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Endocytosis in Secretory Cells

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Endocytosis in secretory cells

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[Plates 1–3]

Membranes of secretion granules inserted during exocytosis into the luminal plasma membrane of glandular cells are retrieved by endocytosis as revealed by electron dense tracers applied selectively to the apical cell surfaces. Two major pathways that endocytic vesicles may take are described: (1) a direct route to the Golgi complex (e.g. in parotid and exocrine pancreas) with later appearance of the tracer in the periphery of mature secretion granules; (2) an indirect route with lysosomes as a first station and the subsequent appearance of tracer in stacked Golgi cisternae. It is presumed that some of the retrieved membrane follows the same pathways and is reutilized in the secretory cycle.

1. Introduction

In eukaryotic cells, the uptake of macromolecular substances is accomplished by endocytosis, which involves the concomitant removal of membrane portions from the cell surface (Silverstein *et al.* 1979). Endocytosis in secretory cells is of particular interest because of its postulated relation to exocytosis. Each exocytotic event results in the temporary enlargement of the plasma membrane by the formation of an exocytotic lacuna (figures 1–3). This paper concentrates on endocytosis in secretory cells as a mechanism to retrieve excess membrane after stimulated exocytosis. It describes the pathways of endocytosis and their relation to membrane recycling as revealed by the use of electron dense tracers.

2. Localization of coated pits

Several observations suggest that the membrane of an exocytotic lacuna (which is largely identical with the membrane of a secretion granule) is retrieved selectively rather than other cell surface areas at random.

1. The membranes of compartments participating in secretion remain constant in their surface area and distinct in their composition (Palade 1975; Meldolesi *et al.* 1978). For example, in the adrenal medulla the major protein of the granule membrane, β-hydroxylase, is absent from the membranes of rough endoplasmic reticulum (r.e.r.), the Golgi complex and the cell surface (Winkler 1977).

2. Freeze–fracture observations by Meldolesi and coworkers on the rat parotid gland indicate that plasma membrane components do not intermix with the granule membrane inserted during exocytosis (De Camilli *et al.* 1976).

3. Coated pits bulging towards the cytoplasm are found on membranes of exocytotic lacunae, for example in the bovine adrenal medulla (Grynszpan-Winograd 1971). In other glands, e.g. the rat parotid gland, coated pits are not commonly observed after the usual fixation (neither

by vascular perfusion or by immersion). However, if the luminal plasma membrane of acini is fixed rapidly by *infusion of the fixative* into the parotid duct, coated pits are regularly visible on the membranes of exocytotic lacunae (figure 4). This points to the rapidity of coated pit formation and detachment at this particular location. Occasionally, larger areas of the apical plasma membrane are coated; they may represent remnants of exocytotic lacunae (figure 5).

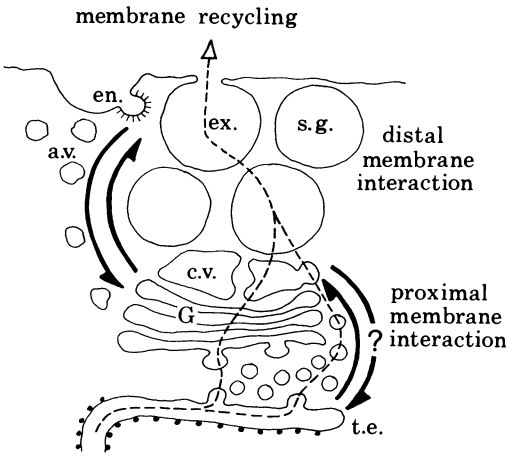
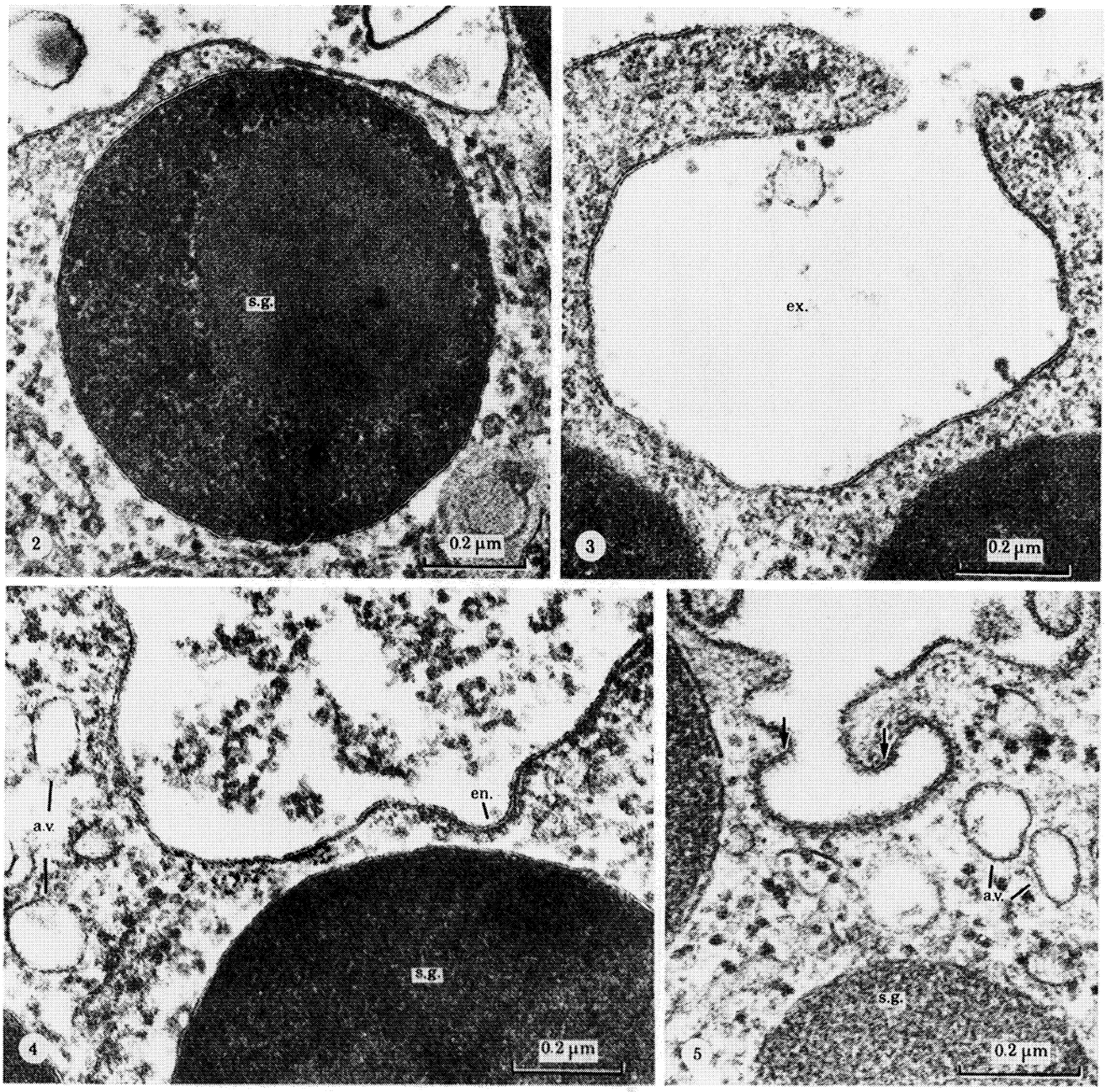


FIGURE 1. Intracellular transport of secretory proteins (thin broken lines) and related membrane interactions (thick arrows) in an exocrine gland cell. Membrane interactions are required at least at two steps where the transport is discontinuous by the formation of vesicles. (1) *Proximal membrane interaction*: peripheral Golgi vesicles which derive from transitional elements (t.e.) carry secretion product to the Golgi complex (G) including condensing vacuoles (c.v.). It is not decided whether vesicles return from the Golgi complex as empty containers to transitional elements as postulated (arrow with question mark). (2) *Distal membrane interaction*: during exocytosis, the membranes of secretion granules (s.g.) are inserted into the apical plasma membrane thereby forming an exocytotic lacuna (ex.). This membrane in excess is removed by the formation of coated pits (en.) and apical vesicles (a.v.). Their membrane is transferred in part to the Golgi complex and reutilized in the secretory cycle.

3. SELECTIVE APPLICATION OF TRACERS TO THE APICAL PLASMA MEMBRANE

In polarized secretory epithelial cells, exocytosis occurs only at the apical cell surface, under normal conditions. To trace the fate of membrane removed after induced exocytosis, tracers have to be applied selectively to the apical plasma membrane. Two systems have been explored. (1) In the parotid and the exocrine pancreas of the rat, tracers are infused through the duct at 15–25 mmHg (*ca.* 2.0–3.3 kPa), which is sufficient to reach the acinar lumina and low enough to avoid the rupture of tight junctions (figure 6) (Herzog & Farquhar 1977; Herzog & Reggio 1980). (2) In suspension cultures of closed inside-out follicles prepared from pig thyroid gland the polarity of the epithelial cell wall is reversed, with the apical plasma membrane facing the culture medium and the basal plasmalemma limiting the central cavity (figures 10 and 11) (Herzog & Miller 1981). For studies on endocytosis, the tracer is added to the culture medium.

