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The fate of the luminal asymmetric unit membrane of the superficial cell of the rat transitional epithelium

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Summary. The fate of the luminal asymmetric unit membrane (AUM) of the superficial cell of the transitional epithelium of the rat urinary bladder was electron microscopically and cytochemically investigated using exogenously administered horseradish peroxidase (HRP) as tracer. HRP-positive discoidal vesicles were formed by the folding of the AUM of the luminal surface plasma membrane. With the passage of time, these vesicles changed their shapes and were finally transformed into lysosomes by the following possible routes: 1) by becoming multivesicular bodies (MVBs); 2) by directly fusing with lysosomes; and 3) by becoming autophagic vacuoles. Another possibility would be reutilization.

Key words: Asymmetric unit membrane, Transitional epithelium, Discoidal vesicles, Horseradish peroxidase, Rat

Introduction

The superficial cells of the transitional epithelium of the mammalian urinary bladder are characterized by their luminal thick asymmetric unit membrane (AUM), which shares the same ultrastructural features with the limiting membrane of the discoidal vesicles in the cytoplasm (Hicks, 1965, 1966; Porter et al., 1965, 1967; Staehelin et al., 1972; Severs and Hicks, 1979; Seguchi et al., 1980; Seguchi, 1982). When the bladder contracts, the surface area of the luminal plasma membrane decreases by the pinching off of adjacent plaque regions, and the excess membrane enters the cytoplasm in the form of discoidal vesicles (Hicks, 1965; Porter et al., 1965, 1967; Noack et al., 1975; Minsky and Chlapowski, 1978). During the expansion-contraction cycle of the urinary bladder, 85-94% of the luminal AUM of the superficial cells has been reported to be translocated into and out of the cytoplasm in the form of discoidal vesicles (Minsky and Chlapowski, 1978). This specialized unit membrane seems to derive from the thickened membrane of Golgi lamellae which ultimately fuses with the luminal surface after travelling through the cytoplasm in the form of discoidal vesicles (Hicks, 1966; Severs and Hicks, 1977, 1979; Alroy et al., 1982). Therefore, it has been suggested that the Golgi complex is the critical site for the morphogenesis of the AUM. The fate of the luminal AUM after being internalized in the form of discoidal vesicles is, however, still poorly understood. The present study was designed to clarify this point with the aid of ultracytochemistry and the use of HRP as tracer.

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Materials and methods

Preparation of tissues

Adult male Sprague-Dawley rats, weighing 200-300 g, were employed. The animals were first stimulated to urinate, and then, under ether anaesthesia, an abdominal incision of 1 cm in length was made from which 1 ml of 1% HRP (type II, Sigma Chemical Co., St. Louis, MO, USA) in 0.85% NaCl solution was injected from the bottom into the empty bladder, and the incision was closed. The animals were perfused through the heart, under sodium pentobarbital anaesthesia, with 0.85% NaCl physiological saline followed by 2% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4, for 10 min, at 15, 30 min, 1, 2, 4, 6, and 8 hr after the administration of HRP. The bladder was excised and fixed in the same fixative for 1 hr at 0-4 °C. Non-frozen tissue slices of 40 μm in thickness were cut on a Microslicer (Dosaka EM Co., Kyoto, Japan) and used for the cytochemical study. In addition, several non-treated bladders were processed for conventional electron microscopy.

All the tissue slices used in the present study were postfixed with 1% OsO_4 in 0.1M cacodylate buffer, pH 7.4, for 1 hr at room temperature, dehydrated in a series of graded ethanols and subsequently embedded in

Spurr's epoxy resin (Spurr, 1969). Ultrathin sections were stained with uranyl acetate and lead citrate. The specimens were examined in a JEM-1200 EX electron microscope (JEOL Co., Tokyo, Japan) operated at 80 kV.

Horseradish peroxidase (HRP) detection

The tissue slices were first treated with 2% H_2O_2 for 10 min in order to inhibit endogenous peroxidase, according to the method of Fahimi (Fahimi, 1970), and then incubated for 1 hr at room temperature in a medium containing 0.1% 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Wako Pure Chemical Industries Ltd., Osaka, Japan) and 0.01% H_2O_2 in 0.05M Tris-HCl buffer, pH 7.6 (Graham and Karnovsky, 1966). As control, tissue slices from bladders that did not receive HRP were incubated in the full medium, and tissue slices from bladders that received HRP were incubated in the medium without H_2O_2, or without DAB. After the incubation step the slices were washed several times with Tris-HCl buffer, pH 7.6, and processed as indicated above.

Results

Many of the multivesicular bodies (MVBs) in the superficial cells were clearly seen to be limited by an AUM of 12 nm in thickness (Fig. 1). In addition, forming and formed autophagosomes with AUM were also observed (Fig. 2).

In the specimens resected 15 min after the HRP injection, the tracer was distributed on the free surface of the superficial cells. Some DAB reaction was also detected in the infoldings of the plasma membrane that extended deep into the cytoplasm, from which discoidal vesicles were beginning to form (Fig. 3). However, no positive structures were found in the cytoplasm.

At 30 min after the HRP injection, discoidal vesicles containing HRP were observed in the cytoplasm beneath the luminal plasma membrane (Fig. 4). Some of these vesicles were seen to fuse with each other.

