Localization of the Epidermal Growth Factor (EGF) Receptor within the Endosome of EGF-stimulated Epidermoid Carcinoma (A431) Cells

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Abstract. We have followed the internalization pathway of both epidermal growth factor (EGF) and its receptor in human epidermoid carcinoma (A431) cells. Using EGF conjugated with horseradish peroxidase and anti-receptor monoclonal antibodies (TL5 and EGFR1) coupled either directly or indirectly to colloidal gold we have identified an extensive elaboration of endosomal compartments, consisting of a peripheral branching network of tubular cisternae connected to vacuolar elements that contain small vesicles and a pericentriolar compartment consisting of a tubular cisternal network connected to multivesicular bodies. Immunocytochemistry on frozen thin sections using receptor-specific antibody-gold revealed that at 4°C in the presence of EGF, receptors were mainly on the plasma membrane and, to a lesser extent, within some elements of both the peripheral and pericentriolar endosomal compartments. Upon warming to 37°C there was an EGF-dependent redistribution of most binding sites, first to the peripheral endosome compartment and then to the pericentriolar compartment and lysosomes. Upon warming only to 20°C the ligand-receptor complex accumulated in the pericentriolar compartment.

Acid phosphatase cytochemistry identifies hydrolytic activity only within secondary lysosomes and *trans* cisternae of the Golgi stacks. Together these observations suggest that the prelysosomal endosome compartment extends to the pericentriolar complex and that the transfer of EGF receptor complexes to the acid phosphatase–positive lysosome involves a discontinuous, temperature-dependent step.

EPIDERMAL growth factor (EGF)[1] is a potent mitogenic polypeptide, which, upon binding to specific receptors on the plasma membrane of its target cell, initiates a chain of intracellular events that have been studied extensively (1, 4–6, 12, 26, 28, 35, 38). By the use of labeled derivatives of EGF it has been shown both biochemically and morphologically that cell-bound EGF is rapidly internalized by its target cells into an endosomal compartment and is subsequently degraded in lysosomes (5, 16, 17, 30, 34). In most cell types continuous incubation at 37°C in the presence of EGF results not only in the degradation of EGF but also in the down-regulation of the EGF receptor. This led to the idea, for which Krupp et al. (29) and Stoscheck and Carpenter (36) have recently provided direct biochemical evidence, that most internalized EGF receptors are degraded along with their bound ligand.

Morphological experiments by McKanna et al. (30) using monomeric ferritin coupled to EGF indirectly demonstrated that the EGF receptor in A431 cells is internalized along with EGF to multivesicular bodies (MVBs) and subsequently to lysosomes. More recently Beguinot et al. (2) provided direct morphological evidence that down-regulation of the EGF receptor in KB cells is due to receptor internalization into an endosomal compartment followed by degradation in lysosomes.

Much of the work on internalization and processing of the EGF receptor and its ligand has been carried out in epidermoid carcinoma (A431) cells, mainly because of the large number of receptors for EGF that these cells express ($1–3 \times 10^6$/cell). However, although EGF receptors have recently been identified by immunocytochemistry in these cells (3), the morphological pathway of internalization of the EGF receptor has so far not been documented. Two monoclonal antibodies, TL5 (35) and EGFR1 (39), have been described that recognize, respectively, the carbohydrate domain and the protein backbone of the EGF receptor. Neither of these antibodies competes for the binding of EGF to its receptor. By using these antibodies and immunogold cytochemical techniques on ultrathin frozen sections we have been able to localize the EGF receptor in EGF-stimulated A431 cells. By incubating A431 cells with conjugates of EGF-horseradish peroxidase (HRP) we have also been able to follow the pathway taken by internalized peptide. Finally, by demonstrating acid phosphatase activity within EGF-stimulated cells, we have examined the relationship between the prelysosomal

[1] *Abbreviations used in this paper:* EGF, epidermal growth factor; HRP, horseradish peroxidase; MVB, multivesicular body.

endosome compartments and lysosomes concerned with processing the internalized receptor complexes.

Materials and Methods

Cell Culture and Incubation with Tracers

A431 cells were cultured in Dulbecco's modified Eagle's medium, 10% fetal calf serum on 3-cm-diam petri dishes. Before each experiment they were rinsed free of serum and incubated for 1 h in serum-free DME. They were incubated with the various tracers as described in the text, and at each temperature change the cells were transferred directly to medium prewarmed or precooled to the appropriate temperature.

For peroxidase immunocytochemistry cells were fixed in dilute Karnovsky fluid (27) and incubated in a diaminobenzidine substrate bath as described by Graham and Karnovsky (14). They were then post-osmicated and embedded in Epon. Sections cut on a Sorvall Porter Blum MT2-B (DuPont Instruments, Newtown, CT) were examined in a Philips 301 electron microscope.

EGF-HRP Conjugate

EGF purified from mouse salivary glands was a gift from Dr. H. Gregory, ICI Pharmaceuticals, Macclesfield, Cheshire, England. HRP (type II) was obtained from Sigma, Poole, Dorset, England. The conjugate was prepared using 200 μg EGF and 2.5 mg HRP using N-succinimidyl 3-(2 pyridyldithio) propionate heterobifunctional reagent as recommended by the manufacturers (Pharmacia, Milton Keynes, England). They were then dialyzed to remove unreacted reagent. The HRP was activated with dithiothreitol and chromatographed on Sephadex G25. The activated HRP and EGF were then reacted together for 2 d at 5°C. Finally, free EGF was removed from the conjugate by filtration on a YM IO Amicon filter (Amicon Corp., Danvers, MA). The preparation of this conjugate is essentially the same as that described and characterized previously (41). Using ^{125}I-EGF to trace label the EGF indicated a molar ratio of EGF to HRP of 1:10 and suggested that there were substantial amounts of free HRP in the preparation. However, previous studies employing free HRP with the protocol used in this study (18) have shown that in A431 cells the binding and uptake of free HRP is negligible.

