extremely unlikely that these cells should be completely impermeable to water. They, however, seem to be structurally much better suited to allow a quick diffusion of water into the haemolymph. If this explanation were to be the correct one, it would provide further support to the idea that the channels for passive diffusion of water from the papillary epithelium into the haemolymph are highly restricted, and also that the water in this tissue can only be routed into the intercellular spaces down an osmotic gradient. The appearance of a non-electrolyte in the intercellular spaces in the event of acute hypotonicity and ion depletion suggests that the rectal lumen is the only major source of ions for the papillary epithelium under normal conditions. Since the predominant cation in the rectal lumen in vivo is potassium (being the predominant cation in the excretory fluid of Malpighian tubules) the proposed solute pump on the stacks probably secretes KCl. Very high concentrations of this salt (up to 2 M KCl) are also found in the lumen of the cryptonephric tubules of Tenebrio and are thought to be concerned with the absorption of water (Ramsay, 1964). It is known (Phillips, 1964b) that in Schistossigma the potassium from the rectal lumen is absorbed 10 times faster than sodium.

The secretion of potassium into the intercellular spaces in large quantities may be expected to cause a depletion of this cation in the cells. It will also draw free anions (probably chloride) from the cells down the electrochemical gradient. Obeying the law of electrical neutrality, free cations from the rectal lumen (perhaps also from the infundibular fluid and the haemolymph to some extent) will move into the cells to balance the fixed anion groups known to be present on cytoplasmic proteins (Davson, 1964). Such a mechanism alone may be responsible for an almost complete removal of all the ions from the rectal lumen. If ions are also available to the cells from the infundibulum or haemolymph, it might enable these papillary cells to pump more water than would be possible if ions originated solely from the rectal lumen. It is evident that the electrochemical potential difference between the haemolymph and rectal lumen measured by Phillips (1961, 1964a, b) may not reveal the actual electrochemical phenomena in rectal papillae.

Control of absorptive processes. It is known that the contents of the rectum may often be very dilute. In Calliphora Phillips (1961) noted that the urine excreted by ‘water-fed’ flies had an osmotic pressure only 17% of that of the haemolymph. Osmotic pressure measurements on the rectal fluid of flies of different ages have also indicated that rectal contents can frequently be very dilute even under normal conditions (unpublished observations). As Phillips (1961) points out, it is not clear whether this hypotonicity is due to a higher reabsorption of ions in proportion to water or only because of the dilution of urine by the water that has moved into the rectum from the crop. Although a large number of flies of different ages were studied during the present investigation, papillae cells were never found in a state of hydration similar to that shown after hypotonic injections. This would suggest that the insects are capable of exercising control over the concentration of urine discharged from the
rectum, possibly by regulating the passive permeability of the apical plasma membrane to water. Such a passive change in permeability of the rectum has already been indicated from studies on *Schistocerca* (Phillips, 1964c) and *Dysdercus fasciatus* (Berridge, 1965). If such control mechanisms prove to be of general occurrence in insects, it will represent a striking analogy with the collecting ducts of the vertebrate kidney (Milne, 1965) and the toad urinary bladder (Leaf & Hays, 1962; Bentley, 1966). Permeability of these vertebrate tissues is controlled by antidiuretic hormone, which seems to act by changing the diameter of the pores in the apical plasma membrane. If it is permitted to carry the analogy between insects and vertebrates further, it is possible that permeability of the apical membrane of papillae cells is controlled by a similar hormone-dependent mechanism. It has been suggested elsewhere (Gupta & Berridge, 1966b) that such an antidiuretic hormone might be released from the neurosecretory axons terminating in the medulla.

Permeability of the apical and lateral plasma membranes might also be varied by regulating the surface area available for diffusion. It is of interest to note that, under conditions of minimal fluid transport, the paired membranes of the leaflets and stacks become closely apposed. The enclosed extracellular channels in such cases are reduced to about 20 Å in width and are mostly filled with the material of the glyco-calyx. By applying the law of diffusion through narrow clefts (Pappenheimer, 1953) it can be calculated that, under such conditions, the diffusion of ions and water will be extremely restricted (see also Nicholls & Kuffler, 1964). Thus the effective surface area of the two membranes will be greatly reduced. Such a state of apposed membranes in fasting flies with free access to water may, therefore, represent a condition of 'closed pores'. It is not, however, understood how, if at all, such a condition is caused by the neurohormones. The possible role of the particulate subunits on the apical plasma membrane in this and other possible functions has already been discussed elsewhere (Gupta & Berridge, 1966a).