In the specimens taken at 1, and 2 hours after the administration of HRP, the discoidal vesicles with tracer had moved to deeper portions of the cytoplasm (Fig. 5). Some of them were enlarged and rounded and inclusion vesicles appeared in their lumen, as if they were beginning to transform into MVBs. Some others got access to lysosomes (Fig. 6).

After 4 hr, some HRP-positive vesicular structures fused with primary or secondary lysosomes (Fig. 7).

After 6 hr, a number of lysosomes containing HRP were observed in the basal cytoplasm, but forming MVBs with HRP labelling were still present near the luminal surface (Figs. 8, 9). MVB-like structures with HRP were clearly seen fusing with other, non-labelled, MVBs (Fig. 10). Some HRP-positive discoidal vesicles, on the other hand, acquired a horse-shoe contour, and wrapped around other organelles, becoming autophagosomes (Figs. 11, 12).

Moreover, at 6 and 8 hr after the injection of HRP,

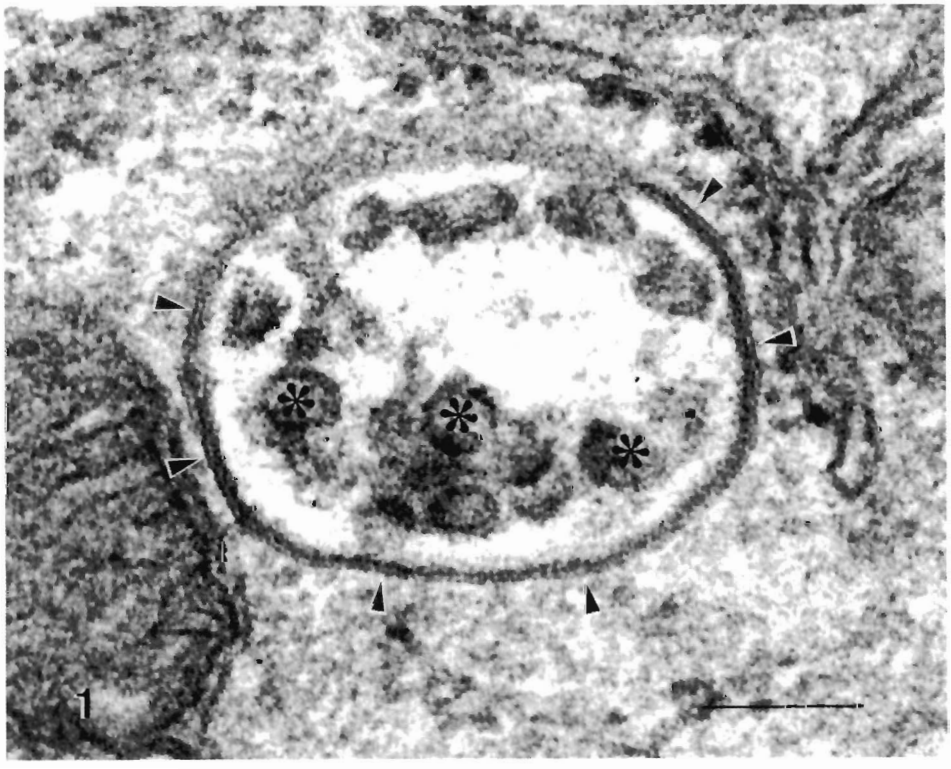


Fig. 1. This high magnification view of a MVB with inclusion vesicles (*), taken by conventional electron microscopy, clearly shows that the MVB is limited by AUM (arrowheads). Bar= 0.1 µm. x 210,000

some positively-labelled discoidal vesicles were again found near the luminal plasma membrane and, occasionally, were seen opening into the lumen of the urinary bladder (Fig. 13). HRP deposition was, however, no longer detectable on the luminal surface of the superficial cells.

The control specimens were completely devoid of HRP labelling (Fig. 14).

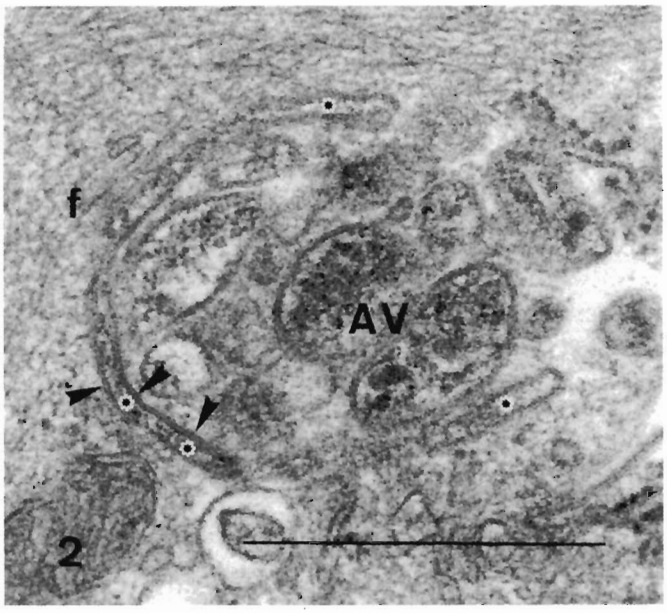


Fig. 2. Electron micrograph of an autophagic vacuole (AV) from normal tissue. The envelope of the forming AV consists of double-membrane AUM (arrowheads), indicating its luminal AUM or discoidal vesicle origin. Lumen of the AV (*); filaments (f). Bar= 0.5 µm. x 96,000

