A COAT OF REPEATING SUBUNITS ON THE CYTOPLASMIC SURFACE OF THE PLASMA MEMBRANE IN THE RECTAL PAPILLAE OF THE BLOWFLY, *CALLIPHORA ERYTHROCEPHALA* (MEIG.), STUDIED IN SITU BY ELECTRON MICROSCOPY

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INTRODUCTION

Structures associated with the rectum of insects, e.g., rectal papillae, rectal glands, rectal pads, are believed to be concerned with an active uptake of water and ions from rectal contents (1–7). In the blowfly, rectal papillae are 4 hollow cones with their tips projecting into the rectal wall and their bases facing the haemolymph. The wall of each cone is formed by a single layer of columnar epithelium, but the concavity is filled with tracheae and connective tissue cells containing many nerve endings (8, 9). Our studies have revealed that, in essence, the epithelial cells have the same organization that Copeland (10) described in the sodium-transporting anal papillae of a mosquito larva. However, the structures he designated as "mitochondrial pumps" are far more numerous and complex in this material and are associated with the lateral plasma membrane of the cells (11, 12). The apical surface (facing the lumen) of the papillae is lined by a cuticular intima, and the underlying plasma membrane of the epithelial cells is highly infolded to form "leaflets" (11). In all our electron micrographs, the cytoplasmic surface of this apical plasma mem-

brane was found to be studded with subunits of regular shape, dimension, and spatial arrangement.

It is now widely accepted that cellular membranes are lipoprotein structures, which, in the electron micrographs of suitably fixed and sectioned material, exhibit a triple-layered "unit membrane" configuration with a total width of about 75 A (13). In spite of this basic similarity

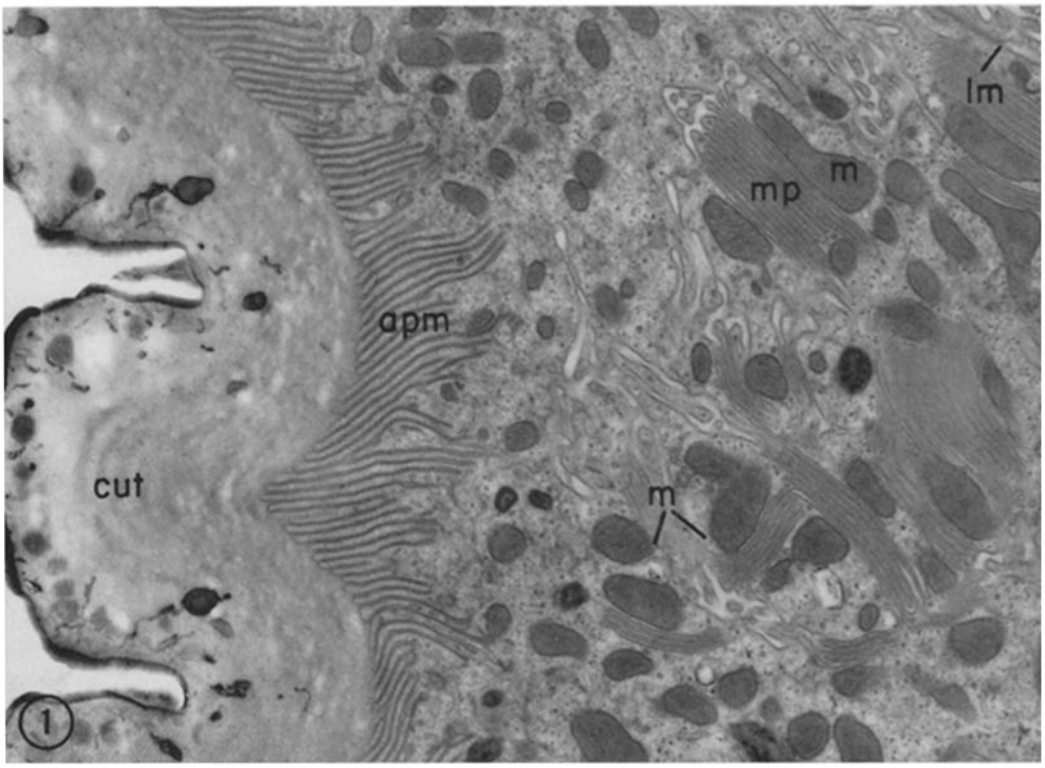


FIGURE 1 A low magnification field from the apical region of the rectal papilla cells, showing a cuticular intima (*cut*), apical leaflets (*apm*), mitochondrial pumps (*mp*), mitochondria (*m*), and a glimpse of the lateral plasma membrane (*lm*). × 20,000.