Manufacture of TL5-Colloidal Gold Complexes

Colloidal gold (~12-nm diam) was made according to the standard methods of Horisberger and Rosset (25) and Frens (10), and titrated to pH 7.5 with aqueous K_2CO_3. To prepare the complex, TL5 was dialyzed overnight against 0.005 M NaCl and mixed with the colloidal gold according to the method described by Geoghegan and Ackerman (11). Carbowax was added at a final concentration of 0.01% before centrifugation at 20,000 *g* for 20 min and resuspension in phosphate-buffered saline (PBS) + 0.01% carbowax. The TL5-gold conjugate was stored at 5°C. For immunocytochemistry, TL5-gold was washed by centrifugation in PBS containing 0.01% carbowax on a Beckman airfuge (Beckman Instruments Inc., Palo Alto, CA) at 100,000 *g* for 1 min and then resuspended in PBS + 0.1% bovine serum albumin (BSA) and 0.01% carbowax. Electron microscope examination of the gold conjugate before use revealed that the gold was monodisperse.

Manufacture of Protein A-Colloidal Gold Conjugates

Colloidal gold (~8-nm diam) was made according to the standard method of Faulk and Taylor (8) and titrated to pH 7.5 with 0.01 M acetic acid. The gold was stabilized by the addition of a solution of protein A (Pharmacia) according to the method of Geoghegan and Ackerman (11). Carbowax was added at a final concentration of 0.01% before centrifugation twice at 50,000 *g* for 60 min and resuspension in 0.01% carbowax after the first wash and 20 mM Tris-buffered saline, pH 8.3, containing 0.1% BSA and 0.02% azide after the second wash. The protein A-gold conjugate was stored at 5°C. For immunocytochemistry, protein A-gold was washed by centrifugation in PBS containing 0.01% carbowax and 0.1% BSA in a Beckman airfuge at 100,000 *g* for 2 min and then resuspended in PBS + 0.1% BSA and 0.01% carbowax.

Immunocytochemistry

A431 cells were fixed for 30 min in 8% paraformaldehyde in phosphate buffer, pH 7.4 (TL5), or for 60 min in 2% paraformaldehyde + 0.05% glutaraldehyde in phosphate buffer pH 7.4 (EGFR1), then scraped off the dish and pelleted in a Beckman microfuge. Small (0.5 mm^3) pieces of the pellet were frozen in liquid nitrogen, cryosectioned, and immunolabeled essentially as first described by Tokuyasu (37). The sections were either labeled with TL5 coupled to colloidal gold for 45 min or incubated with EGFR1 for 30 min followed by protein A-gold for 20 min, then washed for 20 min in five changes of PBS. Staining and embedding were carried out according to the method described by Griffiths et al. (15). The sections were examined at 80 keV in a Philips 301 electron microscope.

For combined acid phosphatase cytochemistry and immunolabeling of EGF receptors on frozen thin sections, A431 cells were fixed in 2% paraformaldehyde/0.05% glutaraldehyde and then processed as described by Robinson and Karnovsky (33) using cerium as the capture reagent. Control preparations in which sodium beta-glycerophosphate was omitted from the substrate bath showed no reaction product even after prolonged incubations (120 min). The acid phosphatase staining was followed by freezing and cryosectioning as described above. Thin frozen sections were incubated with EGFRI and 8-nm protein A-gold and processed for microscopy. Acid phosphatase stained material was also processed for Epon embedding.

Results

In recent studies in which the uptake and processing of transferrin and transferrin receptors were followed in A431 cells, two endosome compartments were described (23, 24). The peripheral compartment lying immediately beneath the plasma membrane consists of a branching network of tubular cisternae connected to vacuolar elements that contain small vesicles. The juxtanuclear compartment consists of a tubular cisternal network connected to MVBs and also includes apparently free lysosome-like bodies. For the present work on EGF-ligand receptor complexes, we made preliminary studies using EGF-peroxidase and anti-transferrin receptor antibody-gold. The extensive co-localization of the two tracers suggested that both of the endosome compartments identified previously were involved in processing EGF.

Since EGF stimulation is known to induce both receptor-mediated uptake and fluid-phase endocytosis in A431 cells we have adopted an experimental protocol that minimizes the fluid phase uptake of EGF. This protocol involves incubation of the cells at 5°C in the presence of EGF, followed by rinsing in the cold and transfer to EGF-free medium at either 20 or 37°C. 20°C incubations were employed because it has been shown in hepatocytes that at this temperature the transfer of endocytosed ligand to lysosomes is inhibited (7). By comparing compartments labeled at 37 and 20°C some indication of the extent of the prelysosomal compartments may therefore be obtained. Before proceeding with tracer experiments we examined the effect of these various temperature changes on the morphological appearance of the two previously described endosome compartments in both unstimulated and EGF-stimulated cells.

Effect of Temperature and EGF Stimulation on the Organization of the Endosome Compartments

In cells incubated at 5°C for 30 min, rinsed at 5°C, and then warmed to 20 or 37°C for up to 60 min, the organization of the peripheral endosome compartment was essentially as described previously. In cells warmed only to 37°C for 60 min, however, the juxtanuclear area became dominated by secondary lysosome-like structures containing myelin figures. In cells warmed to 20°C the number of autophagosome-like structures in the juxtanuclear area was greatly increased.

In cells incubated with EGF-HRP for 30 min at 5°C, rinsed at 5°C, and then warmed to 20 to 37°C, the cell periphery was seen, by light microscopy, to ruffle extensively during the earlier phases of the warm-up period. After this ruffling activity, examination by electron microscopy identified the appearance of large electron-lucent vacuoles in the peripheral cytoplasm and an increase in the number of tubular cisternae and vesicle-containing vacuolar elements. In cells incubated