In both systems, endocytic uptake of tracers occurs only from the apical plasmalemma. Hence, any intracellular occurrence of the applied tracer is indicative of its origin from the apical cell surface. We presume that some plasma membrane follows the intracellular route of the tracer and that its pathway may allow in part conclusions on the concomitant movement of retrieved plasma membrane. The tracers applied to the apical plasma membranes are either markers of the content (e.g. dextran) or non-specific membrane markers (e.g. cationized ferritin).



FIGURES 2–5. Exocytosis and endocytosis in the rat parotid gland. Fixation was performed by infusion of glutaraldehyde containing tannic acid through the parotid duct to achieve rapid fixation of the luminal plasma membrane. The membranes of a secretion granule (s.g.) and of the apical cell surface are in close proximity to each other (figure 2). After insertion of the granule membrane into the apical plasma membrane, an exocytotic lacuna (ex.) is formed (figure 3). Coated pits (en.) are seen in the membrane of the exocytotic lacuna (figure 4). Larger coated areas of the apical plasma membrane (between the two arrows) may represent remnants of exocytotic lacunae (figure 5). Smooth-surfaced apical vesicles (a.v. in figures 4 and 5) are endocytic in nature. Apparently, they are formed from coated pits that lose their coat upon detachment.

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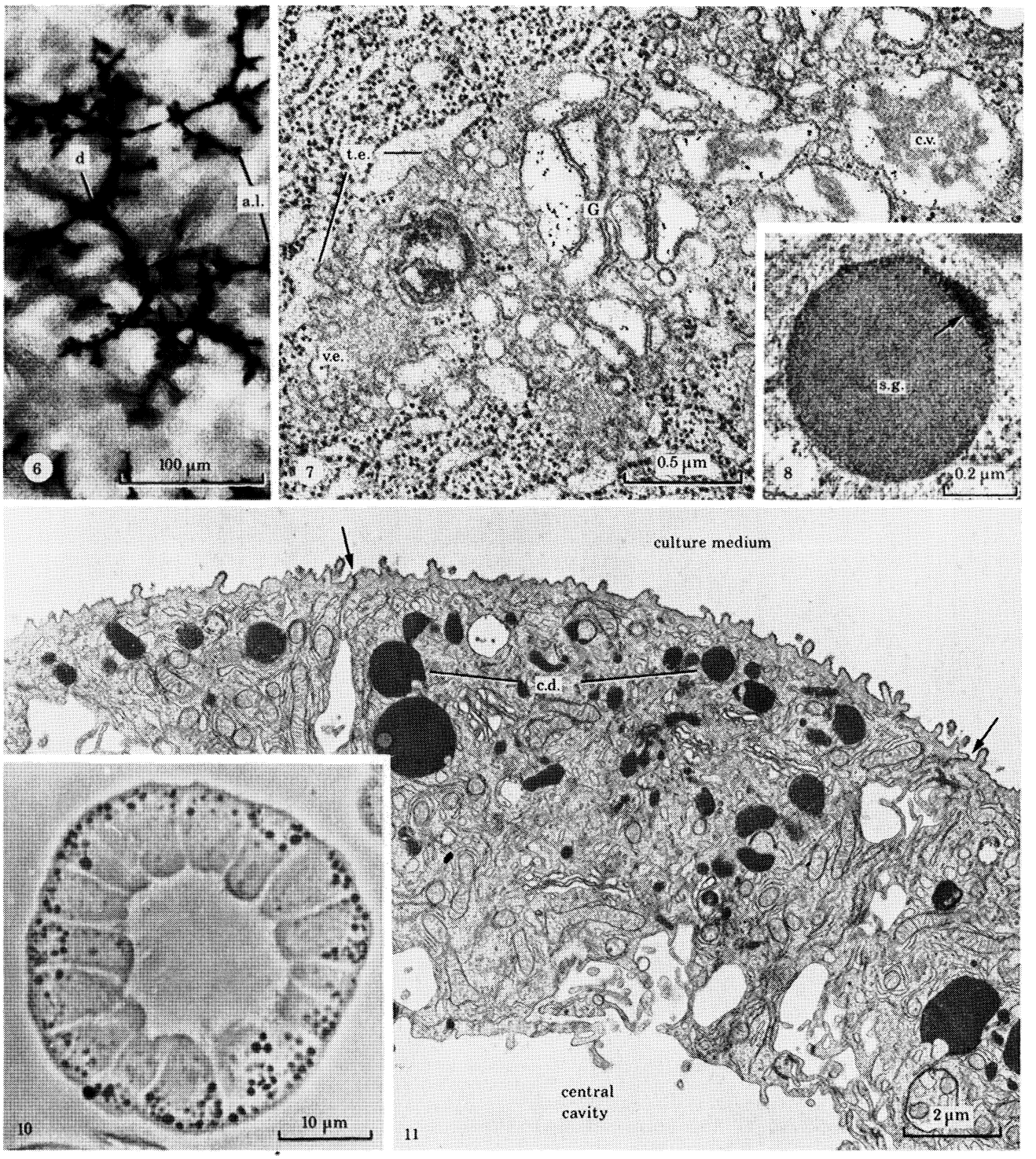

FIGURES 6–8, 10 AND 11. For description see opposite.

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Description of plate 2

Figures 6–8. Pathways of endocytosis from the luminal plasma membrane of rat exocrine pancreas. Dextran ($M_r \approx 44000$) was infused into the pancreatic duct at 15–25 mmHg (*ca.* 2.0–3.3 kPa) because at this pressure, infused india ink marks the lumina of ducts (d.) and acinar lumina (a.l.) with no indication of leakage into the basolateral intercellular space (figure 6). Dextran particles are found in coated pits and apical vesicles (not shown) and, 2 min later, in stacked Golgi cisternae (G) and condensing vacuoles (c.v.). Tracer particles are absent from transitional elements (t.e.) and peripheral Golgi vesicles (ve.) (figure 7); 15 min after infusion, dextran is observed in condensed form in mature secretion granules (s.g.) (figure 8).

Figures 10 and 11. Inside-out follicles prepared from pig thyroid gland in suspension culture. The follicles are formed by a single wall of thyroid epithelial cells which enclose a central cavity (light micrograph, figure 10). The apical plasma membrane is recognized by microvilli and the characteristic localization of tight junctions (arrows). The apical cell surface is directed towards the culture medium and the basal plasma membrane faces the central cavity. Colloid droplets (c.d.) are found preferentially in the cell apex (figure 11).

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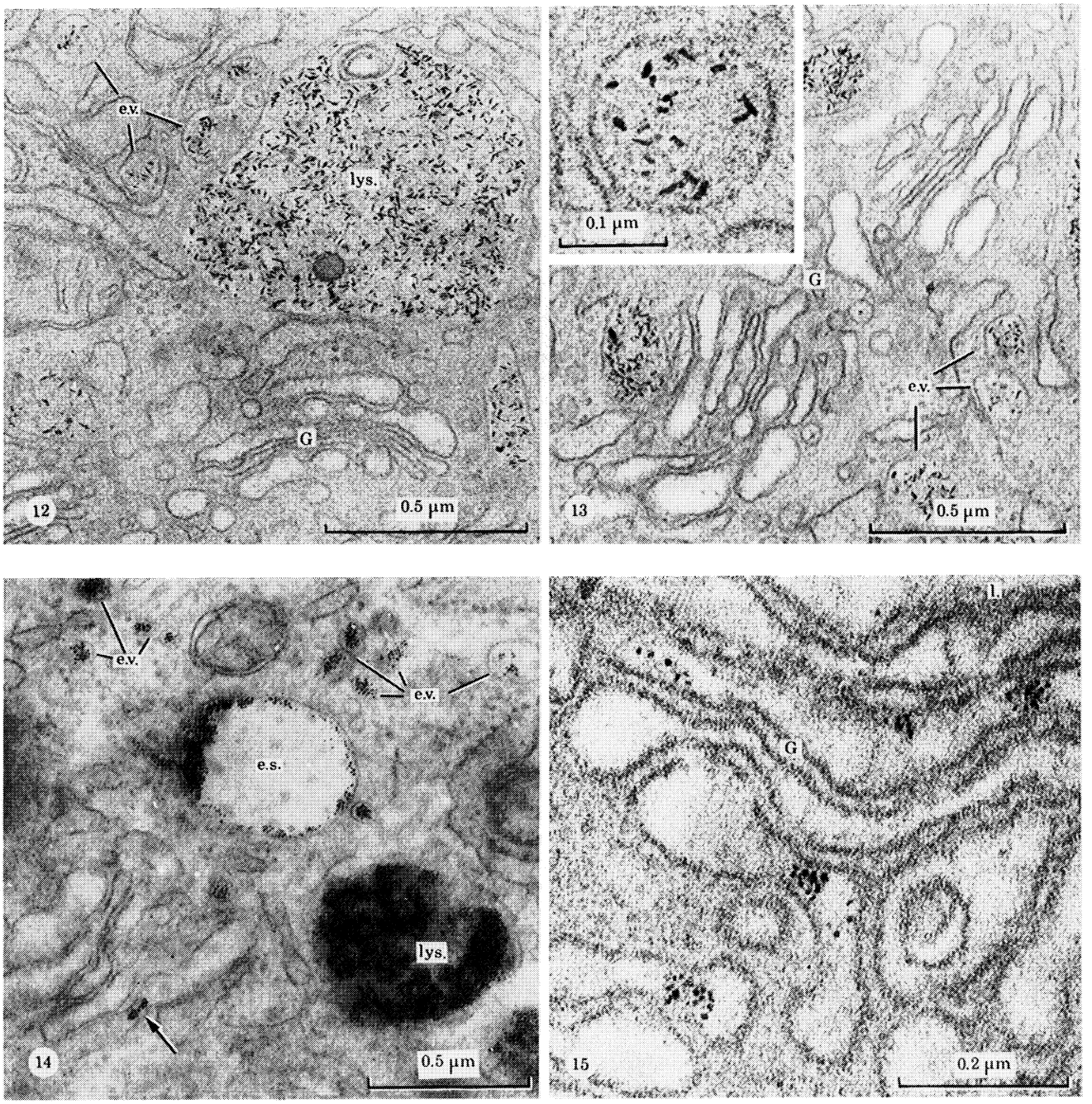