in their molecular architecture, cellular membranes possess a high degree of physiological specialization, such as selective absorption of nonelectrolytes and electrolytes, permeability, and energy-dependent transport processes. Various investigators have suggested that at least some of these properties may be determined by additional coats of macromolecules on one or both surfaces of the bimolecular leaflet.

The presence of an extraneous coat on the plasma membrane, first proposed by Chambers (14), has now been demonstrated by electron microscopy in a variety of systems (15–17). It is thought that this "glycocalyx" (16) is responsible for the selective filtration of molecules and also acts as a selective binding site for proteins (15–17) prior to their incorporation into the cells by micropinocytosis. Its function as a "carrier" in ion pumps has been suggested by Bennett (16) but excluded by Brandt (15). A number of workers have recently demonstrated the presence of micropinocytotic vesicles thought to be concerned with selective incorporation of proteins into cells (17–22). Such micropinocytotic vesicles are characterized by the presence of a coat, about 20 mμ wide, on the cytoplasmic surface of the plasma membrane. In the pericardial cells of an aphid, Bowers (21) has demonstrated that this coat of "bristles" (19) appears to be made up of closely

packed hexagonal subunits. Such particulate coats however, seem to be confined to the surface of pinocytotic vesicles and have not been observed on the rest of the plasma membrane.

Another category of particles, measuring 80 to 100 A in diameter and attached to the membrane of the mitochondrial cristae by "stalks" that are 30 to 50 A in length, has been widely observed in negatively stained preparations of isolated and osmotically disrupted mitochondria (23–26). However, the spatial relationship of such particles to the membrane (about 75 A) of the cristae in situ is not at all clear. Smith (25) has pointed out the difficulty of fitting such 130 to 150 A long particles into a 70 to 100 A wide intercristal space found in the sectioned sarcosomes of the blowfly. Such discrepancies have led some workers to suggest that either these "elementary particles" in mitochondria may be complete artifacts (27) or that they may somehow be fitted into the membranes in vivo. It was originally suggested that these elementary particles in mitochondria contain enzymes of the electron transfer system (24). It has also been proposed that other membranes in cells may contain such particulate packages (24). Recently, Benedetti and Emmelot (28) have reported similar but smaller (50 to 60 A) particles on the surface of negatively stained plasma membranes isolated from rat liver, and suggested tentatively that these particles may contain adenosinetriphosphatases.

METHODS

Rectum of the adult blowfly, *Calliphora erythrocephala* Meig., was fixed in an ice cold 2.5% solution of glutaraldehyde in 0.05 M sodium cacodylate buffer (29) which was made isosmotic with haemolymph by adding 0.15 M sucrose. Pieces of tissue were washed in ice cold buffer containing 0.3 M sucrose and postfixed in 1% OsO_4 in Veronal-acetate buffer. Subsequently, the material was dehydrated in an ascending series of ethanol and embedded in Araldite (30). Further detail on material and methods may be found elsewhere (9, 11). The blocks were sectioned with glass knives on a Huxley-Cambridge ultramicrotome, and sections showing silver and very light gold interference colors were picked up on collodion-coated grids; these were "stained" in a saturated solution of uranyl acetate in 50% ethanol, washed, and restained briefly in Reynold's lead citrate (31). For comparison, some sections were stained either in uranyl acetate or only in Reynold's lead. A thin layer of carbon was deposited on the backs of the grids. The sections were examined under a Philips EM 200 operated at 60 kv. The accuracy of measurements has been checked by comparing micrographs with those of a diffraction grating taken under identical conditions. Negatives made at an instrumental magnification of up to 40,000 were further enlarged photographically.

