THE PERMEABILITY OF DRAGONFLY MALPIGHIAN TUBULE CELLS TO PROTEIN USING HORSERADISH PEROXIDASE AS A TRACER

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Theoretical models developed to explain the coupled solute-water transport in various absorptive epithelia consider the movement of water to be a passive process produced by the localized secretion of ions into channels associated with the epithelium (cf. 6, 9–13, 20, 28). This standing gradient hypothesis has been extended to Malpighian tubules of insects (7, 14). In these particular cells, osmotic gradients are thought to be established within extracellular compartments associated with basal infoldings of the plasma membrane as well as between microvilli on the lumenal surface of these cells (7, 11–14, 17). Essential to the validity of the standing gradient hypothesis is that high molecular weight substances such as blood proteins be prevented from accumulating within

the channels formed by the basal infoldings of the Malpighian tubule cells (7). The permeability of the Malpighian tubule to solutes and water has been the subject of many physiological studies (cf. 4, 5, 7 for review). However, little has been done to demonstrate morphologically the diffusion barrier to proteins inferred by physiologists, largely because of the lack of adequate electron-opaque tracers which are of small enough dimensions and which can be localized precisely enough to reflect physiological events. Horseradish peroxidase (HRP) with a molecular weight of approximately 40,000 permits the localization, movement, and permeability of cells to protein with rather precise localization (19). For this reason, it was felt of interest to determine if an exogenous protein such as HRP when injected into the hemocoel fluid could gain entrance into the extracellular spaces formed by the basal plasma membrane infoldings of the Malpighian tubule cells and, if so, to further determine if this protein could be transported across the plasma membrane and could accumulate within the cytoplasm.

MATERIALS AND METHODS

Female dragonflies, *Libellula pulchella*, were collected in nature during the summer months near Iowa City. Shortly after their capture, a 3.25% solution of horseradish peroxidase (Sigma Chemical Co., St. Louis, Mo., Type II) prepared in insect Ringer's was injected into the hemocoel in quantities ranging from 0.05 to 0.1 ml, depending upon the size of the animal. The dragonflies remained viable and active subsequent to their injection with the peroxidase. At 15-, 30-, and 45-min intervals, portions of the proximal, intermediate, and distal regions of the Malpighian tubules were transferred to a fixative consisting of either 3% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) or a modified version of the glutaraldehyde–paraformaldehyde fixative described by Karnovsky (15, 18, 19) prepared with 0.1 M sodium cacodylate-HCl buffer (pH 7.4). Fixation for a 2 hr period was carried out in the cold (4°C) for the first fixative indicated, whereas fixation occurred at room temperature in the second case. Short segments of the fixed tubules were washed in phosphate buffer (three changes) overnight at 4°C, and the tissue slices were then incubated for 10–30 min at 37°C in freshly prepared Karnovsky medium containing diaminobenzidine and hydrogen peroxide (19). After incubation, the slices of the Malpighian tubules were again rinsed in phosphate buffer and subsequently postfixed for 1 hr in 1% osmium tetroxide buffered at pH 7.4 with 0.1 M phosphate buffer. The tissue was then dehydrated in ethanol and propylene oxide and embedded in Epon 812 (23). Thin sections on naked copper grids (400 mesh) were examined and photographed without further staining or after staining with uranyl acetate (31) and lead citrate (27), in an RCA EMU-3G electron microscope.