To summarize, the fine-structural studies of the rectum of *Calliphora* reported here strongly suggest that the ions and water absorbed in the organ are routed through a hitherto unsuspected system of extensive intercellular spaces in the rectal papillae (Gupta & Berridge, 1966b). The structural design favours the application of the double-membrane model of Curran (1960, 1965) to explain an apparently 'active' transport of water reported in the system (Phillips, 1961, 1964a), although the theory of local osmosis (Diamond, 1965) cannot be excluded on the present evidence alone. The functional interpretations of the fine structure, however, can only be, at best, conjectural. The postulated mechanism will remain to be proved by further experimental evidence. It is suggested that for further physiological studies on insect rectum containing structures like rectal papillae and pads, etc., it will be desirable to treat the organ as composed of two types of epithelia—the rectal and papillary epithelia—the latter capable of creating high osmotic gradients within itself. Thus the final solution as to the exact mechanism may depend on information on the absorbate present in the intercellular spaces.
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REFERENCES

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Fine structure of rectal papillae in blowfly


M. J. Berridge and B. L. Gupta


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ABBREVIATIONS

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<tr>
<td>apl</td>
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Fig. 3. A low-magnification field from the apical region of the cortical epithelium showing the cuticular intima (cut), infoldings of the apical plasma membrane to form leaflets (apl), dense and tortuous profile of apposed lateral plasma membranes (lpm) separating to form the boundaries of a large intercellular space (ics), and stacks (st) of infolded lateral plasma membrane (arrows) closely associated with large mitochondria (m). The cytoplasm also shows abundant ribosomal or polysomal profiles, microtubules and elements of rough and smooth endoplasmic reticulum. A tracheole (t), still enclosed in a process of a tracheoblast, in the intercellular space and profiles of 'coated vesicles' (pv) associated with the leaflets are also to be noted. The thicker regions (sd) of the apposed lateral boundaries have a septate desmosome type of configuration, while the thinner regions (zo) represent zonula occludens. × 25,000.

Figs. 4, 5. Details of the zonula occludens in Fig. 3 showing a typical 5-layered structure believed to arise from the fusion of 2-unit membranes. Such occluded zones alternate with septate desmosomes as the junctional complexes between the cells in the apical region. Transverse profile of a microtubule (mt) is also seen in Fig. 4.

Fig. 4, × 145,000; Fig. 5, × 104,000.
Fig. 6. A portion of the lateral cell boundaries through the main 'body' of the cortica epithelium from a normal (2-day-old) fly, showing highly dilated intercellular spaces (ics) with transparent contents and containing profiles of tracheoles (t). It is to be noted that the tracheoles seem to be 'naked' and are not surrounded by a cell membrane (double arrow). The lateral plasma membranes may either be closely apposed (asterisks) and studded with tiny desmosome-like densities (pdm), form occluded zones (zo), line the large intercellular spaces (ics), or be infolded (single arrows) to form sacs piled in stacks (st). × 27,000.

Fig. 7. A field corresponding to that in Fig. 6, from a starved fly but also representing the condition in fasting flies (3-4 days old), showing that the larger intercellular spaces are now completely obliterated. The lateral plasma membranes (arrows) are separated by a narrow space of 100-200 Å and tightly surround the tracheolar tubes (t). The membranes in the stacks (st) are unaffected in starved flies (compare Fig. 12 from a fasting fly). Notice the profile of a Golgi dictyosome (g); these are not common in this material. × 25,000.

Fig. 8. As in Figs. 6, 7, but from a freshly emerged imago used as control for the starvation experiment, showing microvillus-like projections of the cytoplasm into the intercellular spaces (ics). × 25,000.
Fig. 9. A field from the basal region of the papillae cells showing the junctional complexes at the lateral boundaries (Ip). In the portion terminating at the basal surface (bpm) the lateral boundaries are formed by a continuous collar of septate desmosomes (sd), followed by an occluded zone (zo). The rest of the lateral plasma membranes up to the junctional complexes in the apical region (Fig. 3) follow a course described in Fig. 6. Further to be noticed in this figure are profiles of dilated blind diverticula (bd) in communication with the lumina of the sacs (arrows) which form stacks (st) with an orderly arrangement incorporating large mitochondria (m). The cytoplasm reveals scattered profiles of ribosomes, microtubules (mt) and occasional dictyosomes (g). Only a small part of the basal lamina (blm) is seen on the basal surface that faces the haemolymph. × 57,500.
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Fig. 10. A low-magnification field from the cortex of the papillae showing the basement lamella (blm), with incorporated radial cells (rc), covering the basal plasma membrane (bpm) of the epithelial cells. Taken from a normal fly (2 days old) the micrograph shows the abundance of stacks (st) and the dilatation of the intercellular spaces (single arrows), including their blind diverticula (bd). The double arrows point to the opening of the sacs into larger intercellular spaces (ics). $\times 12500$.