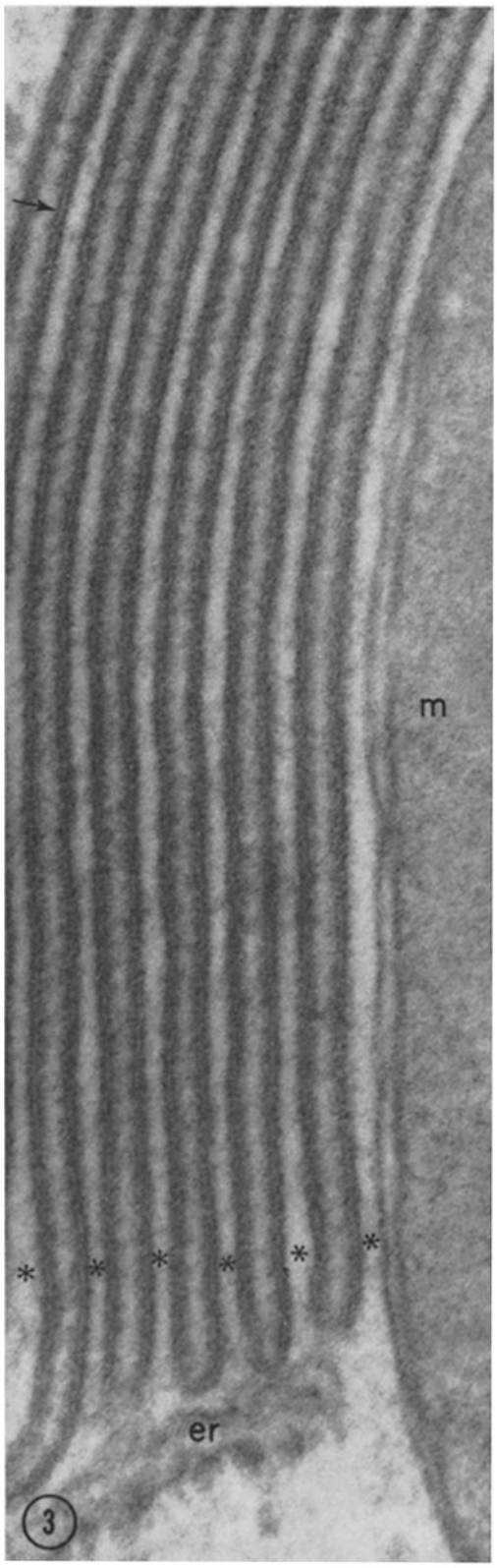
RESULTS

Fig. 1 is a survey micrograph of a portion of the apical part of the papilla cell showing the cuticular intima, apical leaflets, and a few mitochondrial pumps. The stacks of membranes (*mp*), which are intimately associated with mitochondria (*m*), arise as a complex infolding of the lateral membrane (*lm*) of the cells (9, 11). A magnesium- and pH-specific adenosinetriphosphatase has been localized on the stacked membranes of the mitochondrial pumps (12). No noticeable concentration of this enzyme was found on the membranes of the apical leaflets.

The leaflets are broad, sheetlike infoldings of the apical plasma membrane and do not have the discrete, circular profiles of the microvilli found on other excretory and absorptive surfaces. It has been shown elsewhere that the subcuticular space enclosed by the membrane of the leaflets varies in the degree of dilatation that is related to the change in the rectal contents (11). However, the cytoplasmic layer separating the two membranes has a fairly constant width of about 200 to 300 A. At suitable magnifications, a continuous and un-

FIGURE 2 A field showing a few apical leaflets with unit membranes uniformly covered on the cytoplasmic side (asterisks) by a regular array of particulate subunits (arrows), and a mitochondrion (*m*). × 150,000.

FIGURE 3 A part of the stacked, infolded lateral membrane forming the mitochondrial pump, to show the absence of surface subunits. Asterisks mark the cytoplasm; *er*, cisterna of rough endoplasmic reticulum; and *m*, a small portion of a mitochondrion. Arrow indicates the site where the three layers of unit membrane are best visible. × 175,000.



interrupted plasma membrane, 75 to 80 A in width, can be followed through large fields of the leaflets. In section, the plasma membrane exhibits a well defined tripartite structure with two dense lines 25 A wide separated by a clear space 25 to 30 A wide (Figs. 2 and 4). These values are in close agreement with those given for a unit membrane in other cells (13). The outer surface of these membranes (i.e. the surface facing the subcuticular space) is covered by very loose, feltlike material composed of thin filaments of moderate electron opacity (Figs. 2 and 4). This is probably comparable to the glycoprotein coat on the surface of membranes in other systems (15–17, 32).