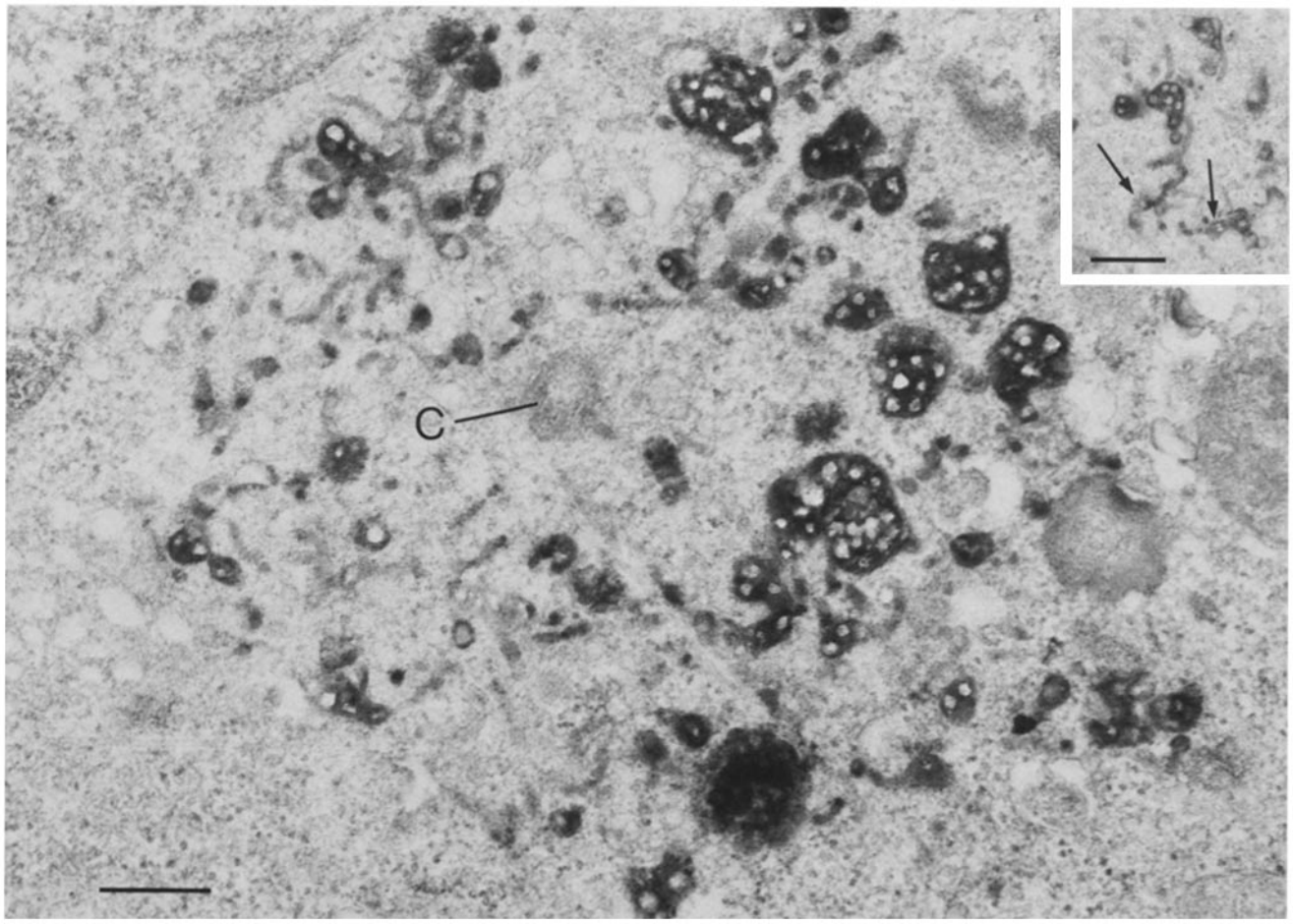

Figure 1. A431 cell incubated with EGF-HRP at 5°C for 30 min and then transferred to EGF-free medium at 20°C for 60 min. EGF-HRP reaction product is seen in the cisternal and MVB elements of the pericentriolar endosome compartment. The endosomal elements surround the centrioles (*C*). *Inset:* Pericentriolar endosome compartment with EGF-HRP reaction product in distended cisternae containing small vesicles (arrow). Bar, 200 nm.

for 30 min at 5°C in the presence of EGF-HRP, reaction product was distributed evenly over the plasma membrane. Upon transfer to warm medium, reaction product became concentrated within the tubular cisternae and vesicle-containing vacuolar elements of the peripheral endosome compartment. If instead of with EGF-HRP the cells were preincubated with free EGF at 5°C and then transferred to warmed medium containing free HRP, reaction product was observed in the larger electron-lucent vacuoles. This result confirms the earlier observations of Haigler et al., who used essentially the same protocol with EGF-ferritin and HRP (17) and showed that, whereas EGF induces fluid-phase endocytosis in these cells, the prebound EGF ligand is preferentially sequestered within endosomes derived by receptor-mediated uptake.

After incubation in EGF-HRP at 5°C, rinsing, and warming to 37°C, peroxidase reaction product began to appear in the juxtanuclear area after 15 min. At 20°C this distribution was not observed until after 30 min. Under the 37°C incubation conditions HRP reaction product in the juxtanuclear area was distributed throughout a branching smooth-surfaced reticulum of tubular cisternae, which was occasionally distended and contained small vesicles. Many MVBs also became labeled. These cisternal and vacuolar structures lay within the vicinity of the Golgi area as defined by the characteristic stacks of flattened cisternae, but their most obvious relationship was with the centriolar complex (Fig. 1). In appropriate section planes, the tubular cisternae and MVBs were seen to encircle the centrioles, the microtubules that radiate from the center of the complex extending between them. Lysosome-like structures also occurred in the pericentriolar region, and after incubations of 60 min at 37°C many of them contained peroxidase reaction product.

At 20°C and with incubations of up to 120 min, the number and distribution of MVBs in the pericentriolar area were similar to that observed at 15 min, but the number of autophagosome-like structures significantly increased. In preparations incubated at 20°C for ≥30 min EGF peroxidase in the pericentriolar area appeared to be confined to the MVBs and their associated cisternae.

Distribution of TL5 Antibody/Gold and EGFR1-Protein A-Gold Complexes in A431 Cells

In general, all of the morphological features identified in conventional thin sections were identifiable in ultrathin frozen sections, and both antibodies localized the EGF receptor to identical cellular organelles. However, a difference in the density of labeling was observed between the two antibodies in that TL5 labeled the plasma membrane more heavily than

did EGFR1, and this level remained high even after the EGF receptor had been induced to internalize. This will be discussed further below. In cells incubated at 5°C with EGF and fixed without warming, labeling was heaviest along the plasma membrane (Fig. 2). Within these cells peripheral vacuoles containing vesicles and occasional MVBs and lysosomes were also labeled but at a low level. Very occasionally gold particles were also observed over the flattened cisternae of the Golgi complex.