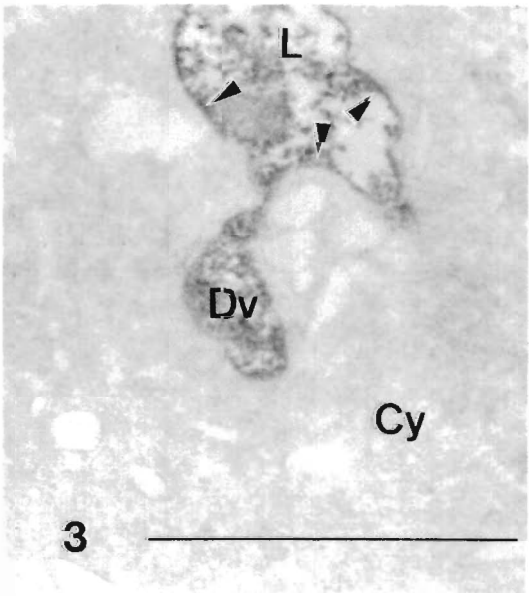
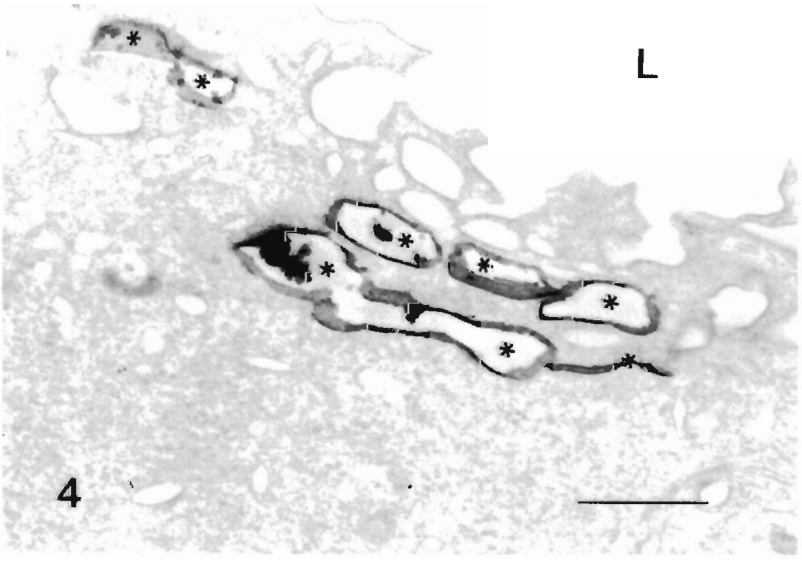


Fig. 3. Superficial cells after 15 min of HRP administration. Positive labelling is found on the luminal surface (arrowheads) and within the forming discoidal vesicles (Dv). Cytoplasm (Cy). Lumen (L). Bar= 1.0 µm. x 49,000



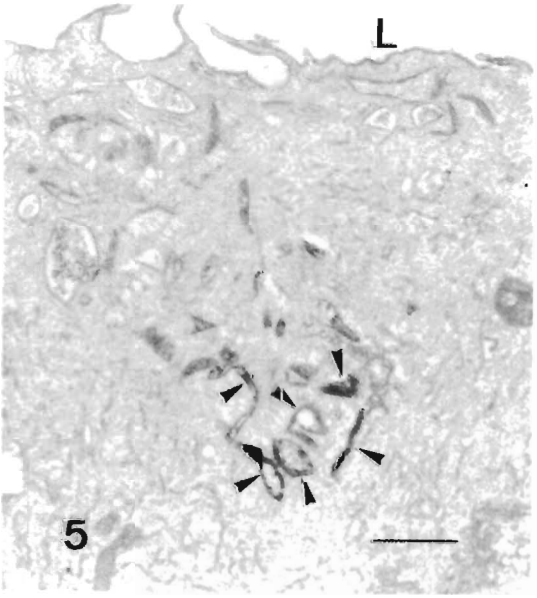


Fig. 4. The superficial cells after 30 min of HRP administration. Discoidal vesicles with attached HRP (*) are seen in the cytoplasm. Lumen (L). Bar= 1.0 µm. x 17,000

Fig. 5. Superficial cells after 1 hr of HRP administration. HRP-positive discoidal vesicles (arrowheads) are located in deeper regions of the cell. Lumen (L). Bar= 1.0 µm. x 11,000