OBSERVATIONS

The cytoarchitectural organization of Malpighian tubule cells has been described in a wide variety of insects (1–3, 8, 16, 24–26, 29, 30, 32, 33). In general, the basic features of the structure of these cells is so similar that a complete morphological description of them in the dragonfly is unnecessary. Rather, the preliminary observations reported here will deal only with the distribution of the horseradish peroxidase. The basal portion of a Malpighian tubule cell is illustrated in Fig. 1. The black deposits which represent the reaction product indicate the location of the protein after a 30-min in vivo exposure to horseradish peroxidase. From this figure, it is apparent that the protein fills the basal lamella surrounding the cells so that the structure of the lamella is completely obscured. Further, all of the narrow extracellular spaces formed by the highly invaginated plasma membrane in this region of the cell are filled with the reaction product. In some areas, localized expansions of the plasma membrane extend into the adjacent cytoplasm. These specialized areas of the invaginated plasma membrane also contain reaction product, as do a number of small and apparently isolated vesicles in the cytoplasm. These features suggest that the horseradish peroxidase is incorporated into the cell by the formation of micropinocytotic vesicles associated with the invaginated plasma membrane. Larger vesicles located farther in the interior of the cytoplasm also contain the reaction product, in which location the reaction product appears to constitute a dense rim around the periphery of the vesicles. The distribution of HRP such as illustrated in Fig. 1 was found in cells comprising the proximal, distal, and intermediate portions of the tubule.

Berridge and Oschman (7) have suggested that the basement lamella associated with the basal infoldings of the plasma membrane in Malpighian tubules of *Calliphora* is important in preventing the accumulation of such substances as proteins within these channels which might interfere with the mechanisms suggested for the standing osmotic gradient hypothesis. In their studies on the Mal-