FIGURES 12–15. Pathways of endocytosis from the apical plasma membrane of inside-out thyroid follicles suspended in a medium containing a content marker (iron dextran, figures 12 and 13) or a non-specific membrane marker (cationized ferritin, figures 14 and 15). Internalized iron dextran is restricted to endocytic vesicles (e.v.) and to lysosomes (lys., figure 12). Cationized ferritin is found in endocytic vesicles (e.v.), in endosomes (e.s.) and, 30 min later (figure 14), also in Golgi cisternae (arrow, figure 14). Stacked Golgi cisternae are more frequently labelled with cationized ferritin at 90 min (figure 15) whereas iron dextran (Imposil) remains limited to lysosomes and vesicles close to the Golgi complex without reaching its stacked cisternae (figure 13). Iron dextran is rod-shaped with a length of *ca.* 21 nm (inset, figure 13). It is therefore morphologically distinct from the spherical ferritin particle (figure 15).

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4. Formation of endocytic vesicles

After infusion of dextran into the duct of the parotid or the exocrine pancreas, the tracer is confined to the acinar lumina including exocytotic lacunae and – occasionally visible – the coated pits. Tracer particles are also observed in smooth-surfaced apical vesicles, thus proving their endocytic nature (Herzog & Farquhar 1977; Herzog & Reggio 1980).

Cationized ferritin (CF) has been found to interact with anionic components of the secretion product present in the ducts and acinar lumina of rat parotid and exocrine pancreas. By this interaction, CF may lose its ability to bind to the apical plasma membrane. The advantages of CF as a non-specific membrane marker are its distinct electron microscopical visualization and its mild charge interaction with the plasmalemma. The disadvantage is that CF particles may dissociate upon internalization when other anionic sites become available, e.g. in lysosomes. Stable, covalently linked and non-perturbing membrane markers are needed but are not yet available.

Suspension cultures of inside-out thyroid follicles can be freed of their secretion product. This allows CF particles to bind to the apical cell surface. At 37 °C, CF particles concentrate in coated pits and are also found in mainly smooth-surfaced endocytic vesicles (Herzog & Miller 1979).

In all gland cells studied so far, coated pits detach, rapidly losing their coat and forming smooth-surfaced endocytic vesicles. Similar dissociation of the coat has been described before (Roth *et al.* 1976). This dissociation of the coat may be required for the ability of vesicle membranes to fuse with the membranes of other compartments (Pearse 1980).

5. Pathways of endocytosis in secretory cells

(*a*) *Exocrine gland cells*

Endocytic vesicles derived from the apical cell surface of rat parotid and the exocrine pancreas carry dextran as a tracer rapidly, within 2–5 min, to stacked Golgi cisternae (Herzog & Farquhar 1977; Herzog & Reggio 1980). In the exocrine pancreas, dextran is found simultaneously in condensing vacuoles (Herzog & Reggio 1980). Fusion of incoming vesicles is highly specific and does not occur with the membranes of the rough endoplasmic reticulum, transitional elements and peripheral Golgi vesicles (figure 7). Furthermore, the approach of filling all Golgi cisternae with tracer has not so far revealed the postulated recycling of peripheral vesicles in the proximal membrane interaction (figure 1).

At times later than 15 min, dextran particles are located in condensed form in the periphery of the secretion granule matrix (figure 8). The internalization of a content marker like dextran suggests that small amounts of the secretion product are taken up again and repackaged together with newly synthesized secretory protein (Herzog & Reggio 1980). In contrast, horseradish peroxidase (HRP) appeared to adhere in part to both the luminal cell surface and the membranes of exocytotic lacunae (Herzog & Reggio 1980). This binding apparently changed the fate of internalized apical plasma membrane, which was internalized and transported exclusively to lysosomes. Peroxidase was not recovered in Golgi cisternae, condensing vacuoles or mature secretion granules (Herzog & Reggio 1980). This observation raises the question of whether both tracers are transferred to their distinct intracellular destinations by different vesicular carriers (for further discussion see §6).

The intracellular route of HRP to lysosomes does not necessarily imply that the membranes

segregating the internalized tracer also end in lysosomes. In the rat exocrine pancreas, lysosomes constitute only a minor component among the membrane-bounded compartments. The potential recovery of membrane from the lysosome surface has therefore, been investigated in a cell type in which the endocytic pathway to lysosomes is obligatory, i.e. the thyroid follicle cell.

(*b*) *Thyroid follicle cells*

The secretion of thyroid hormones requires the bidirectional transport of newly synthesized thyroglobulin into the follicle lumen for temporary storage (figure 9) and its subsequent endocytosis and transfer to lysosomes, where thyroid hormones are freed from their peptide linkages (van den Hove-Vandenbroucke 1980). An *in vitro* system of closed inside-out thyroid follicles (figures 10 and 11) has been used to study endocytosis from the apical cell surface.

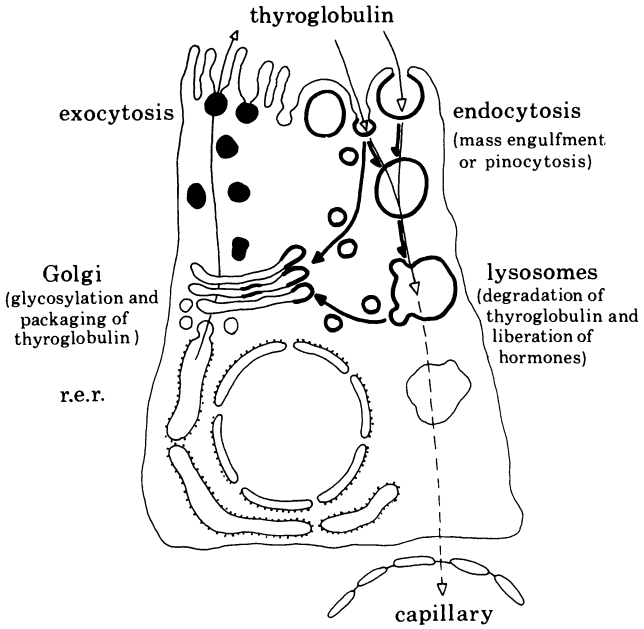

FIGURE 9. Bidirectional transport of thyroglobulin (thin arrows) and the membrane interactions during endocytosis (thick arrows). Newly synthesized *thyroglobulin* is transported via the Golgi complex to secretion granules and released by exocytosis. Under the right conditions, thyroglobulin is internalized by endocytosis and transferred to lysosomes where thyroid hormones are freed from their peptide linkages and released by an unknown mechanism into the bloodstream. The *membranes* retrieved during endocytosis are marked with thick contours. Whereas thyroglobulin is degraded in lysosomes, the concomitantly transferred membrane may be recovered, transferred to the membrane of the stacked Golgi cisternae, and reutilized for granule formation. A direct but slow endocytic route from the apical cell surface to Golgi cisternae cannot be excluded.

Regardless of charge of composition of the tracer, lysosomes are reached within 5 min (Herzog & Miller 1979, 1981). They derive from a prelysosomal compartment, the endosome (figure 14). Here, lysosomal enzymes are absent and cationized ferritin (CF) is still bound to the inner surface of the membrane. In secondary lysosomes, however, the particles detach and collect in their matrix (figure 14). Native (anionic) ferritin (Herzog & Miller 1979) and uncharged iron dextran (Herzog & Miller 1981) remain in the lysosomal matrix (figures 12 and 13), whereas CF is found later, beginning at 30 min, also in stacked Golgi cisternae (figures 14 and 15).

Similarly, if CF-coated latex beads are internalized, the CF particles dissociate from the latex

beads after entering the lysosomes and are found 30 min later also in Golgi cisternae, whereas the latex beads remain within the lysosomes (Herzog & Miller 1979). These observations mirror thyroid function, when thyroglobulin is taken up from the lumen for lysosomal degradation and liberation of thyroid hormones, and they suggest that small portions of the lysosomal membrane are removed and transferred to Golgi cisternae (figure 9) (Herzog & Miller 1979).

6. Multiple pathways of endocytosis

The results with tracers indicate that the Golgi complex is reached by two distinct endocytic routes:

(1) a direct route: cell surface → Golgi complex (2–5 min) (Herzog & Farquhar 1977; Herzog & Miller 1979);

(2) an indirect route: cell surface → lysosomes (5 min) → Golgi complex (30 min) (Herzog & Miller 1979; Ottosen *et al.* 1980).