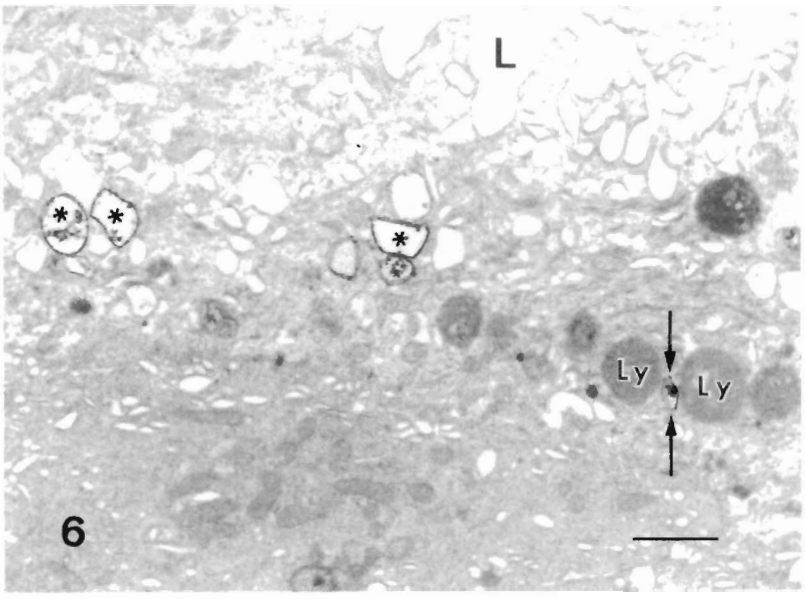


Fig. 6. Superficial cells after 2 hr of HRP administration. Some of the discoidal vesicles with HRP (*) are enlarged and some of them (arrows) can be seen close to lysosomes (Ly). Lumen (L). Bar= 1.0 µm. x 11,000

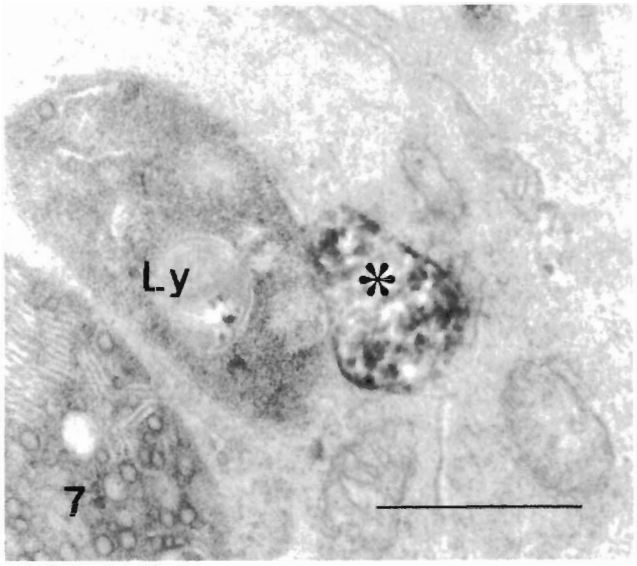


Fig. 7. Superficial cells after 4 hr of HRP administration. HRP-labelled vacuoles (*) are seen fusing with lysosomes (Ly). Bar= 0.5 µm. x 54,000