In cells preincubated at 5°C with EGF and then warmed to 37°C for 15 min the extensive peripheral endosome compartment observed in EGF-HRP-stimulated cells was the most heavily labeled compartment. Gold particles were distributed throughout the peripheral cisternae and were associated with both the limiting membrane and the vesicles of the vacuolar structures (Figs. 3 and 4).

After 30 min at 37°C, the cisternal elements, MVBs, and lysosome-like structures that characterize the pericentriolar area became the most heavily labeled compartments. In frozen thin section preparations the morphology of the MVB population appeared much more heterogeneous than in conventional resin-embedded sections. Some MVBs contained many tightly packed vesicles, and others, more similar to those of the peripheral compartment, contained fewer, usually larger vesicles (Fig. 4). Within these labeled MVBs gold particles were predominantly associated with the internal vesicles.

With incubations at 20°C pericentriolar MVBs were very heavily labeled, most containing many densely packed vesicles. Again, most of the labeling was associated with the internal vesicles of these MVBs.

Distribution of Lysosomal Hydrolases within the Endosome Pathway

Although incubations at 20°C suggest that the prelysosomal endosome extends to the MVB-cisternae of the pericentriolar complex it was important to identify the distribution of acid hydrolase–containing compartments in this area. Acid phosphatase activity was therefore displayed using the cerium capture method of Robinson and Karnovsky (33). In Epon sections of A431 cells acid phosphatase activity was distributed throughout the *trans*-most cisternae of the flattened Golgi stack and within well-defined secondary lysosomal elements. However, many of the vacuolar elements in the pericentriolar area were acid phosphatase negative. In thick (800 nm to 1 μm) sections, it was clear that these elements, which were probably endosomal, were not connected to the acid phosphatase–positive elements, even though they often lay immediately adjacent to them (Fig. 5).

Labeling EGF-stimulated acid phosphatase–stained preparations with the EGFRI-protein A gold technique demonstrated that at 20°C most of the MVBs in the pericentriolar area that contain EGF receptors lack acid phosphatase reaction product. Conversely, the acid phosphatase–positive lysosomes rarely label with EGFRI. At 37°C and after incuba-

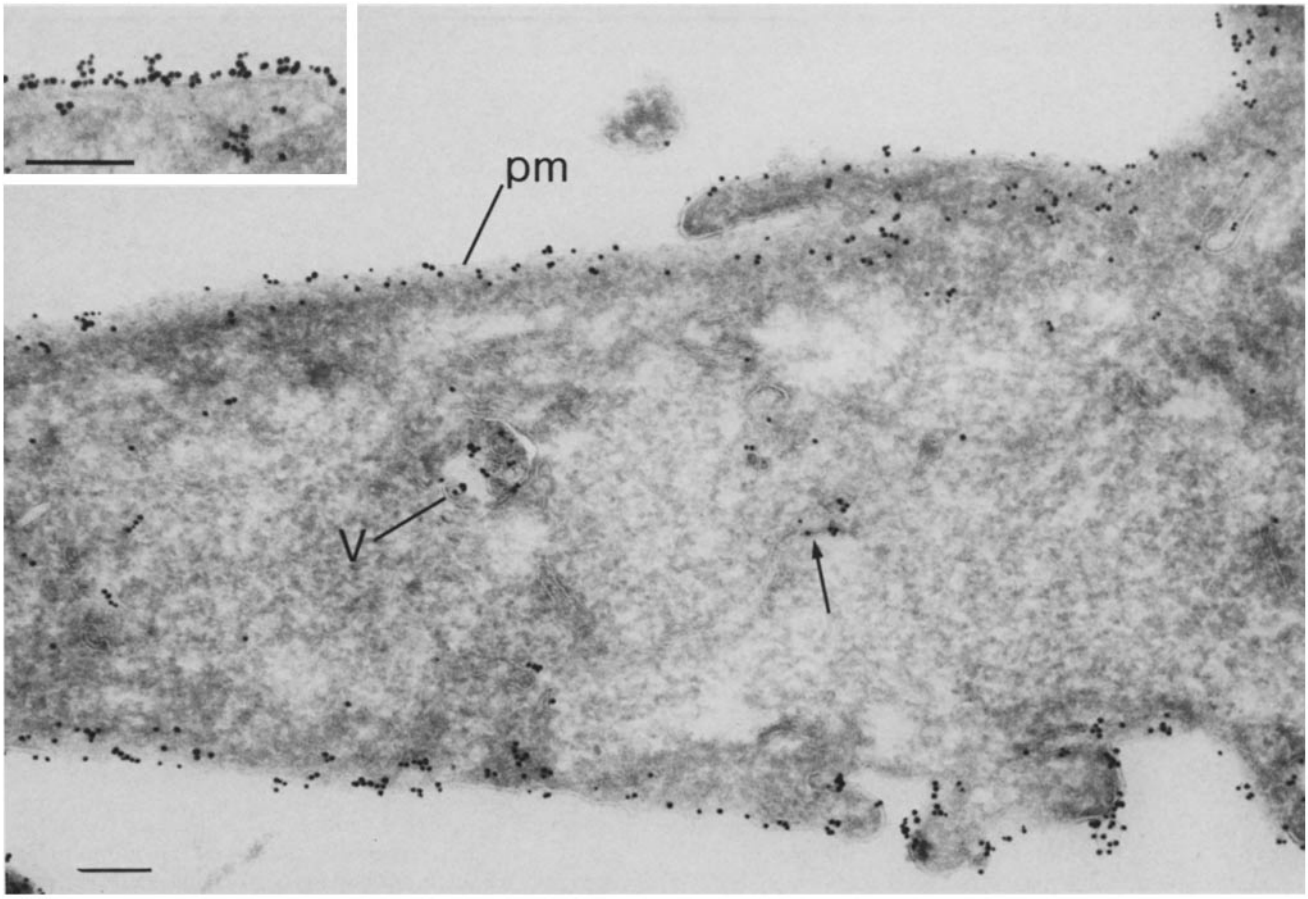

Figure 2. A431 cell incubated with EGF-HRP for 30 min at 5°C. Thin-frozen section labeled with TL5 antibody-gold. Labeling is heaviest along the plasma membrane (*pm*). A peripheral vacuole containing vesicles (*V*) and membrane-bound cisternal elements within the cytoplasm (arrows) are also labeled. *Inset:* TL5 antibody-gold labeling of the plasma membrane. Bar, 200 nm.

