Effect of Colchicine on the Golgi Complex of Rat Pancreatic Acinar Cells

MARGIT PAVELKA and ADOLF ELLINGER
Institute of Micromorphology and Electronmicroscopy, University of Vienna, Austria

ABSTRACT Colchicine administered to adult rats at a dosage of 0.5 mg/100 g of body weight effected a disorganization of the Golgi apparatus in pancreatic acinar cells. The results obtained after various periods of treatment (10 min to 6 h) showed (*a*) changes in all components of the Golgi complex, and (*b*) occurrence of large vacuoles that predominated in cytoplasmic areas outside the Golgi region.

The alterations in Golgi stacks concerned elements of the proximal and distal side: (*a*) accumulation of transport vesicles, (*b*) formation of small, polymorphic secretion granules, and (*c*) alterations in the cytochemical localization of enzymes and reaction product after osmification. Transport vesicles accumulated and accompanied short, dilated cisternae, which lack mostly the reaction products of thiamine pyrophosphatase, inosine diphosphatase, and acid phosphatase, and osmium deposits after prolonged osmification. After 4 to 6 h of treatment, accumulated transport vesicles occupied extensive cellular areas; stacked cisternae were not demonstrable in these regions. The changes on the distal Golgi side included GERL elements: condensing vacuoles were diminished; they were substituted by small, polymorphic zymogen granules, which appeared to be formed by distal Golgi cisternae and by rigid lamellae. Unusually extended coated regions covered condensing vacuoles, rigid lamellae, and polymorphic secretion granules. A cytochemical distinction between Golgi components and GERL was possible neither in controls nor after colchicine treatment. The cytochemical alterations in Golgi components were demonstrable 20–30 min following administration of colchicine; at 45 min, initial morphological changes—augmentation of transport vesicles and formation of polymorphic zymogen granules—became apparent.

20 min after administration of colchicine, conspicuous groups of large vacuoles occurred. They were located mostly in distinct fields between cisternae of the endoplasmic reticulum, and were accompanied by small osmium—reactive vesicles. Stacked cisternae were not demonstrable in these fields. Vacuoles and vesicles were devoid of reaction products of thiamine pyrophosphatase, inosine diphosphatase, and acid phosphatase.

The results provide evidence that formation of stacked Golgi cisternae is impaired after colchicine treatment. The colchicine—induced disintegration of the Golgi complex suggests a regulatory function of microtubules in the organization of the Golgi apparatus.

Antimicrotubular agents have repeatedly been shown to interfere with the intracellular transport of secretory material in various secreting cells, thus suggesting a role of microtubules in the regulation of the secretory pathway (1–17). In pancreatic acinar cells, from radiochemical and radioautographical studies an interference at the level of the Golgi apparatus has been proposed (1–4); reports on morphological alterations of the Golgi region after microtubule disruption (1–4, 18) are in accordance with similar descriptions in other tissues (5–9, 15–17). The Golgi apparatus has been shown to be displaced and fragmented into smaller units exhibiting conspicuous accumulations of vesicles. However, the connection between these alterations, and the disturbance of the intracellular transport of secretory material is not yet clear. In the exocrine pancreas (2) and in the lacrimal gland (17) the delay of the intracellular transport has been demonstrated as early as 20

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min (2) to 25 min (17) after administration of antimicrotubular agents, whereas alterations of the Golgi apparatus were not existent before 70 min of treatment (17).

We were interested in the alterations occurring in the individual Golgi components after various periods of treatment with colchicine (19). For characterization of proximal and distal Golgi elements (20–23), we made use of prolonged osmification and of localization of thiamine pyrophosphatase (TPPase[1]), inosine diphosphatase (IDPase), and acid phosphatase (AcPase), respectively.

The analysis of distal Golgi components posed questions as to those structures that have been interpreted as Golgi-associated endoplasmic reticulum lysosomes (GERL; [24]). In pancreatic acinar cells, GERL has been defined as consisting of condensing vacuoles, rigid lamellae, and coated vesicles (25). The origin of the elements referred to as GERL is not described convincingly. These elements have been proposed to represent endoplasmic reticulum (24, 25); on the other hand, several morphological and cytochemical findings (26–30) indicate that they should be seen as Golgi components. It was interesting to see whether, in addition to other elements of the Golgi region, those structures that are defined as GERL would be affected by colchicine treatment as well.

MATERIALS AND METHODS

Female albino rats weighing 200–250 g were fasted overnight. Colchicine (Fluka AG, Basel, Switzerland; >98% purity), dissolved in 0.9% sodium chloride immediately before use, was injected intraperitoneally at a dosage of 0.5 mg/100 g of body weight. Time of administration was consistently 9 AM. Control animals received 0.9% sodium chloride. 10, 20, 30, 45 min, and 1, 2, 4, 5, or 6 h after administration of the drug, laparatomy was performed under anaesthesia with pentothal; pancreatic tissue was fixed in situ by injecting 2.5% glutaraldehyde (electron microscopy grade, Merck AG, Darmstadt, Federal Republic of Germany; in 0.1 M sodium cacodylate buffer) into the pancreatic region. Subsequently, the tissue was excised, cut into small pieces, and immersed in the same fixative.