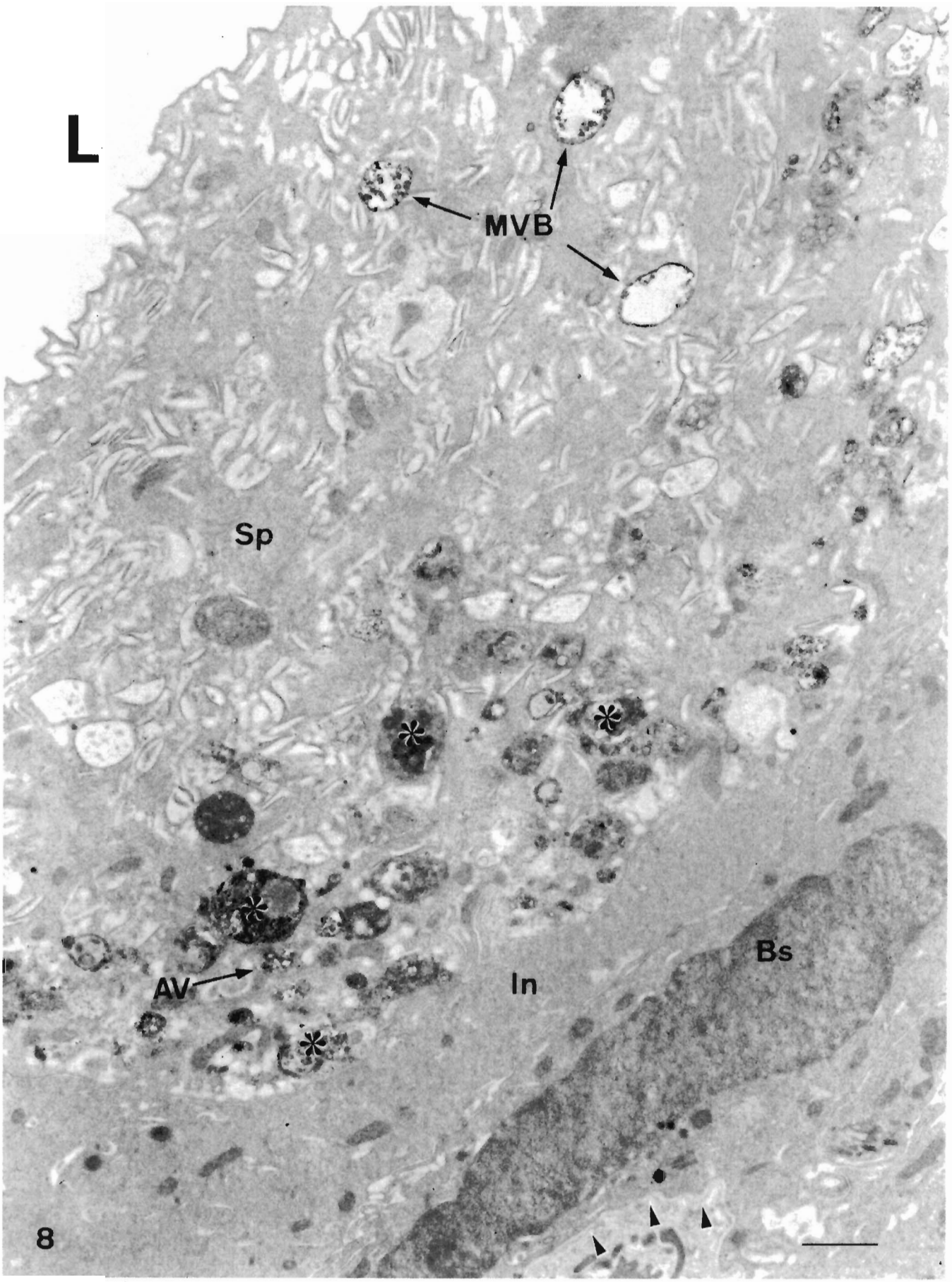
Discussion

Exogenously-administered HRP has been previously reported to appear in some MVBs but not in the discoidal vesicles (Amano et al., 1991). In contrast to the findings of these authors, we have observed that HRP entered the cytoplasm along with the AUM of the luminal surface of the superficial cells, which was internalized in the form of discoidal vesicles derived from the infoldings of the plasma membrane. The discrepancy between the present findings and the observations of Amano et al. may be due to the different methods of administering HRP. Moreover, the HRP employed in the present study was type II which is much more active than type I (presumably used in the study of Amano et al.) and remained easily on the luminal surface during excision and fixation.

The discoidal vesicles marked by HRP fused with each other and became larger in size. Some of them reached deeper portions of the cytoplasm and gradually transformed into MVBs, as indicated by the fact that the limiting membrane of many MVBs was identical with the luminal AUM. This is consistent with the mechanism proposed by Hicks (Hicks, 1966).

We observed that this kind of MVB finally fused with lysosomes. As in other cell types (Nilsson et al., 1989), the formation of MVBs from luminal AUM through the discoidal vesicle stage, and their fusion with lysosomes, suggest the final destination of the AUM to the lysosomes for degradation. In a previous experiment we noted that MVBs fused with lysosomes positive for acid phosphatase (Zhang et al., 1992). With other authors we share the view that the MVBs might be considered as a kind of endosome or prelysosome which become secondary lysosomes after receiving lysosomal enzymes (Jost-Vu et al., 1986; Gruenberg and Howell, 1989). These observations enable us to suggest that this may be one of the possible ways adopted by the superficial cells

Fig. 8. After 6 hr, most of the HRP is localized in the basal part of the superficial cells (Sp), especially in the lysosomes (*), multivesicular bodies (MVB) and autophagocytic vacuoles (AV). The intermediate (In) and basal (Bs) cells show no HRP labelling. Arrowheads indicate the basement membrane. Lumen (L). Bar= 1.0 µm. x 14,000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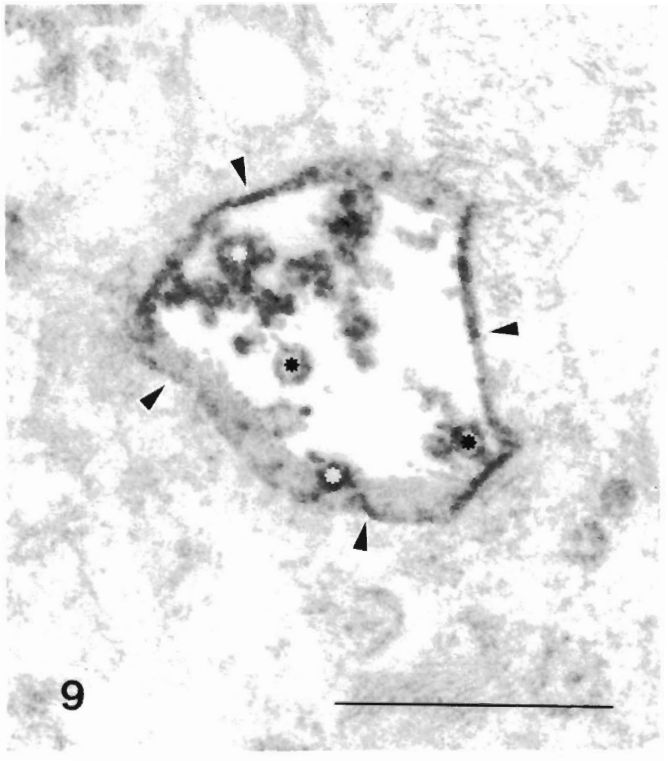

Fig. 9. A forming multivesicular body with HRP attached on its limiting membrane (arrowheads), indicative of its discoidal vesicle origin. Inclusion vesicles (*). Bar= 0.5 µm. x 74,000