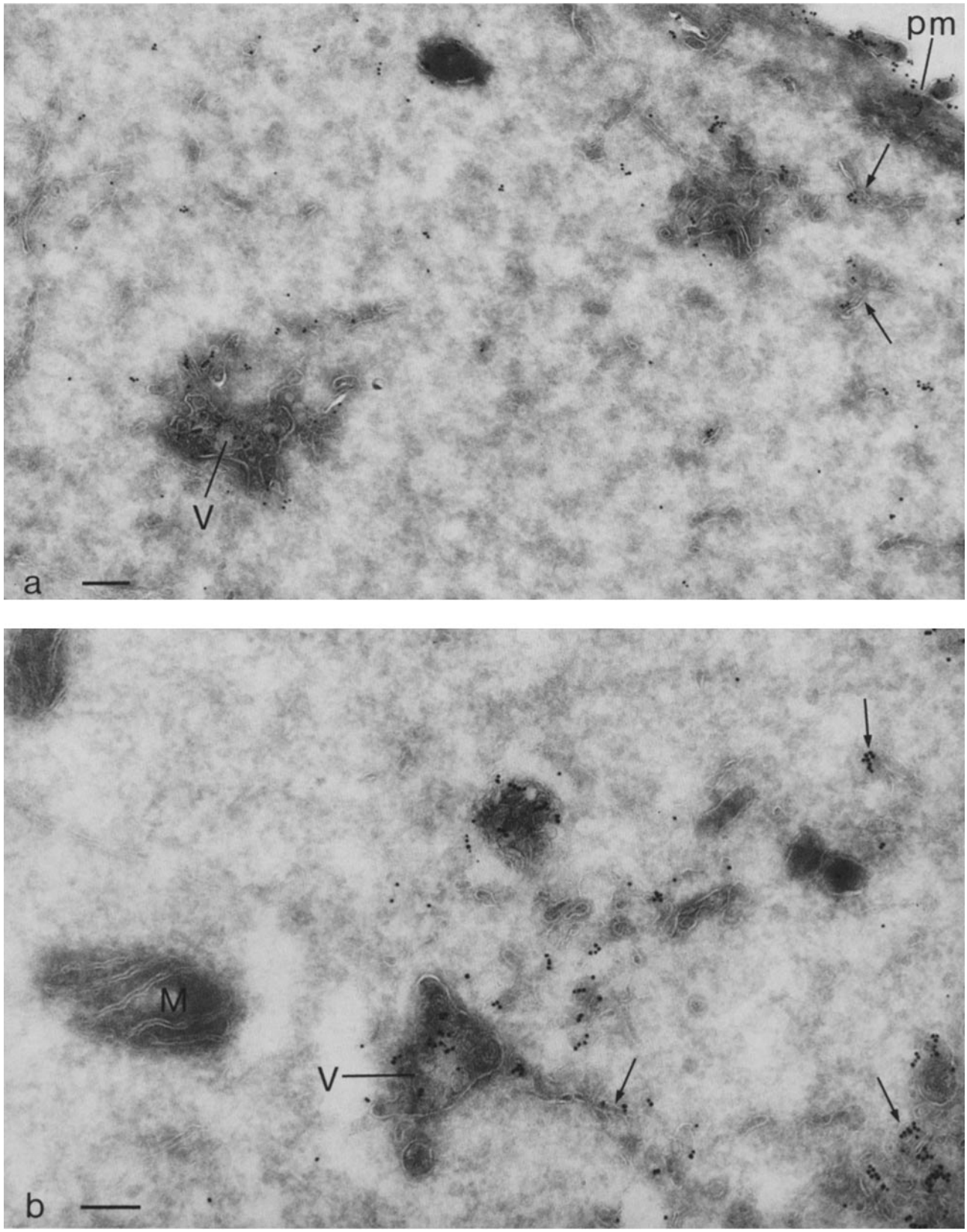

Figure 3. (*a* and *b*) Cells incubated with EGF-HRP at 5°C for 30 min and then transferred to EGF-free medium at 37°C for 15 min. Frozen sections were labeled with TL5 antibody-gold. The plasma membrane (*pm*) and peripheral endosome compartment, consisting of tubular cisternae (arrows) and vacuoles containing vesicles (*V*), are labeled. *M*, mitochondrion. Bar, 200 nm.