On the cytoplasmic side, the apical plasma membrane is covered by a layer of very uniform width (about 150 A), which is further resolved into particulate subunits or densities. Fig. 2 shows, in profile, parts of several membrane pairs (leaflets) covered with uniformly spaced subunits. The center-to-center spacing for these subunits is about 150 A. Although the exact shape of these subunits is difficult to determine from the material in hand, each of them appears to have at least in places a polygonal head attached to the inner leaflet of the unit membrane by a thick stalk. The stalk seems to be about 30 to 50 A long and of indeterminate width. The possibility is not excluded that the subunits may in fact be dumb-bell-shaped structures. In any case, a constriction generally demarcates the "head" from the "base" attached to the membrane. There is a further suggestion that each head may be composed of 6 or 7 subparticles about 50 A wide. It should be borne in mind, however, that some of these values may be spurious, because a section 500 to 800 A thick could accommodate profiles of several particles overlapping each other. In the fields where the membranes have been cut tangentially, thus offering a surface view of the particulate sub-

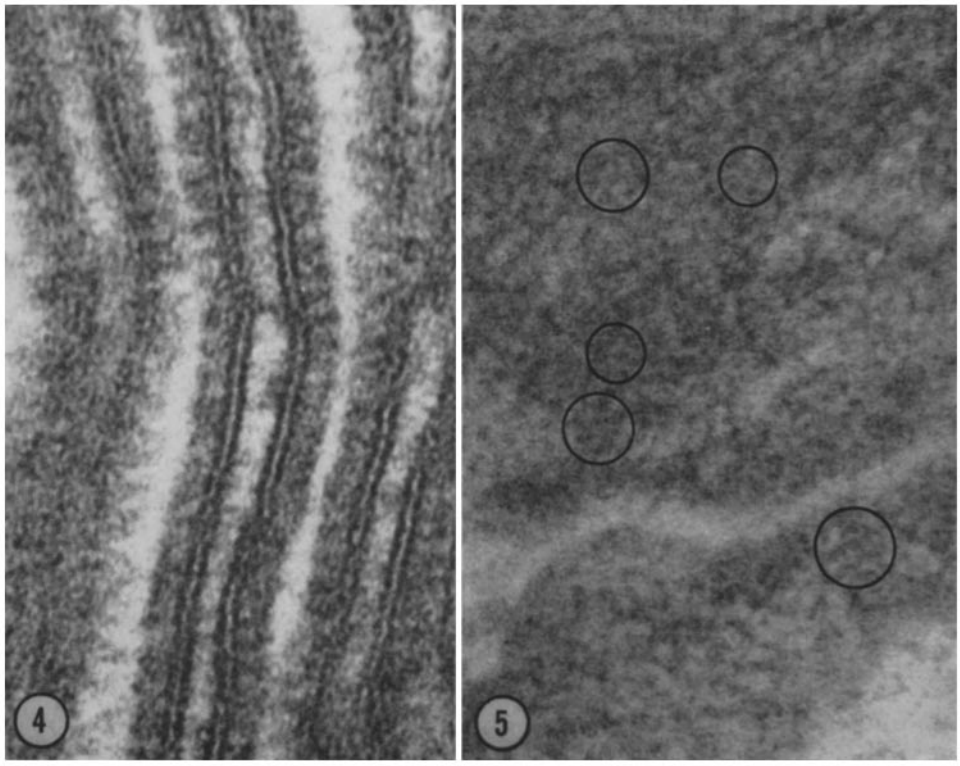


FIGURE 4 A small field from Fig. 2, shown at higher magnification. × 300,000.

FIGURE 5 A portion of a tangential section of the apical membranes, presenting a surface view of the subunits, to show the possibility of a polygonal or hexagonal array (circles). × 175,000.

units, there is some suggestion that the latter may be packed in a two dimensional polygonal array (Fig. 5). While these subunits are seen to the best advantage in "double-stained" sections, they seem to have a much greater affinity for uranyl acetate than for Reynold's lead.