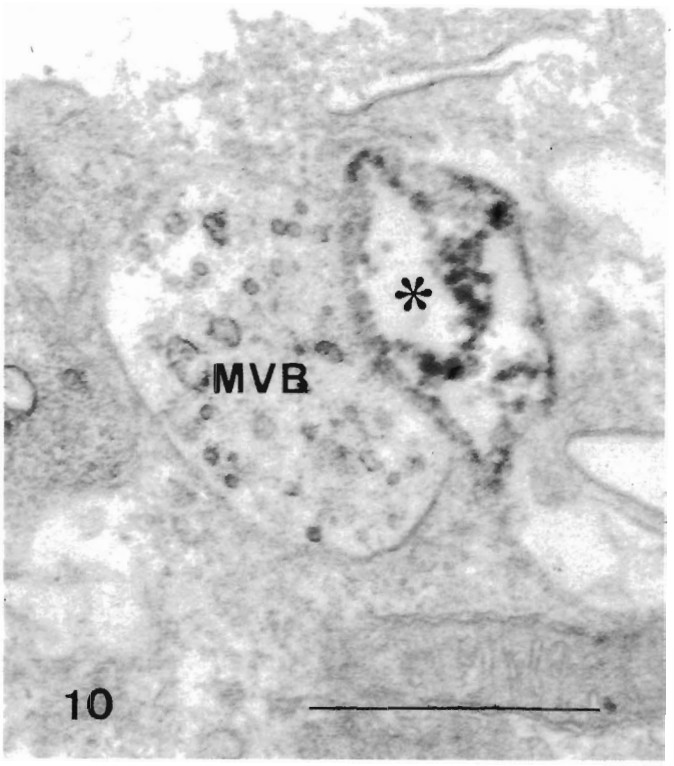

Fig. 10. A HRP-positive MVB (*) fusing with another MVB without HRP (MVB). Bar= 0.5 µm. x 77,000

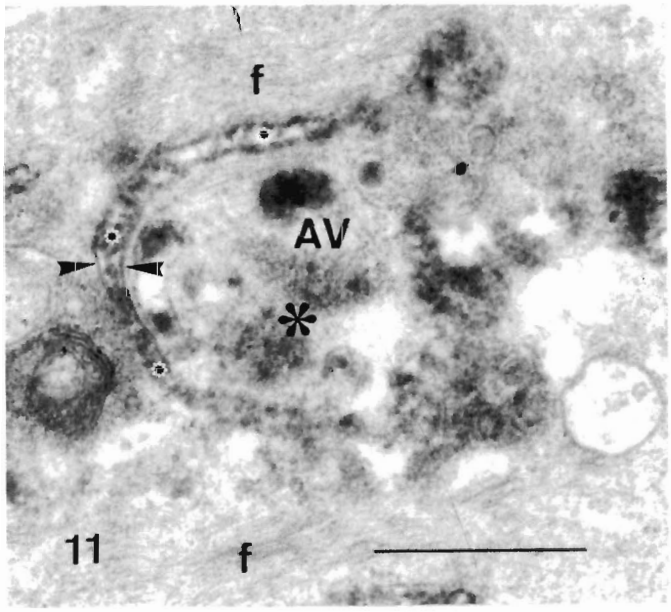

Fig. 11. Forming autophagic vacuole (AV). The HRP labelling can be seen within the lumen (*) and in the part of cytoplasm being wrapped by the forming autophagic vacuole (AV). Arrowheads indicate AUM. Filaments (f). Bar = 0.5 µm. x 56,000

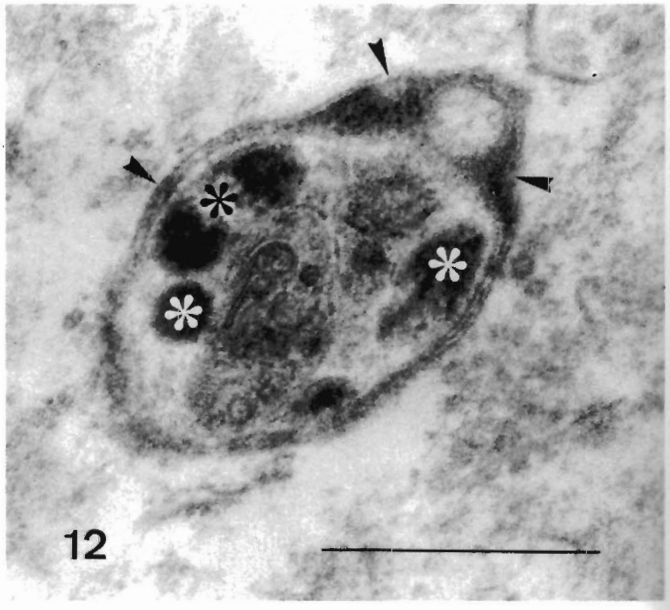

Fig. 12. A formed autophagic vacuole displaying AUM envelope (arrowheads) and HRP labelling within the wrapped material (*). Bar= 0.5 µm. x 74,000

to destroy the old or excess AUM during the membrane cycle. Apart from this, we have also found that some HRP-labelled discoidal vesicles and vacuoles fuse directly with lysosomes, suggesting another pathway of AUM degradation.

Unlike most cell types in which the envelopes of autophagic vacuoles originate from segments of sER (Weiss, 1983), in the superficial cells of the urinary bladder, the AUM has been found involved in the formation of autophagic vacuoles (Hicks, 1966).