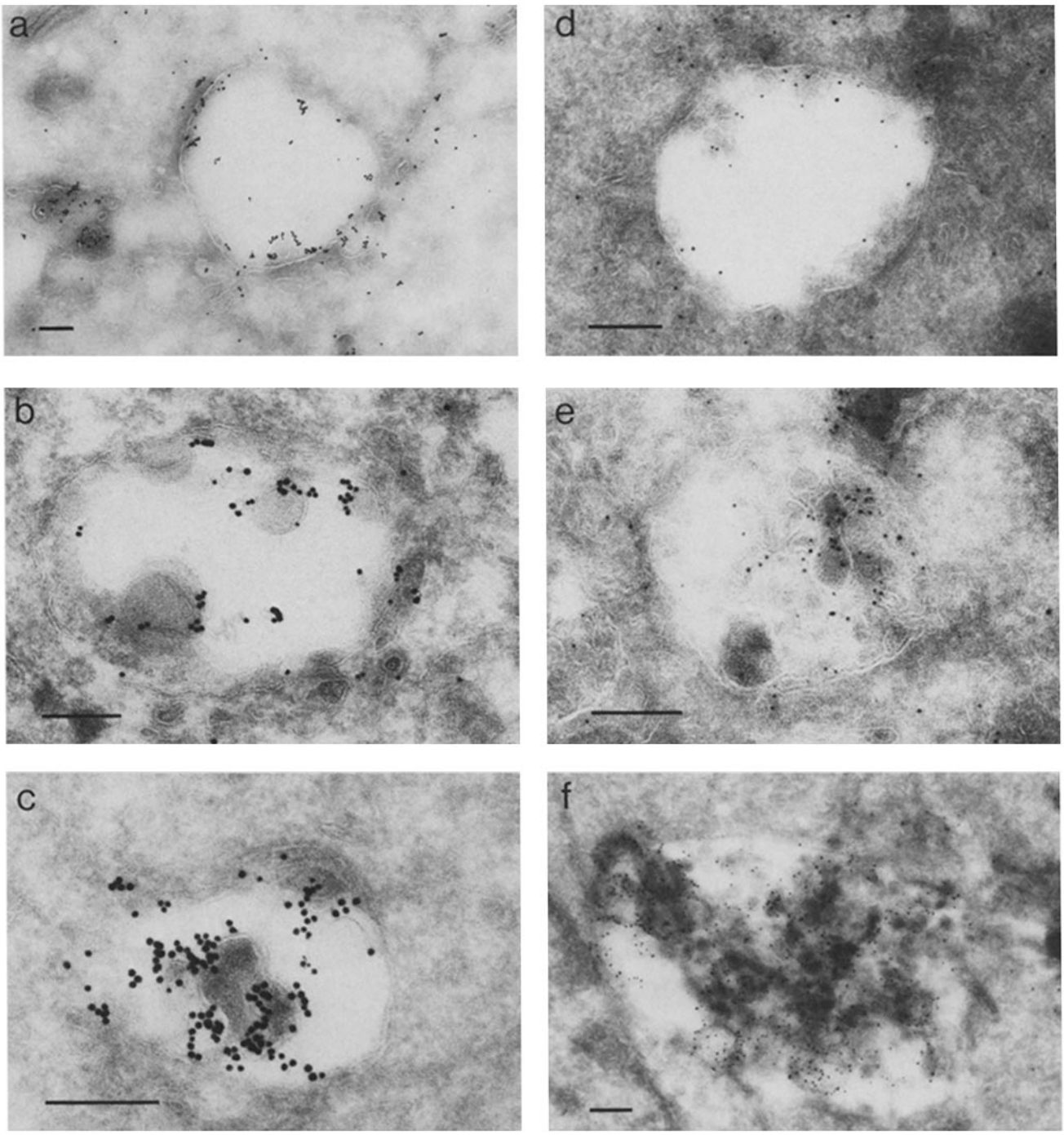

Figure 4. Cells preincubated with EGF-HRP at 5°C for 30 min and then transferred to EGF-free medium at 37°C for 30 min. (*a–c*) Frozen sections labeled with TL5 antibody-gold; (*d–f*) frozen sections labeled with EGFR1 followed by protein A-gold. Appearance of vesicle-containing vacuolar structures in the peripheral (*a* and *d*) and pericentriolar (*b*, *c*, *e*, and *f*) cytoplasm. In the peripheral elements gold particles label the limiting membranes and internal vesicles, whereas in the pericentriolar area labeling is usually associated with the internal vesicles. Bar, 200 nm.

tions of ≥30 min after EGF binding, EGFRI labels receptors within the acid phosphatase–negative MVBs and the acid phosphatase positive lysosomes (Fig. 6).

Discussion

In previous reports the uptake and processing of EGF-coupled ligands by the A431 cell has been studied extensively (16, 17, 30, 41). These studies have concentrated upon the peripheral elements of the endosome system, and in general our observations with EGF-HRP confirm these earlier reports. Comparing the distribution of transferrin-HRP (and transferrin receptor antibody-gold) (23) with the results obtained in the present study, it is clear that upon internalization both of these ligands become distributed throughout the same peripheral endosome compartment.