Fig. 11. As in Fig. 10 but from a starved fly: all the intercellular spaces (single arrows) are now reduced to less than 200 Å in width. The complex distribution of spaces and their interconnexion is seen to better advantage in this state of the cells. Identical conditions are found in the fasting flies. $\times 23000$. 
Figs. 12, 13. Two corresponding fields from the papillary epithelial cells of fasting (Fig. 12) and normal flies (Fig. 13), showing the differences in the degree of dilatation of the larger intercellular spaces as well as the lumina of the sacs in the stacks. In Fig. 12, arrows indicate the sites where the membranes of the sacs have become tightly apposed (see Fig. 14). Fig. 13 also shows the stacks and the infolded nature of the component sacs (arrows) in greater detail. The general appearance of the mitochondria and the ground cytoplasm is also to be compared in these 2 figures. Fig. 12, ×35,000; Fig. 13, ×45,000.
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Figs. 14, 15. Small fields from Figs. 12 and 13 respectively, shown at higher magnifications to reveal the detailed structure of the membranes in the stacks. Arrows indicate the sites where the triple-layered unit-membrane configuration is seen to the best advantage. Asterisks mark the cytoplasmic regions alternating with the lumina of the sacs lined by the membranes. Fig. 14 also shows the details of the sites of tight apposition of the membranes marked by arrows in Fig. 12. At these sites the two membranes are separated by only a narrow space of $< 25 \text{ Å}$, but they retain their unit-membrane configuration. Fig. 14, $\times 250000$; Fig. 15, $\times 220000$. 
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Fig. 16. An area of the rectal papillae corresponding to Figs. 10 and 11, representing the condition of the epithelial cells in the flies dissected 20 min after injection of hypotonic media into the rectum. Note the gross distension of the intercellular spaces (ics) with transparent contents and apparently swollen mitochondria. The stacked infolds are largely unaffected and maintain their normal (compare Fig. 10) arrangement and spacing of the membranes. Part of a nucleus (n) and the glycogen particles (gl) in the cytoplasm of the epithelial cells, and the collagen fibres (col) in the basement lamella (blm) are also seen. × 12,500.
Fig. 17. A small portion of a field similar to that in Fig. 16 but taken from a fly dissected 30 min after hypotonic injection. Aggregates of highly dense material have now appeared between the laminae of the basement lamella. It seems that such aggregates are phagocytosed into the blind diverticulae of epithelial cells. Aggregates 1 to 3 may represent stages of the transfer of material through the laminae (bln) of the basement lamella to the cell surface. A confluence of the basal plasma membrane with the membrane of the blind diverticulae (4) could provide this material with access to the intercellular space (5) where it is finally dispersed as a flocculent material of moderate density (Fig. 18). $\times 16000$.

Fig. 18. As in Fig. 17, showing a large intercellular space (ics), with several profiles of tracheoles (t), filled with uniformly dispersed flocculent material (compare Fig. 16). A large dense body in the cytoplasm (asterisk) probably represents a cytolsome-like structure, while the smaller ones (mvb) are multivesicular bodies (Gupta & Berridge, 1966b). $\times 20000$. 
Figs. 19–22. Portions near to the apical surface of the rectal papillae showing the arrangement of apical leaflets under different conditions: Fig. 19, normal condition; Fig. 20, beginning of the fasting period; Fig. 21, fasting fly; and Fig. 22, starved condition. The subcuticular spaces (asterisks) show a variable distension under different conditions and are more or less completely obliterated in the fasting flies. Close association of the elements of endoplasmic reticulum (er) with the leaflets, profiles of 'coated' vesicles (pv), a dictyosome (g), and sectioned microtubules (mt) are also to be noticed. Fig. 19, × 31,000; Fig. 20, × 32,000; Fig. 21, × 28,000; Fig. 22, × 77,000.
Fig. 23. A small portion from Fig. 21 magnified to show the detailed structure of the leaflets in fasting flies. Like the stacked infolds of the lateral plasma membrane (Fig. 14), the infolded apical membrane forming the leaflets also becomes tightly apposed in places (arrows). The cytoplasmic surface of the apical membrane is covered by uniformly spaced and apparently stalked particulate subunits. \( \times 77000 \).

Fig. 24. A small field from Fig. 23 enlarged to reveal the unaffected unit-membrane structure (arrows), in spite of a very close proximity (separated by 25 Å) of the facing membranes, and the particulate coat on the cytoplasmic surface (asterisks). \( \times 170000 \).
Fig. 25. A low-magnification field from the rectal epithelium of a fasting fly, showing the general organization of the tissue. The rectal wall is thrown into villus-like folds separated by narrow channels (asterisks) lined with a thin basement lamina and presumably containing haemolymph. The apical membrane of the cells is contiguous with the cuticular intima (cut), thus almost completely obliterating the subcuticular space. The muscular coat is also visible (mus). \( \times 23000 \).

Fig. 26. A small area of the rectal epithelium from a fly injected with a hypotonic medium. The subcuticular space (scs) is now prominent. The rest of the structures are unaffected. The cells do not show any ‘dilution effect’ comparable to that in the epithelium of the papillae (Fig. 16). \( \times 30000 \).