The presence of tracer in elements of the Golgi complex does not prove that the membrane inserted is still the same as that retrieved during endocytosis. Compositional changes, e.g. during the temporal fusion with lysosomes, along the indirect route cannot be excluded.

Parameters such as the cell type, the physiological state of the cell and the nature of the tracer may influence the traffic of endocytic vesicles and steer their movement to lysosomes or to Golgi cisternae.

Observations with rat parotid acinar cells indicate that the *physiological state* of the cell may influence the traffic of endocytic vesicles which transfer dextran mainly to Golgi cisternae *in vivo* but increasingly to lysosomes when isolated acini were exposed to the tracer *in vitro* (Herzog & Farquhar 1977).

In the exocrine pancreas, endocytic vesicles deriving from the luminal plasma membrane carry dextran ($M_r \approx 44000$) selectively to the Golgi complex. Horseradish peroxidase ($M_r = 44000$), which binds to the luminal plasma membrane including the membranes of secretion granules inserted during exocytosis, is exclusively transferred to lysosomes (Herzog & Reggio 1980). In β-cells of the endocrine pancreas, HRP does reach the Golgi cisternae (Orci *et al.* 1978), thus underlining the variability in the handling of the same tracer by different secretory *cell types*.

The results with HRP and dextran, two tracers of similar molecular mass, in the same cell type (exocrine pancreas) indicate distinct routes for vesicles carrying tracers of different composition (Herzog & Reggio 1980). Furthermore, the *net charge* of the same tracer may determine its intracellular route in a given cell type; in anterior pituitary cells, polycationic ferritin was found within several stacked Golgi cisternae, secretion granules and lysosomes, whereas native (anionic) ferritin was taken up in bulk phase and shed exclusively into lysosomes (Farquhar 1978).

7. 'Membrane recycling' and 'membrane flow'

Careful biochemical analyses revealed that membrane proteins of secretion granules are synthesized about ten times more slowly than the secretory proteins (for review see Meldolesi *et al.* 1978). This was the first indirect evidence available for the view that membranes of secretion granules are reutilized during successive secretory cycles (Palade 1975).

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Others have assumed, however, that newly synthesized cell membranes and their enclosed contents move and differentiate concomitantly from the r.e.r. through the stacked cisternae of the Golgi complex, from where they reach the plasmalemma. This concept of 'membrane flow and differentiation' (Morré *et al.* 1974) is supported by observations on the mammary gland, where the exocytotic insertion of the casein granule membrane is though to compensate for the membrane lost by apocrine secretion of milk fat globules (Franke *et al.*, 1976). On this view, the membrane of secretion granules would function in part in the biogenesis of the plasma membrane by the transfer of membrane constituents concomitantly with the transport of exportable proteins.

Obviously, this concept does not apply to gland cells without apocrine secretion mechanism. Membrane recycling appears to predominate in cells that release their secretion product by exocytosis and which may therefore not have such a great need for new synthesis of cell surface membrane.