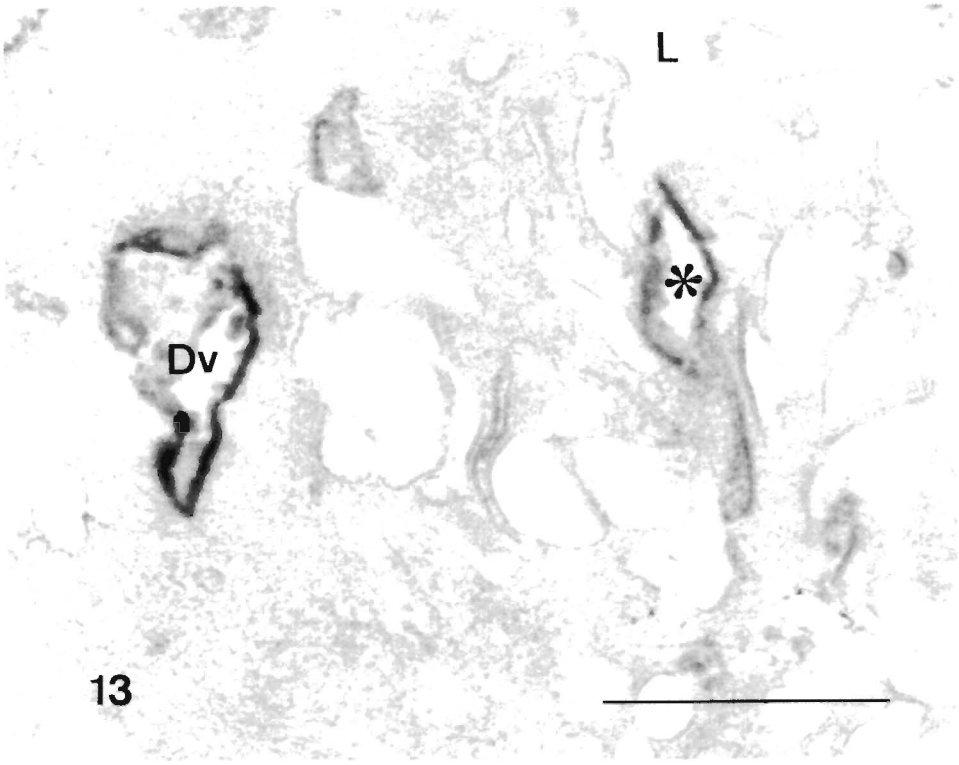

Fig. 13. After 6 hr, HRP-positive discoidal vesicles (*) are seen opening into the lumen (L) of the bladder. Note that no more HRP remains on the luminal surface. Discoidal vesicle (Dv) with HRP labelling. Bar= 0.5 µm. x 76,000

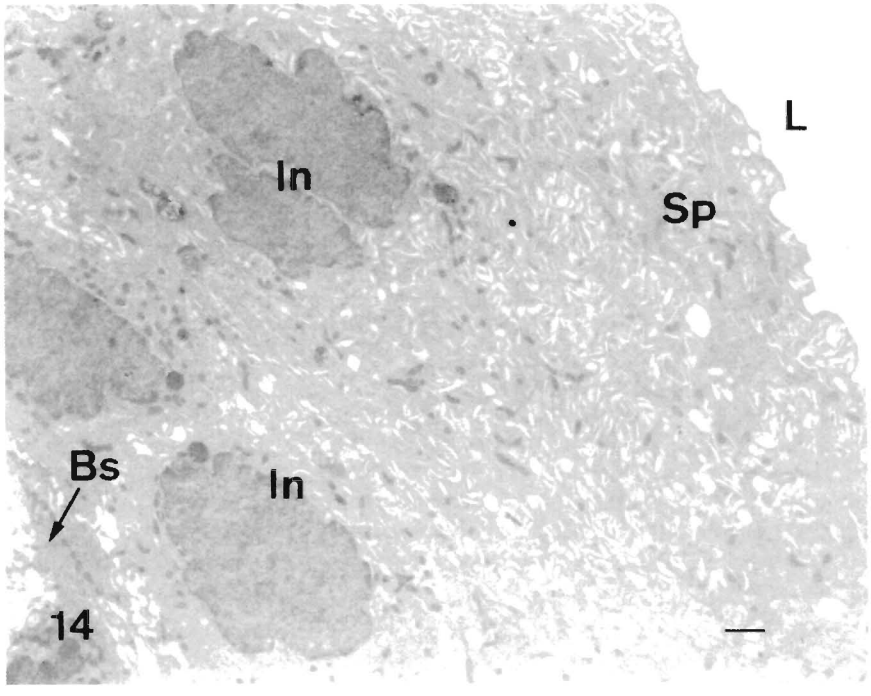

Fig. 14. Control experiment. No HRP labelling is detected in either superficial (Sp), intermediate (In) or basal (Bs) cells when the tissue is incubated in a DAB-containing medium without H_2O_2. Lumen (L); cytoplasm (Cy). Bar= 1.0 µm. x 5,000

Morphologically, the limiting membrane of both forming and formed autophagic vacuoles was clearly demonstrated to be an AUM (Fig. 2). Furthermore, HRP labelling was found inside the lumen of the double-membrane envelope of the autophagic vacuoles (Figs. 11, 12), strongly implying that this limiting membrane is derived from the luminal AUM. In this tracer experiment we observed that some discoidal vesicles fused with one another, enclosed a part of cytoplasm which contained organelles such as ER, ribosomes, vesicles, etc, and finally became autophagic vacuoles. Although the exact mechanism responsible for the formation of this kind of autophagic vacuole is still unknown, this may be an alternative way to deal with unnecessary AUM.