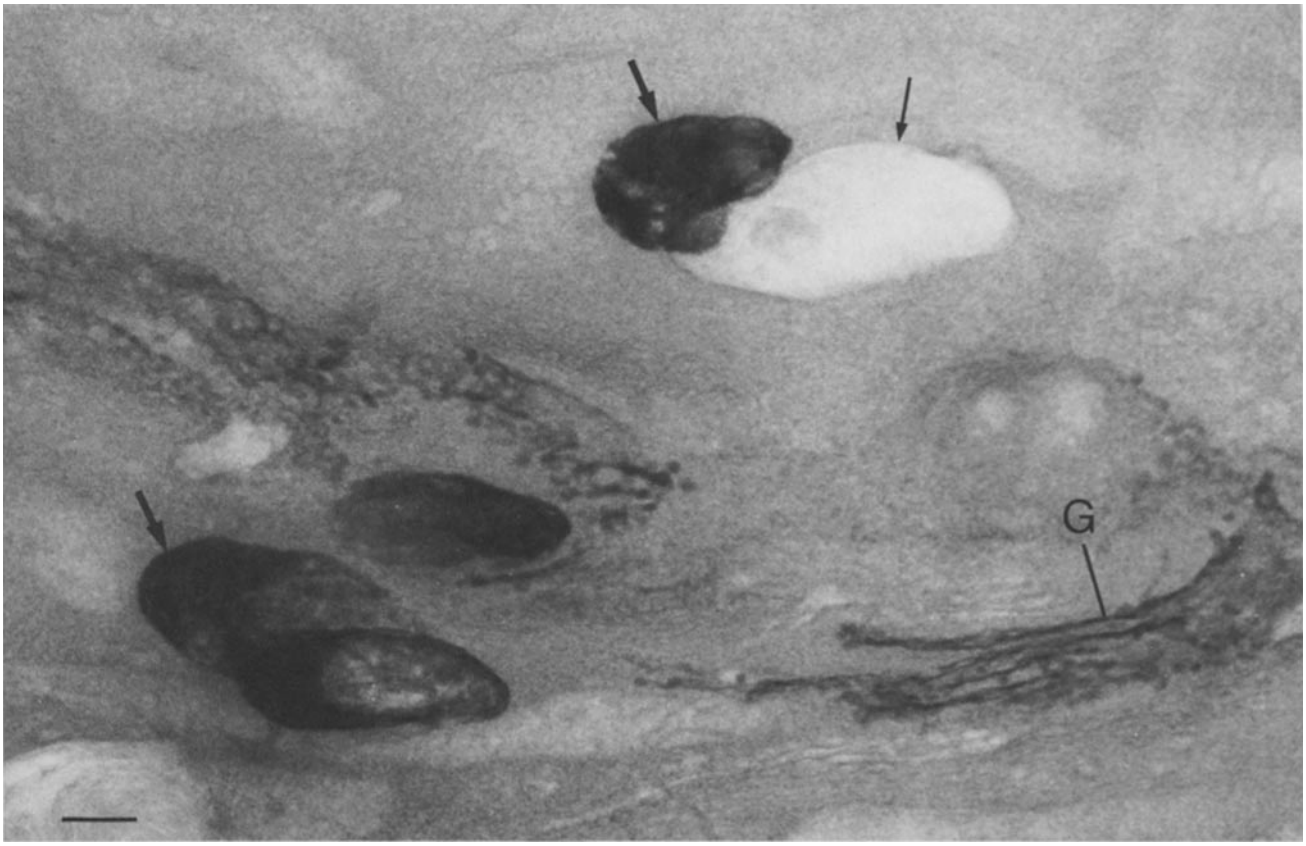

Figure 5. Thick (~1.0 μm) Epon section of an EGF-stimulated cell demonstrating the relationship between acid phosphatase–containing compartments of the Golgi area. The acid phosphatase–positive Golgi cisternae (*G*) and lysosomes (large arrows) are shown. The section is thick enough to accommodate the full thickness of a lysosome and demonstrates that although these acid phosphatase–positive structures lie close to Golgi cisternae and other vacuolar elements, they are separate from them. One kidney-shaped lysosome bends around an acid phosphatase–negative vacuole, which is probably an endosomal element (small arrow), but no connection between them is evident. Bar, 200 nm.

After an incubation of 15–60 min at elevated temperature the EGF-HRP was transferred to elements in the juxtanuclear area surrounding the centrioles. These elements included an elaborate reticulum of smooth-surfaced cisternae and many MVBs, and although similar structures occurred in the Golgi area before the tracer arrived, they were much less numerous and did not so obviously encircle the centrioles. The arrangement of these juxtanuclear elements is the same as that identified in a previous study using transferrin-HRP (23), and it is unlikely, therefore, to have arisen specifically in response to EGF. However, since this kind of arrangement is not nearly as extensive in untreated cells it is probably elaborated by the experimental protocol, which introduces a pulse of ligand into the endosome. In a recent study using ferritin-conjugated transferrin in Chinese hamster ovary cells, Yamashiro and Maxfield (42) identified a juxtanuclear complex of smooth-surfaced cisternae that was also adjacent but separate from the cisternal stacks of the Golgi complex. A similar juxtanuclear elaboration of the endosomal compartment over the same time scale has been described in cultured granulosa cells (22). In this system the movement of endosomes to the juxtanuclear area was shown to be inhibited by microtubule-disrupting agents (21).

In recent studies on KB cells HRP-EGF and HRP-transferrin internalization was followed (40), and the EGF receptor was identified by immunocytochemistry (2). In these studies the labeled ligands and receptors were also shown to be transferred via a common endosome compartment to the juxtanuclear area. A close relationship between the labeled elements and microtubules in the centriolar region was also noted. However, in these studies on KB cells, tracers were found within the Golgi area within 10 min of uptake and they were contained within a "*trans* Golgi reticulum" of cisternae rather than a cisternal-MVB complex. The authors suggested that the *trans* Golgi reticulum, identified by its content of internalized receptors, may correspond to the Golgi-endoplasmic reticulum-lysosome (GERL) compartment identified in a variety of cell types by Novikoff and others (31, 32). A common characteristic of GERL compartments is their acid phosphatase activity. Our observations on the compartments involved in the intracellular processing of EGF-receptor complexes in A431 cells suggest that the pericentriolar compartments we have identified are separate and different from the acid phosphatase–positive elements lying at the *trans* face of the Golgi stack.

In addition, our data on the distribution of acid phosphatase activity suggest that internalized ligand-receptor complexes transfer to a lysosomal compartment only after they have passed through the pericentriolar complex. The morphological insularity of the acid phosphatase–positive lysosomal elements and the inhibitory effects that lowering the temperature to 20°C have on the movement of EGF and EGF receptors