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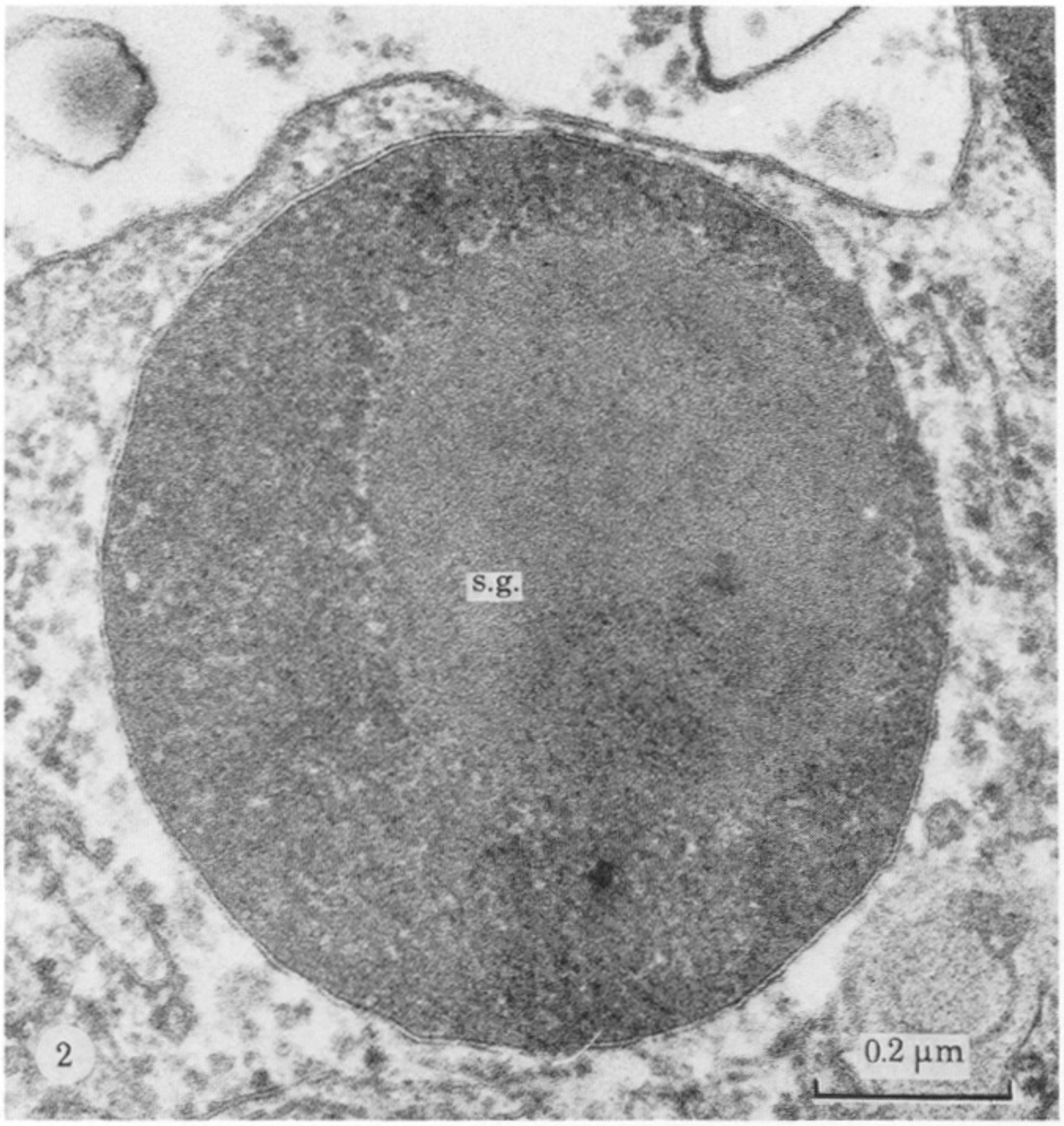
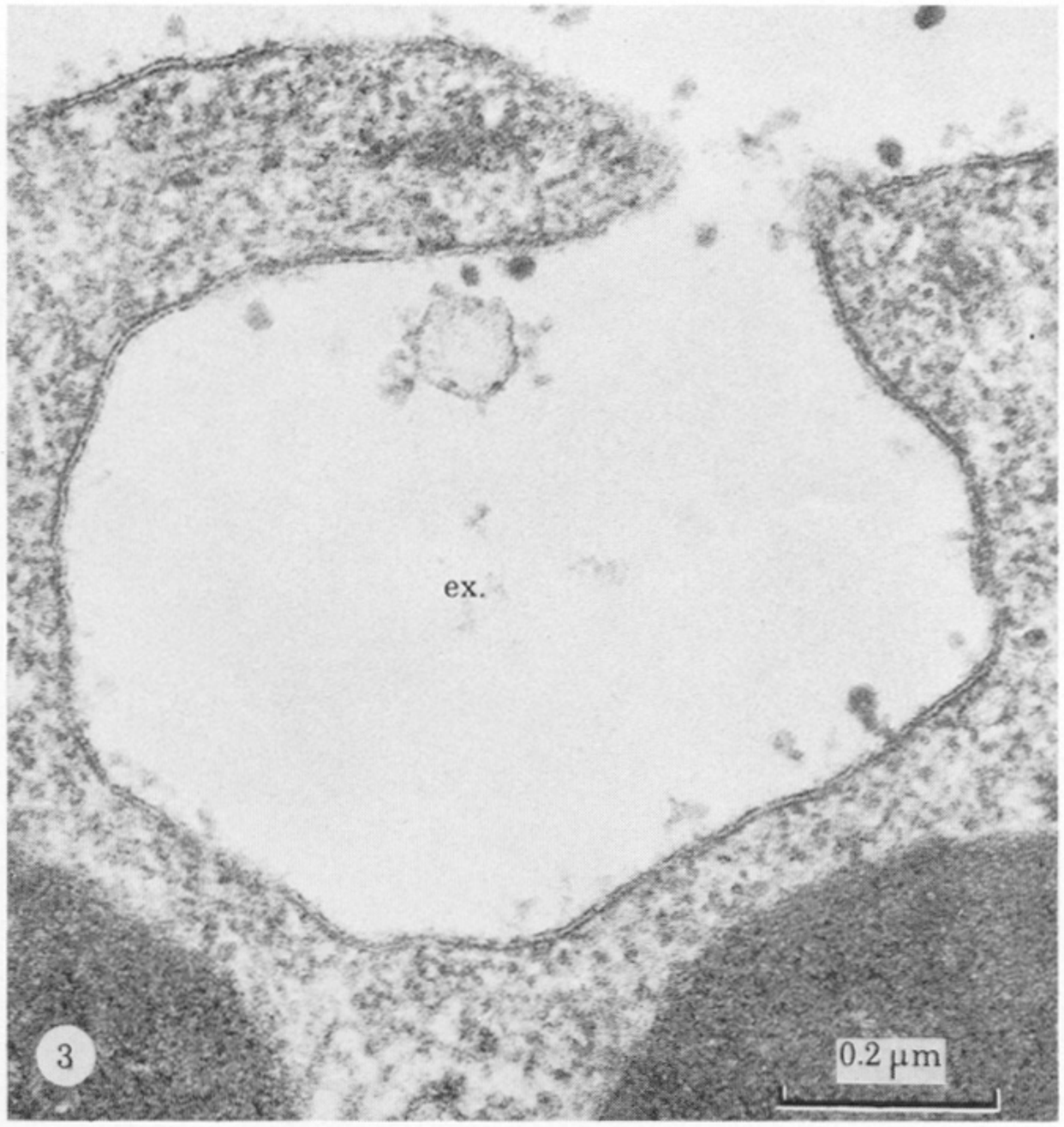