HRP-positive discoidal vesicles were still observed beneath the luminal surface even 6 to 8 hr after the HRP

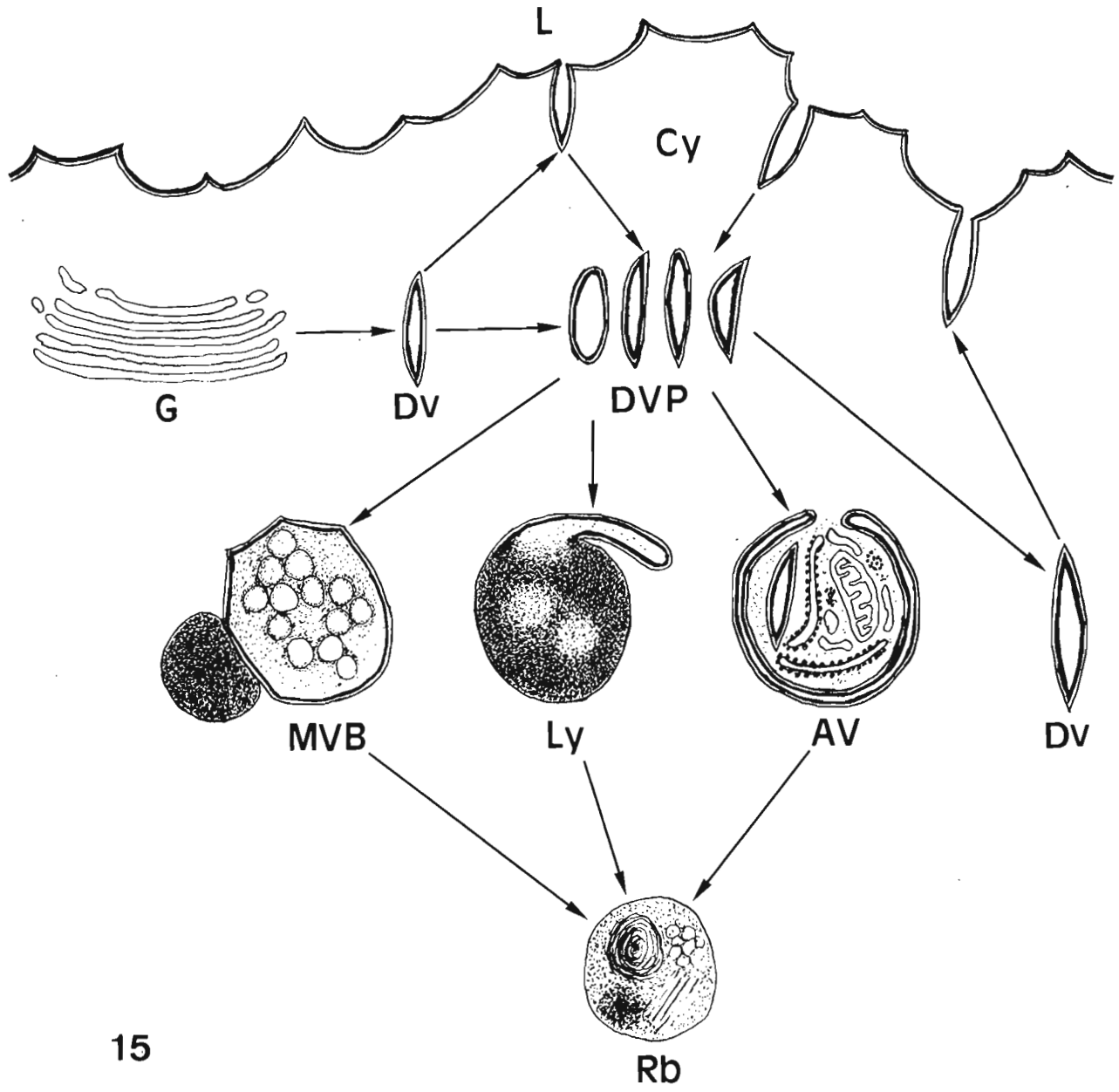

Fig. 15. Schematic representation of the four possible pathways followed by the AUM of the luminal surface of the superficial cells after entering the cytoplasm in the form of discoidal vesicles (Dv). Lumen (L); cytoplasm (Cy); Golgi complex (G); discoidal vesicle pool (DVP); lysosome (Ly); multivesicular body (MVB); autophagic vacuole (AV); residual body (Rb).

injection, and sometimes they were seen fusing with the luminal AUM and opening into the lumen of the bladder in spite of the fact that, by then, no more HRP remained on the luminal surface. This seems to indicate that the discoidal vesicles derived from the luminal surface to which HRP attached, return to the luminal surface when they are needed, after being stored for a certain period of time in the cytoplasm. The discoidal vesicle pool might thus consist of both maturing and matured vesicles (Porter et al., 1965; Severs and Hicks, 1979; Alroy et al., 1982); the former derived from the Golgi complex and the latter from the luminal surface, which is in agreement with the results of a recent immunocytochemical study (Yu et al., 1990). Therefore, we consider that, if those vesicles formed from the luminal AUM are still physiologically active and do not need to be degraded, it would be economical and logical for them to be reutilized, i.e., to become part of the luminal membrane again in the following expansion cycle.

Taking these findings together, we suggest that after entering the cytoplasm in the form of discoidal vesicles, the AUM is transferred to the lysosomes for degradation along the following different pathways: 1) by transforming into MVBs and fusing with lysosomes; 2) by fusing directly with lysosomes; and 3) by becoming autophagic vacuoles with double-membrane envelope. In addition, some of these discoidal vesicles could be reutilized by fusing with and becoming part of the luminal surface again (Fig. 15).

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