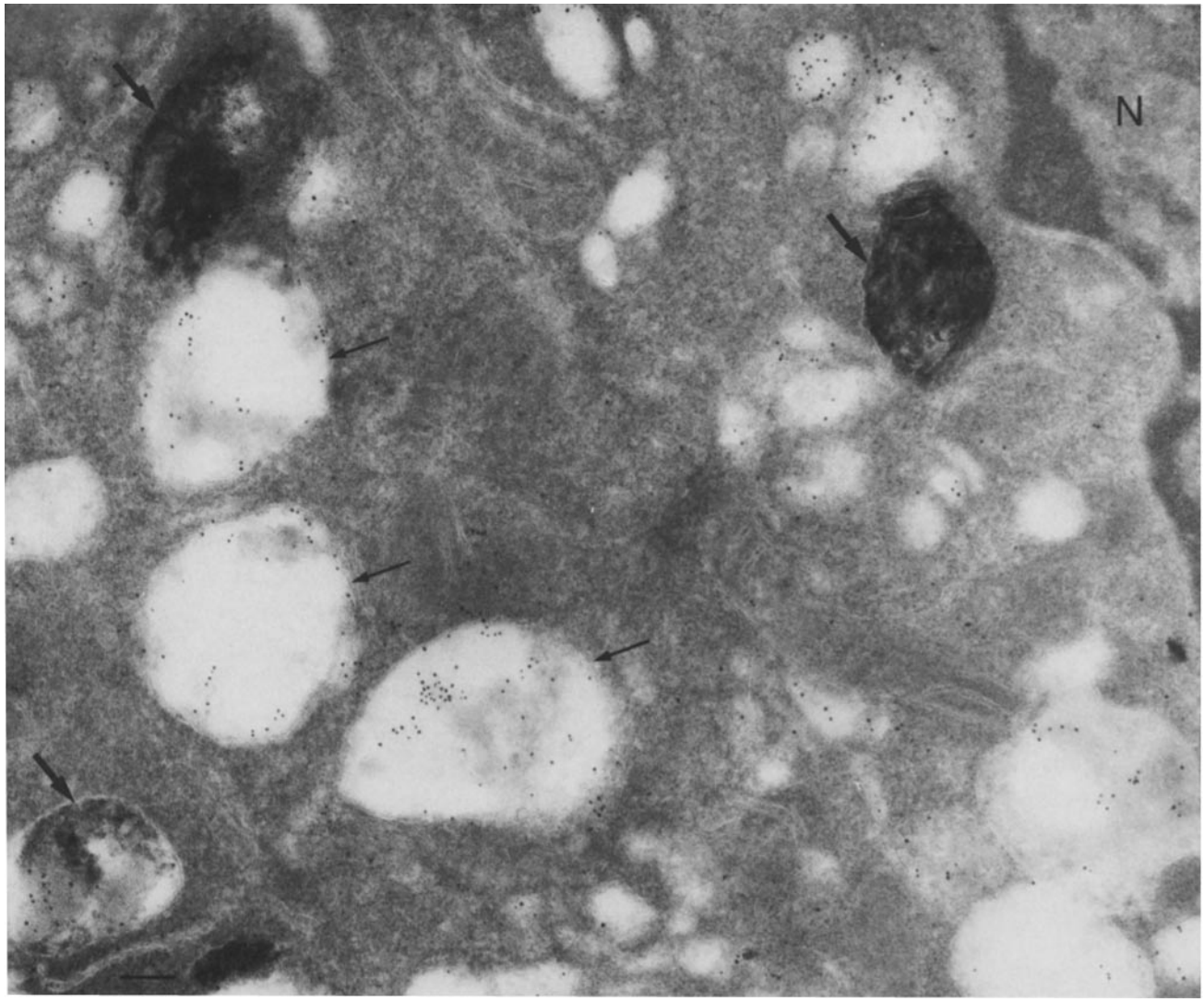

Figure 6. Cryosection of juxtanuclear area in cell stimulated with EGF for 30 min at 37°C demonstrating acid phosphatase activity and the distribution of EGF receptors. Gold particles demonstrate distribution of EGF receptors within multivesicular endosome elements that are acid phosphatase negative (small arrows) and lysosome elements containing acid phosphatase reaction product (large arrows). *N*, nucleus. Bar, 200 nm.

support earlier suggestions (7) that transfer from the endosome to the lysosome is a discontinuous step.

The TL5 antibody used in this study has been shown to identify a blood group antigenic determinant (13). It identifies the EGF receptor in A431 cells, because, unlike in other cell types, this receptor bears the same blood group antigen (9). However, this antigen is not specific for the EGF receptor in these cells because it is also found on other plasma membrane components such as glycolipids and possibly glycoproteins. A proportion of the labeling we have observed using this antibody on unstimulated cells must identify sites other than those of the EGF receptor. On unstimulated cells most of this label was concentrated on the plasma membrane with little internal labeling, and only in EGF-stimulated cells was there a very significant increase in the amount of label on intracellular boundaries. The available information on the selectivity of receptor-mediated uptake mechanisms does not, of course, allow us to exclude the possibility that antigenic sites on membrane components other than the EGF receptor may be internalized in response to EGF stimulation. However, a much higher level of labeling of the plasma membrane was obtained with TL5 at all time points than with EGFR1, perhaps suggesting that most of the non-EGF receptor binding sites are on the plasma membrane and are not internalized along with the EGF receptor. In addition, since the intracellular label coincided in all respects with the EGF-induced redistribution of EGFR1 binding sites, and of EGF-HRP, we believe that most of the label observed in intracellular compartments identifies the internalized receptor.

The distribution of the EGF receptor within the A431 cell during the earlier phases of EGF stimulation closely parallels that of EGF ferritin described by McKanna et al. (30). Within the MVBs and vesicle-containing vacuolar elements EGF-ferritin, TL5-gold, and EGFR1-protein A-gold complexes label the lumenal vesicles, a distribution distinctly different from that which has been observed for the transferrin receptor in this system (23). Harding et al. have, however, localized transferrin receptors to the internal vesicles of multivesicular

endosomes in reticulocytes (19, 20). In contrast to those in A431 cells, however, the vesicles of reticulocyte endosomes are destined for release into the extracellular space when the endosome fuses with the plasma membrane. The different localizations of these various receptor populations within multivesicular endosomes would seem, therefore, to be related to their destinations in the different cell types.

Throughout the endosome system of the A431 cell, the kinetics of transport and the distribution within the various intracellular compartments of TL5 and EGFR1 binding sites and EGF-HRP reaction product are the same. Our observations therefore agree with earlier biochemical studies that showed that a significant proportion of the EGF ligand-receptor complex remains intact throughout its intracellular processing. Taken together, our studies show that in A431 cells EGF binding induces EGF receptors to internalize to elaborate prelysosomal endosome compartments in the peripheral and pericentriolar cytoplasm, and subsequently to the lysosome. Within the MVBs of the pericentriolar compartment the receptors are located predominantly upon lumenal vesicles. This location is different from that of internalized transferrin receptors, which are distributed preferentially in the cisternae and on the limiting membranes of the pericentriolar elements.

We thank Angela Brennan, Carole Thomas, Adrian Walsh, Jenny Willcock, and Adele Gibson for their excellent technical assistance. The EGF-R1 antibody was kindly provided by Peter Goodfellow, Imperial Cancer Research Fund Laboratories, London.

K. Miller was supported by a grant from the North West Cancer Research Campaign, Liverpool.

Received for publication 3 December 1984, and in revised form 30 October 1985.

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