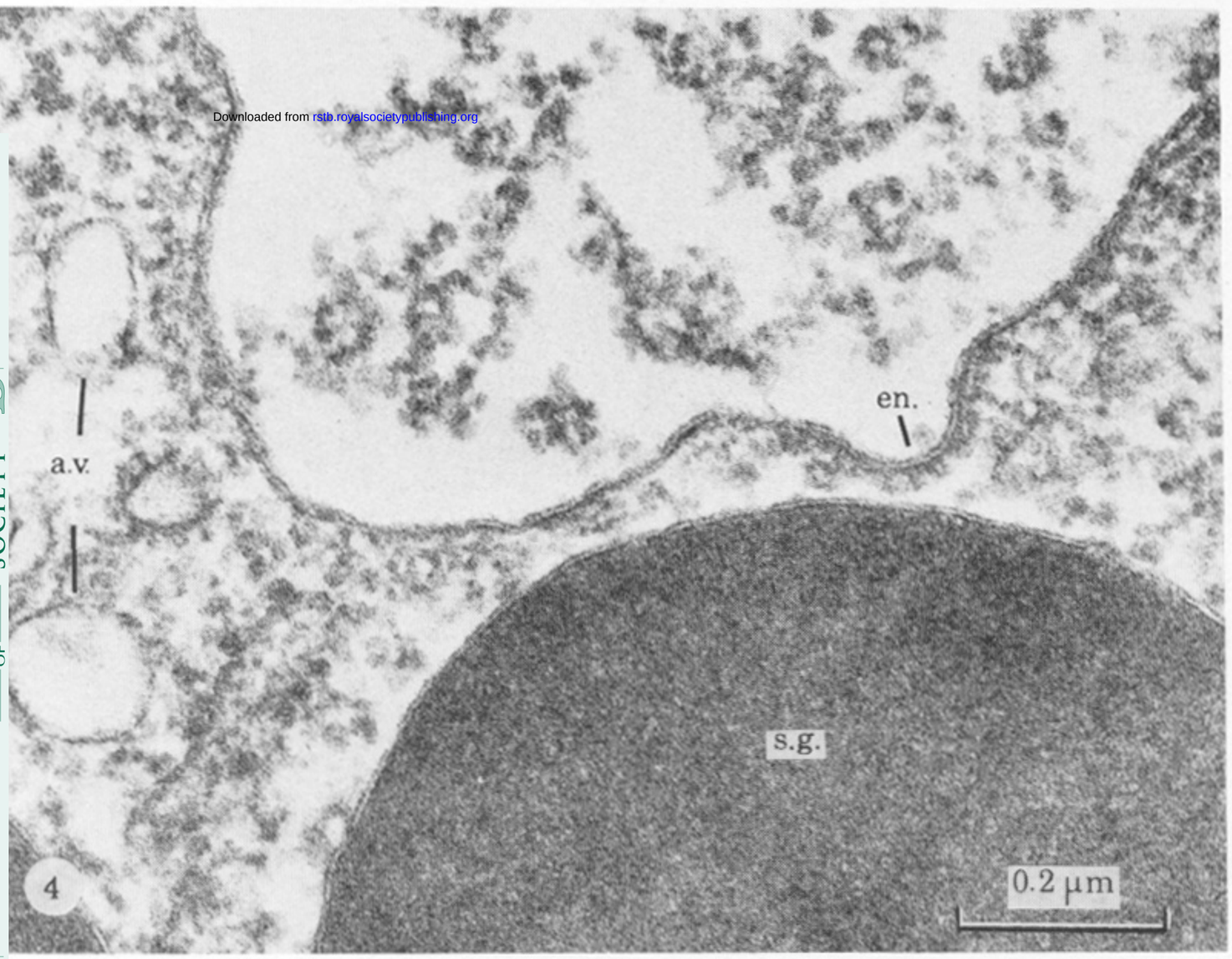
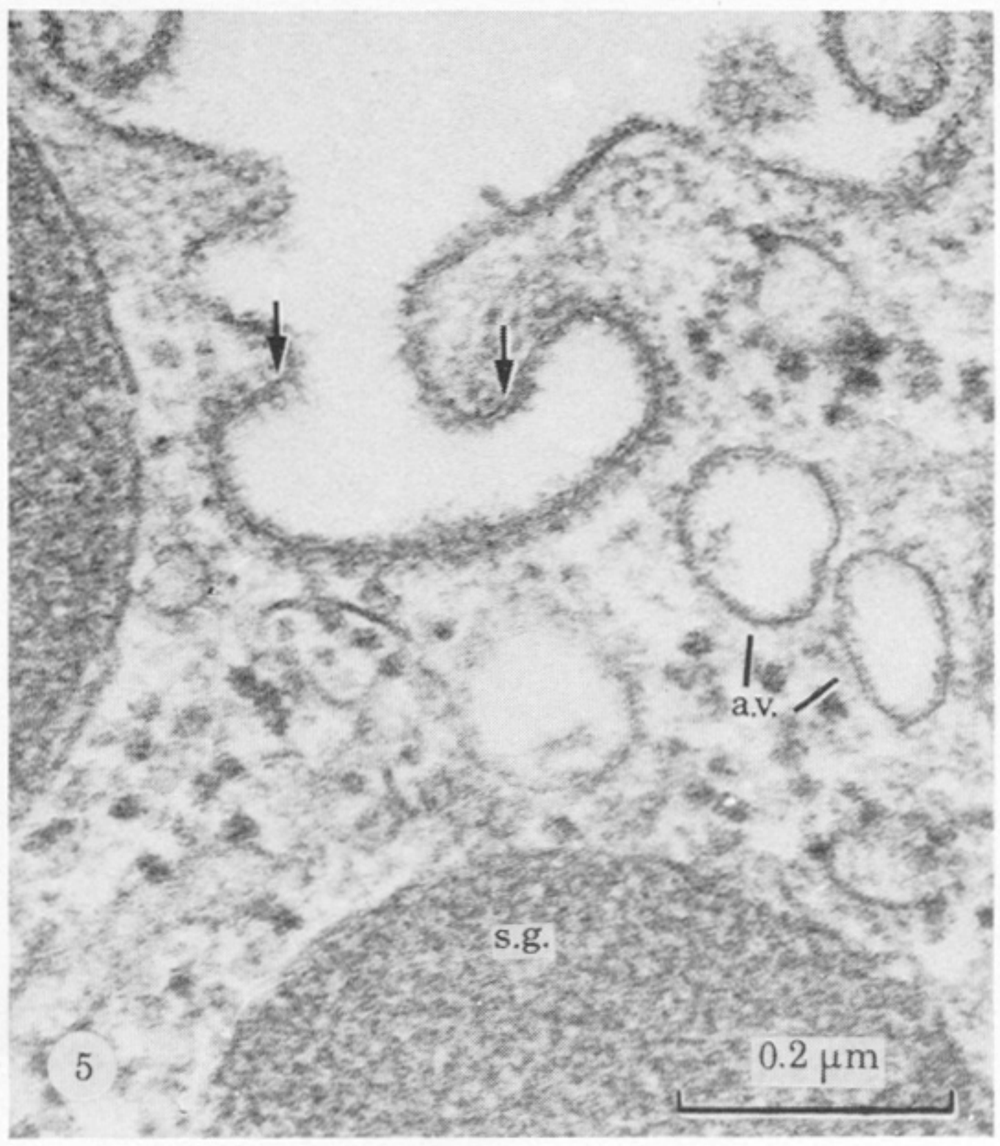
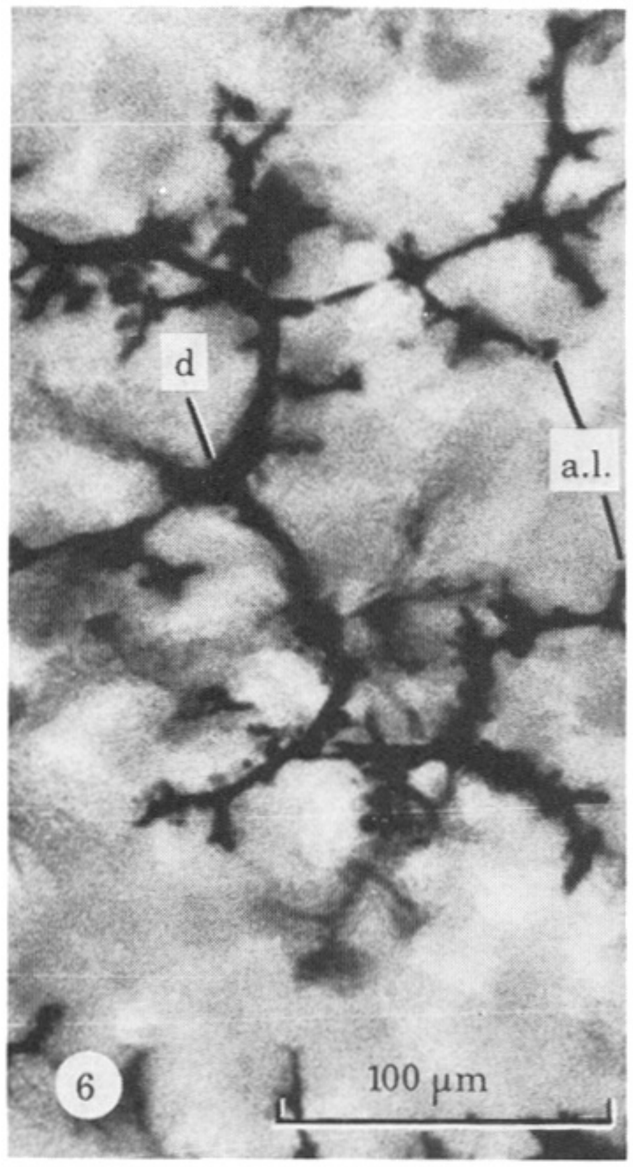