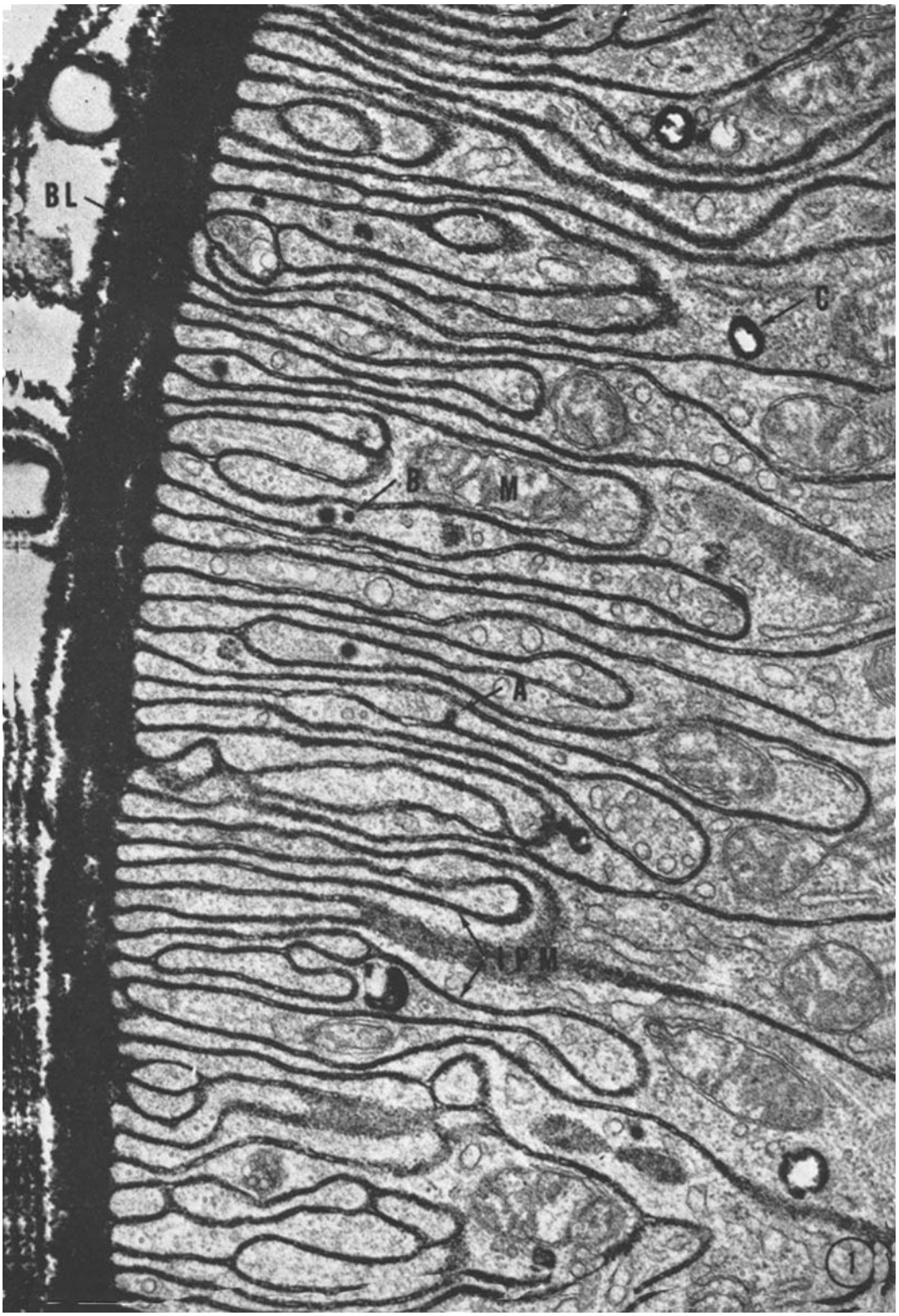

FIGURE 1 Electron micrograph illustrates the basal portion of a Malpighian tubule cell from a dragonfly injected with horseradish peroxidase. The reaction product fills the basal lamella (*BL*) and is present in the extracellular spaces formed by the highly invaginated plasma membrane (*IPM*). Expanded portions of the invaginated plasma membrane are illustrated at (*A*), and isolated vesicles of different size in the cytoplasm at (*B*) and (*C*), all of which contain reaction product. Mitochondrion (*M*). × 31,500.

pighian tubules of *Calliphora*, the authors indicate that the basal infoldings remain clear and do not concentrate protein. Further, they indicate that there is no evidence of extensive macromolecular uptake (micropinocytosis) from the channels into the cells. Locke and Collins (21, 22) found that many tissues of *Calpodes* were capable of taking up HRP when this tracer protein was injected into the blood, but the Malpighian tubules consistently failed to take up the tracer protein. This observation led Berridge and Oschman (7) to suggest that the basement lamella surrounding the Malpighian tubules has different properties than other basement lamellae in insects and prevents the tracer molecule from entering the spaces formed by the infoldings of the plasma membrane in this region of the cell. Thus, the plasma membrane elaborations associated with the lumen and basal portions of the Malpighian tubule cells not only provide amplification of surface area, but produce compartments where local osmotic gradients can be established (cf. 7). The distribution of HRP in the dragonfly Malpighian tubule demonstrates that exogenous protein of the size represented by the horseradish peroxidase is capable of traversing the basal lamella of the Malpighian tubule cells and thereby gains access to the extracellular compartments formed by the extensively invaginated plasma membrane. The results further demonstrate that the protein can be incorporated into the cytoplasm of the cells by a process of micropinocytosis. Fusion of individual micropinocytotic vesicles probably results in the formation of larger vesicles which also contain reaction product. The latter are always located in more internal regions of the cytoplasm. These results, as such, do not contribute information toward the validity of the standing gradient hypothesis proposed for Malpighian tubules in insects.

It would now be of interest to determine the specific concentration of horseradish peroxidase that would be required in the hemocoel fluid to enable this protein to become localized within the various structural areas of the Malpighian tubule cell described. It is obvious that the horseradish peroxidase not only represents foreign protein for this insect, but is probably present in the hemocoel fluid in quantities not normally experienced under routine physiological conditions. It would be of further interest to determine, under conditions in which HRP is located in the extracellular compartments, if the Malpighian tubule is capable of producing urine.

This investigation was supported by a research grant from the National Institutes of Health, United States Public Health Service.

Received for publication 5 March 1970.

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