In the present material, these particles are confined to the apical plasma membrane of the cells and have not been observed on any other membrane. As an example, Fig. 3 shows a part of the infolded lateral plasma membrane at a comparable magnification; the component layers of the unit membrane have the same values as given above for the apical membrane (i.e. 25-25-25 A). There is a suggestion of a coat of feltlike material on both surfaces of these membranes, but there is no indication of any particulate array. Fig. 3 also illustrates the close association between the stacked lateral plasma membrane and mitochondria (*m*) which forms Copeland's (10) so called mitochondrial pump.

DISCUSSION

In many respects the particulate array on the cytoplasmic surface of the apical plasma membranes reported here appears to be very similar to the particulate coat on the cytoplasmic surface of the micropinocytotic vesicles in the pericardial cells of an aphid (21). A more commonly reported occurrence of such "coated vesicles" is in the developing oocytes of insects (18–20, 22), where they are believed to be involved in selective uptake of ovoproteins from the haemolymph. Similar micropinocytotic vesicles are also found associated with the particle-studded membranes in the present instance. However, a selective absorption of proteins or other large molecules has negligible significance in what is known about the function of rectal papillae. Moreover, the present particulate array differs from that on the coated vesicles in that its distribution is over the entire cytoplasmic surface of the infolded apical membrane. Since nothing is known about the biochemical nature of such subunits, it may be relevant to speculate summarily on their functional significance in the present material.

In a functional consideration of the fine structure of rectal papillae, we have pointed out elsewhere (11) that, in addition to the aforementioned pinocytosis, at least three physiological properties, not mutually exclusive, may be localized in the apical membranes of the epithelial cells in these organs: (*a*) secretion of hydrogen ions either alone or as part of (*b*) an ion exchange pump and (*c*) an energy expending ion and/or water transport system. The particulate array on these membranes may be responsible for any or all of these postulated functions. These possibilities will be considered briefly.

The rectal content of the blowfly, as in many other insects, can be extremely acidic (33), and this acidity is presumably achieved by an active secretion of hydrogen ions, possibly as part of an exchange diffusion mechanism for the uptake of potassium. There is some evidence that insects may possess an ion exchange pump which utilizes hydrogen ions instead of sodium (34, 35). Carbonic anhydrase is known to play an important part in providing hydrogen ions to acidify urine in vertebrate kidney (36), and this enzyme may be located in the particulate subunits. Alternatively, hydrogen secretion may be coupled to an electron transport system as is believed to occur in bacteria (37), in which case the particulate subunits contain components of electron and hydrogen transport systems.

In a postulate (11), we have tried to explain the reported absorption of water and ions from the rectum of these insects by an active secretion of ions by the mitochondrial-associated membrane stacks localized on the lateral plasma membrane. On the basis of present evidence alone, however, an active (energy-dependent) movement of water and/or ions at the apical membrane, particularly under extreme conditions, is not totally excluded. Phillips (3–5, 38) and Beament (39) postulate an "active transport of water" in the rectum of insects, and, if such a process exists, it is presumably localized on the apical plasma membrane. A sustained source of energy may thus be required to drive an ion exchange and/or a water pump. Unlike the stacked lateral membranes, the apical leaflets do not show any persistent association with mitochondria. It is conceivable, therefore, that on these membranes the particulate subunits are the seats of complex enzyme systems, generating energy for transport processes. Extramitochondrial electron transport systems have been reported in vertebrate cells (40), and their existence in insects is now generally accepted (41). Indeed, Siekevitz (42) has proposed that the energy generated by such a system may be coupled directly to transport processes without the intermediation of ATP. If a supply of energy is the motive, the alternative

possibility that these particles contain glycolytic enzymes may also be considered, as Fernández-Morán et al. (24) proposed for the particles they observed on the membrane of red blood cells in situ.

A revelation of the true significance of this regular array of particulate subunits on the plasma membrane must await further analysis. There is some evidence (Dr. David S. Smith, personal communication) that similar particulate coats exist on the cytoplasmic surface of cellular membranes in other insect systems, where the presence of energy-expending processes may be suspected.

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