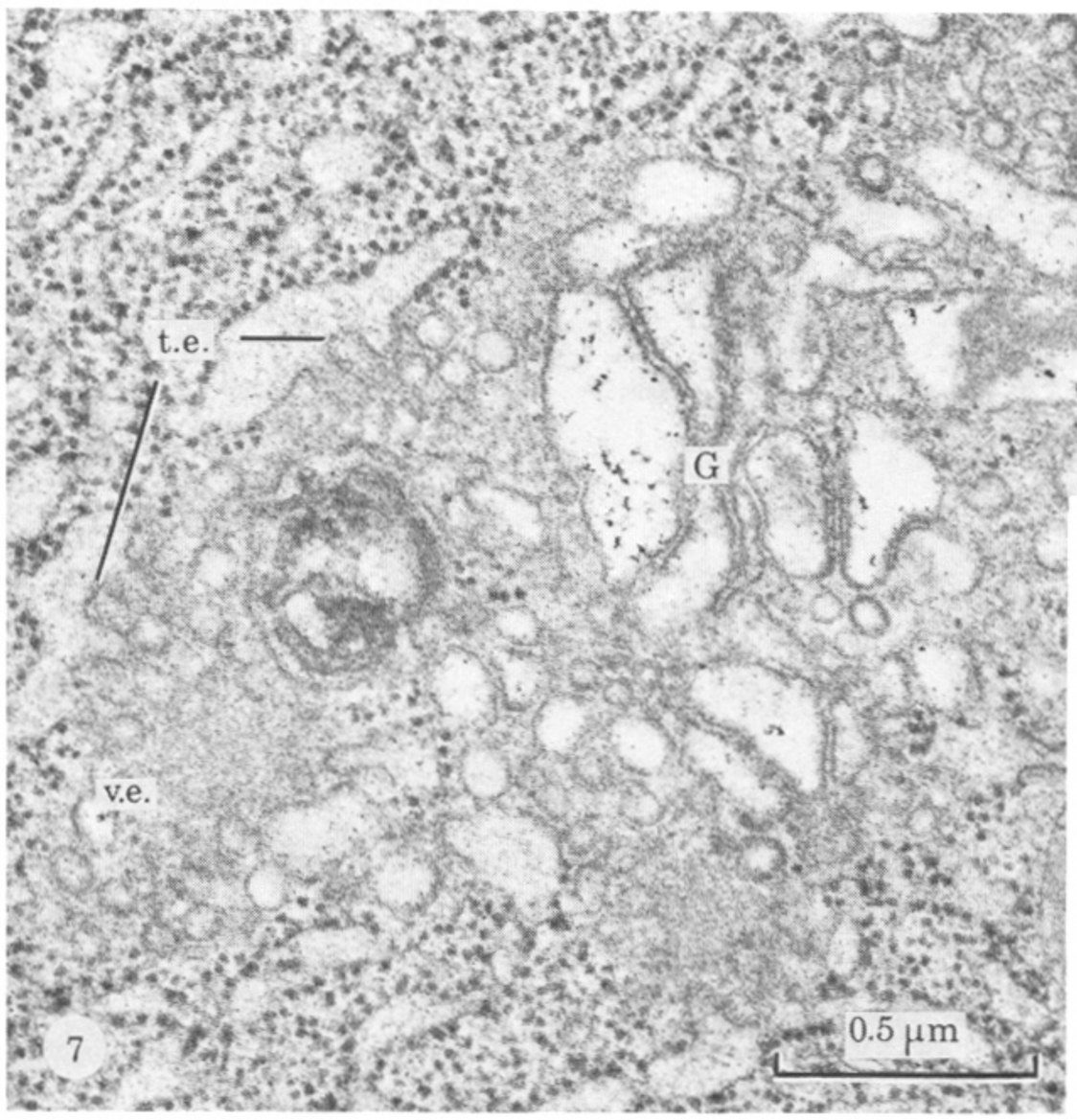


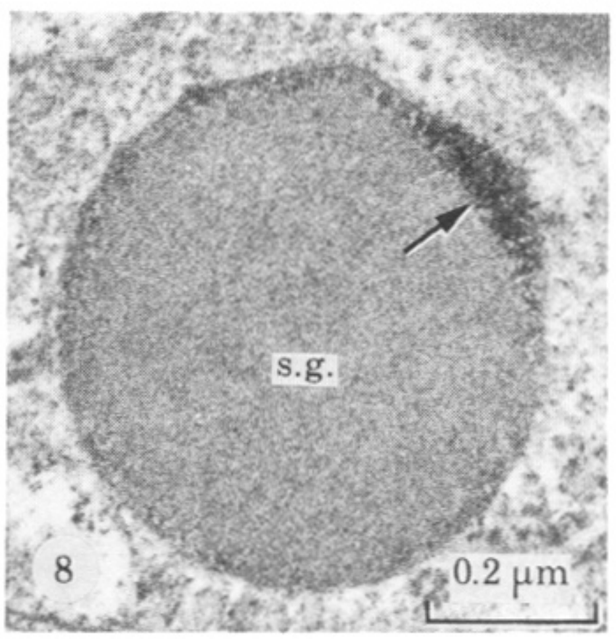


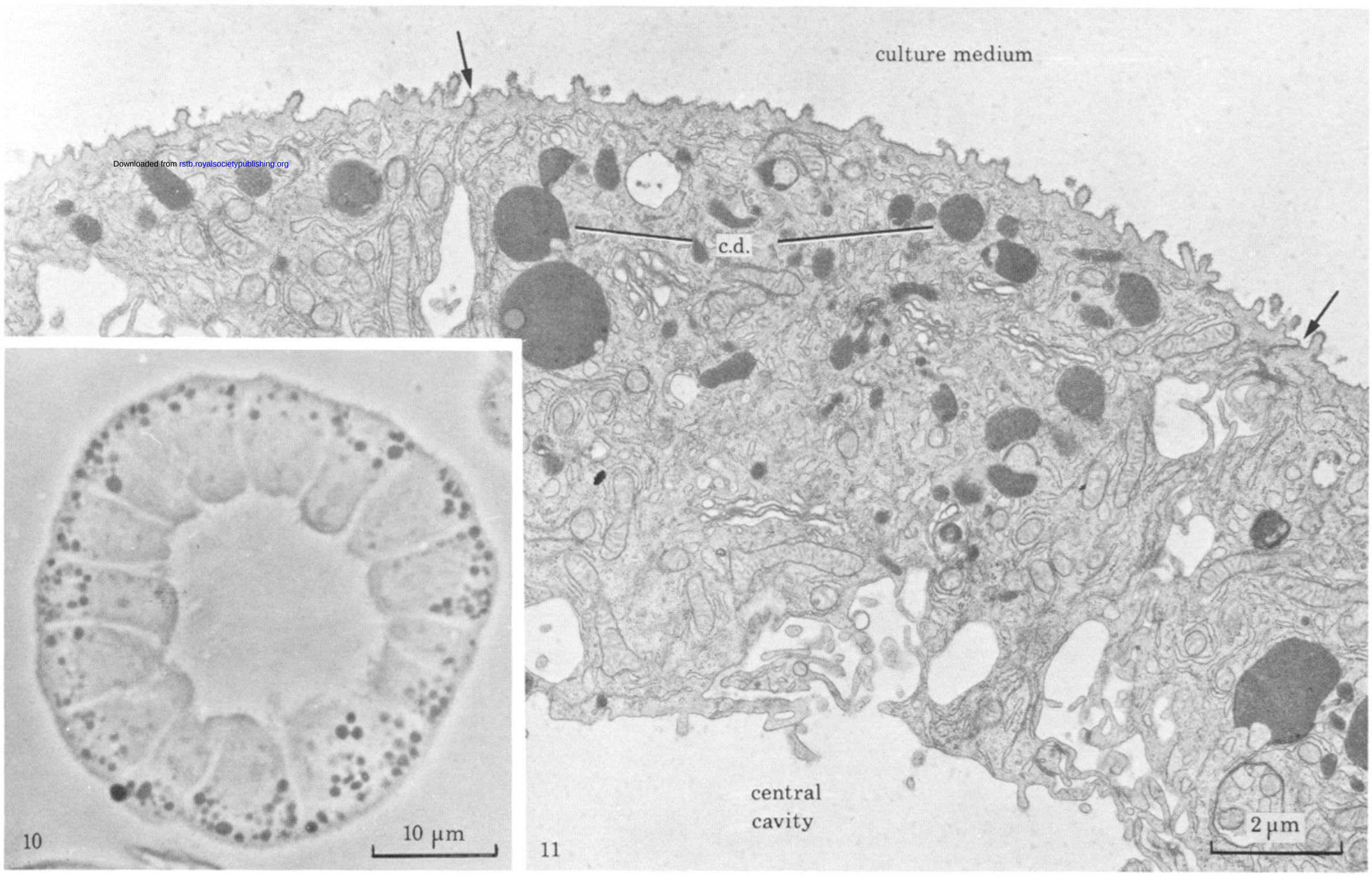


FIGURES 2–5. Exocytosis and endocytosis in the rat parotid gland. Fixation was performed by infusion of glutaraldehyde containing tannic acid through the parotid duct to achieve rapid fixation of the luminal plasma membrane. The membranes of a secretion granule (s.g.) and of the apical cell surface are in close proximity to each other (figure 2). After insertion of the granule membrane into the apical plasma membrane, an exocytotic lacuna (ex.) is formed (figure 3). Coated pits (en.) are seen in the membrane of the exocytotic lacuna (figure 4). Larger coated areas of the apical plasma membrane (between the two arrows) may represent remnants of exocytotic lacunae (figure 5). Smooth-surfaced apical vesicles (a.v. in figures 4 and 5) are endocytic in nature. Apparently, they are formed from coated pits that lose their coat upon detachment.









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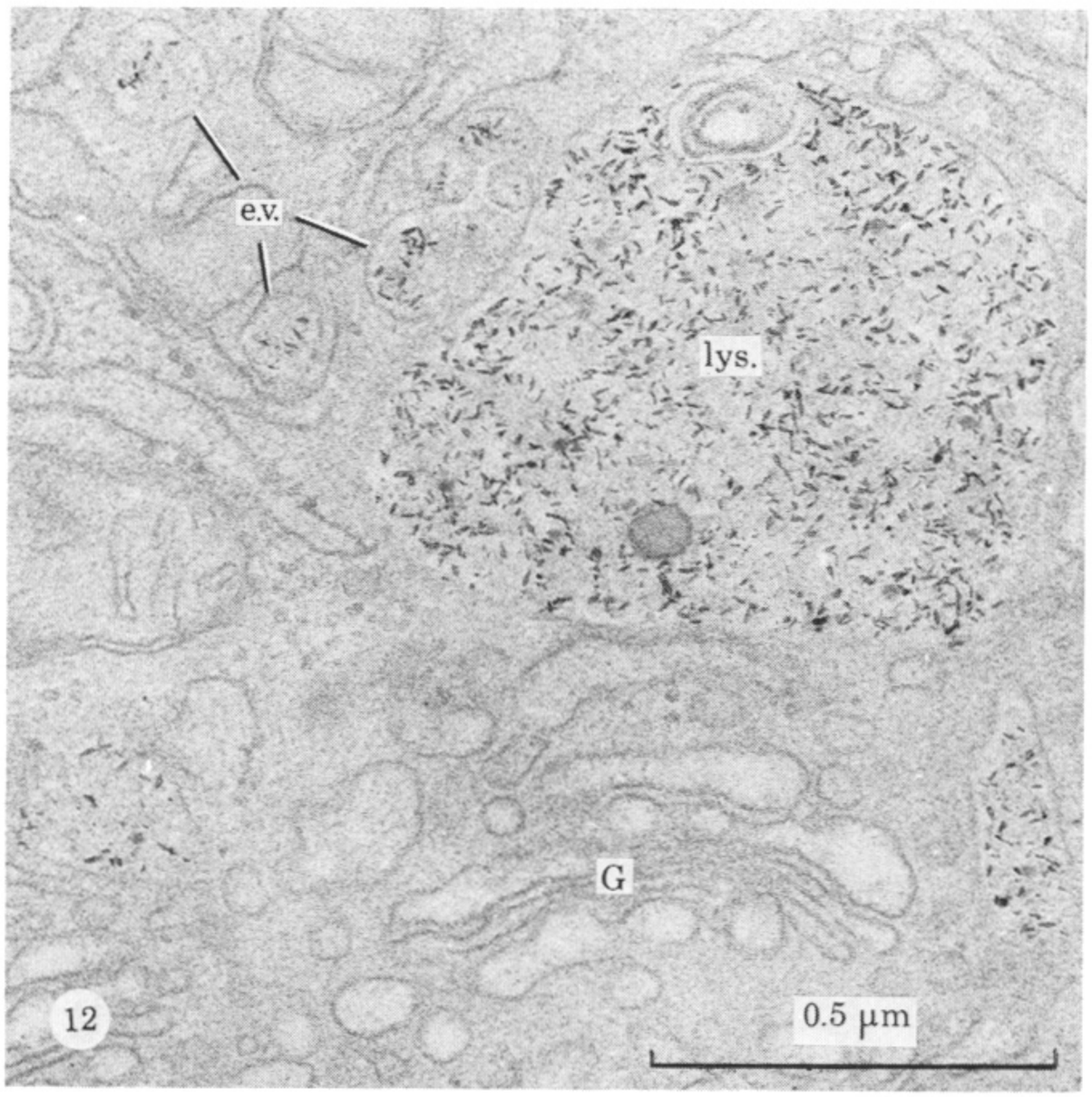
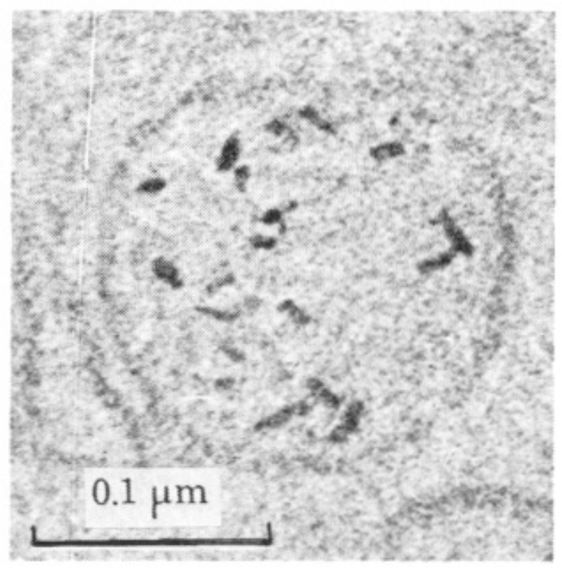
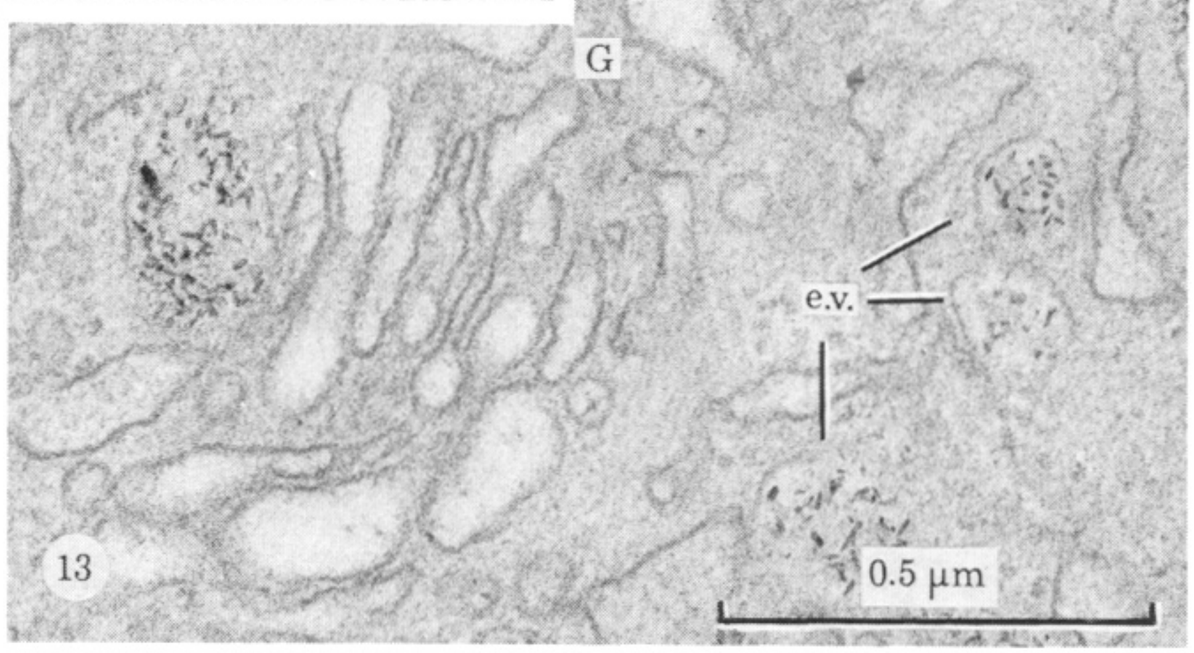
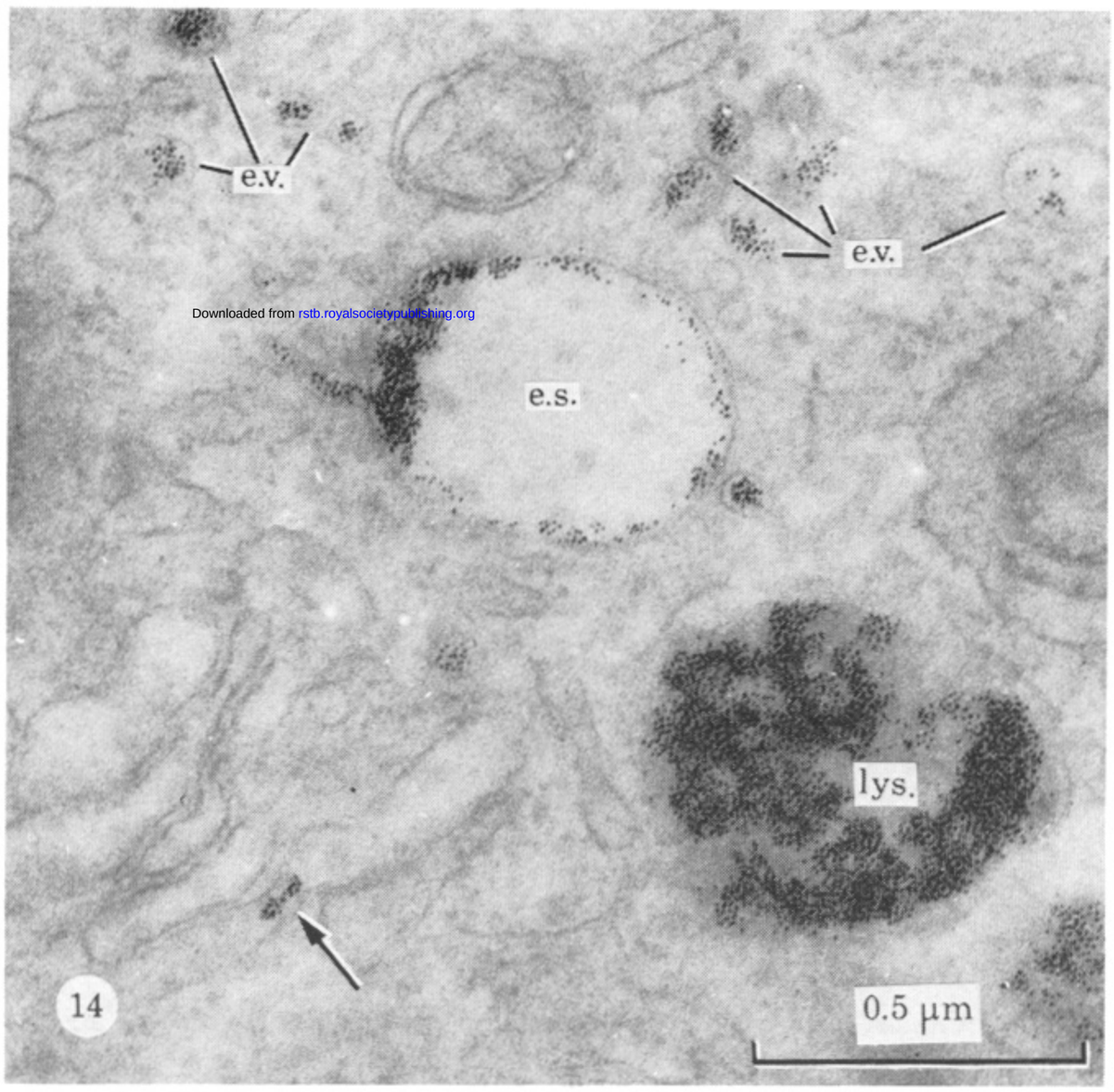
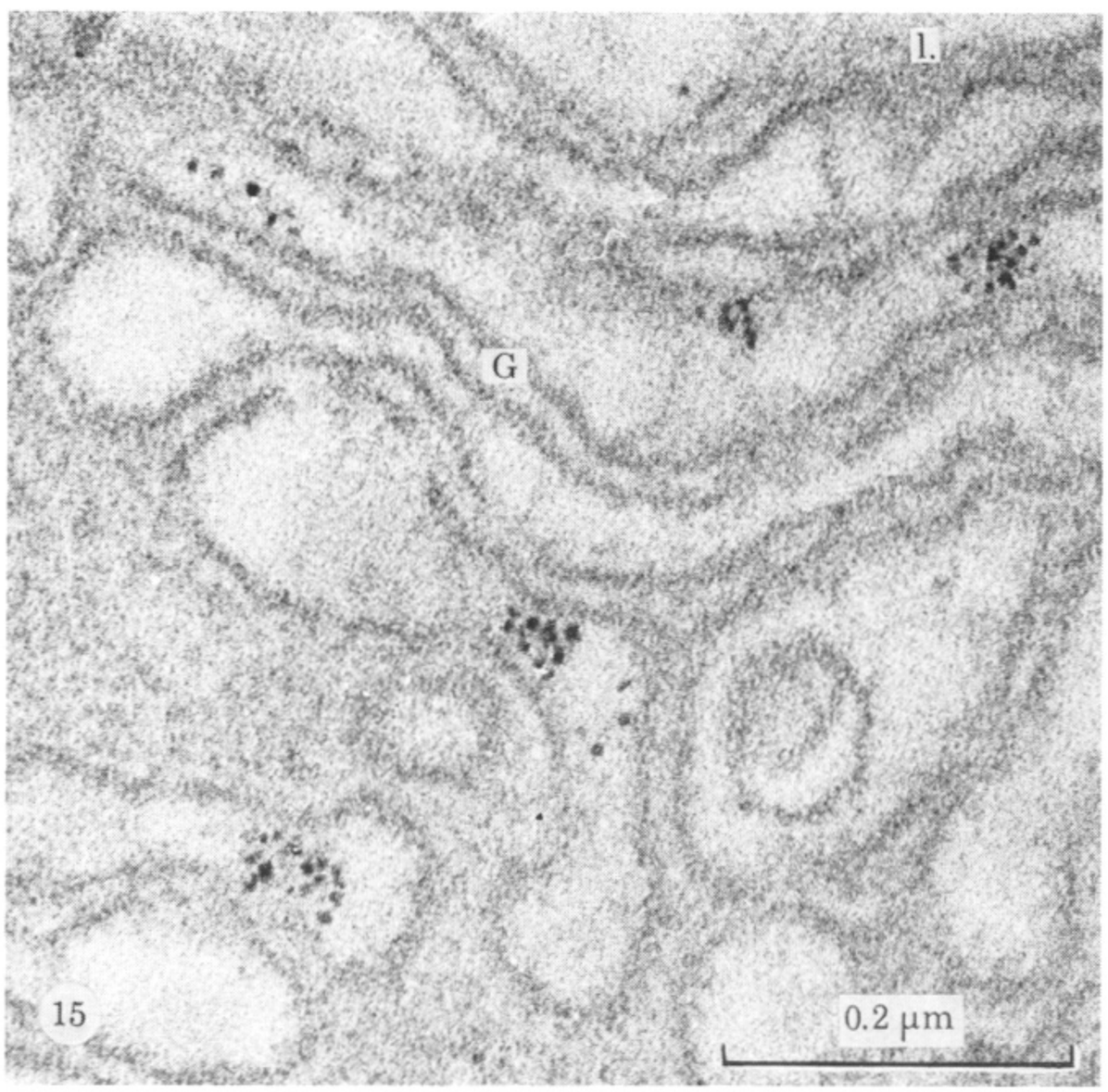
FIGURES 6–8, 10 AND 11. For description see opposite.











FIGURES 12–15. Pathways of endocytosis from the apical plasma membrane of inside-out thyroid follicles suspended in a medium containing a content marker (iron dextran, figures 12 and 13) or a non-specific membrane marker (cationized ferritin, figures 14 and 15). Internalized iron dextran is restricted to endocytic vesicles (e.v.) and to lysosomes (lys., figure 12). Cationized ferritin is found in endocytic vesicles (e.v.), in endosomes (e.s.) and, 30 min later (figure 14), also in Golgi cisternae (arrow, figure 14). Stacked Golgi cisternae are more frequently labelled with cationized ferritin at 90 min (figure 15) whereas iron dextran (Imposil) remains limited to lysosomes and vesicles close to the Golgi complex without reaching its stacked cisternae (figure 13). Iron dextran is rod-shaped with a length of *ca.* 21 nm (inset, figure 13). It is therefore morphologically distinct from the spherical ferritin particle (figure 15).