For morphological studies, specimens were fixed in glutaraldehyde for 2, 4, or 6 h at 4°C; after an overnight rinse in 0.1 M sodium cacodylate buffer, postfixation in 1% veronal acetate-buffered OsO_4 for 1 h, and dehydration in a graded series of ethanol, the tissue was embedded in EPON. Ultrathin sections stained with alcoholic uranyl acetate and alkaline lead citrate were examined with a Philips 400 electron microscope. For cytochemical studies, fixation in glutaraldehyde was performed for 1 h at 4°C; after an overnight rinse in 0.1 M sodium cacodylate buffer containing 10% DMSO and 7.5% sucrose, specimens were sectioned on a freezing microtome (30–40 μm).

TPPase and IDPase were demonstrated according to Novikoff and Goldfischer (31) in a medium containing 25 mg of thiamine pyrophosphate or inosine diphosphate, respectively, 7 ml of aqua bidestillata, 10 ml of 0.2 M Tris maleate buffer pH 7.2, 5 ml of 0.025 M manganese chloride, 3 ml of 1% lead nitrate, and 1.25 g of sucrose for 70 min at 37°C. For localization of AcPase (32), sections were incubated in a medium consisting of 10 ml of 1.25% sodium-β-glycerophosphate, 10 ml of 0.1 M Tris maleate buffer pH 5.0, 10 ml of aqua bidestillata, 20 ml of 0.2% lead nitrate, and 7.5% sucrose for 30 min at 37°C. Cytochemical controls were performed in equivalent media lacking the substrate. After postfixation either in 1% veronal acetate-buffered OsO_4 or in 1% osmium ferrocyanide, specimens were dehydrated and embedded as described above. For prolonged osmification, small pieces of tissue were immersed in 2% aqueous OsO_4 and incubated at 40°C for 40 h (33); the osmium tetroxide solution was renewed after 24 h. Dehydration and embedding followed the procedures described above.

The results reflect examinations of three blocks (in the average) from 20 morphological and 12 cytochemical preparations each from controls and animals treated with colchicine for 6 h, and from five morphological and five cytochemical preparations each from animals treated for 10, 20, 30, 45 min, and 1, 2, 4 and 5 h. For the quantitative analysis of the cytochemical alterations after short periods of colchicine treatment, 400 Golgi stacks each from controls and animals treated for 20 and 45 min were examined for localization of TPPase, IDPase, and AcPase.

[1] *Abbreviations used in this paper:* AcPase, acid phosphatase, GERL, Golgi-associated endoplasmic reticulum lysosomes; IDPase, inosine diphosphatase; and TPPase, thiamine pyrophosphatase.

RESULTS

Controls (Figs. 1 and 2)

The Golgi apparatus of pancreatic acinar cells has been extensively described (25, 27, 34–36); thus, in the following description we concentrate on those details that are especially affected by colchicine treatment.

Transport vesicles 40 to 60 nm in diameter were gathered predominantly at the proximal face of Golgi stacks, and close to their poles. The individual stacks were composed of four to six cisternae of an irregular luminal width; their finely, granular content increased in density from the proximal to the distal side. Condensing vacuoles are shown to form from distal Golgi cisternae (Fig. 1, *a* and *c*). Rigid lamellae characterized by their regular luminal width of 20 to 30 nm and by "mid-line densities" (36; Fig. 1, *c* and *d*) were frequently, but not regularly, located near distal Golgi cisternae.

Coated areas were demonstrable on condensing vacuoles and on rigid lamellae (Fig. 1 *b*) but were rare on mature zymogen granules. Segments of rough endoplasmic reticulum, and transition elements with ribosomes attached to one surface only, could be shown near the distal Golgi components; we were not able, however, to demonstrate membrane continuities between endoplasmic reticulum and condensing vacuoles or rigid lamellae. Microtubules were apparent at both faces of Golgi stacks but were especially numerous at the proximal side (Fig. 1, *b* and *c*). Reaction products of TPPase (Fig. 2 *a*), IDPase (Fig. 2 *b*), and AcPase (Fig. 2 *c*) were observed over cisternae and vesicles at the distal Golgi face. Rigid lamellae and condensing vacuoles reacted strongly for AcPase; they were clearly reactive for IDPase as well, and showed weak reaction for TPPase. After prolonged osmification (Fig. 2 *d*), transport vesicles and proximal Golgi cisternae were reactive, whereas distal cisternae, rigid lamellae, and secretory granules lacked reaction product.

Colchicine Treatment (Figs. 3–9)

Administration of colchicine caused reduction of microtubules. 10–45 min after treatment microtubules were still visible, but after 1 h and after longer periods of treatment most exocrine pancreatic cells lacked microtubular profiles. 10 min after administration of colchicine the Golgi complex was morphologically and cytochemically unchanged in comparison to the controls.

20–30 AND 45 MIN OF TREATMENT (FIG. 3): The Golgi regions appeared morphologically unaltered but the number of stacks that exhibited one or more distal cisternae reactive for AcPase was diminished to 60% as compared with the controls.

The more spectacular finding after this period of treatment was the occurrence of large vacuoles which, arranged into groups, predominated in cellular areas outside the Golgi region (Fig. 3, *a–c*). These vacuoles were located in distinct fields between cisternae of the rough endoplasmic reticulum. They contained a finely granular material and were accompanied by small vesicles that resembled transport vesicles. Thus, these entities are somewhat similar with Golgi components; cisternae in stacked organization, however, were never demonstrable. Cytochemically, these vacuoles and vesicles

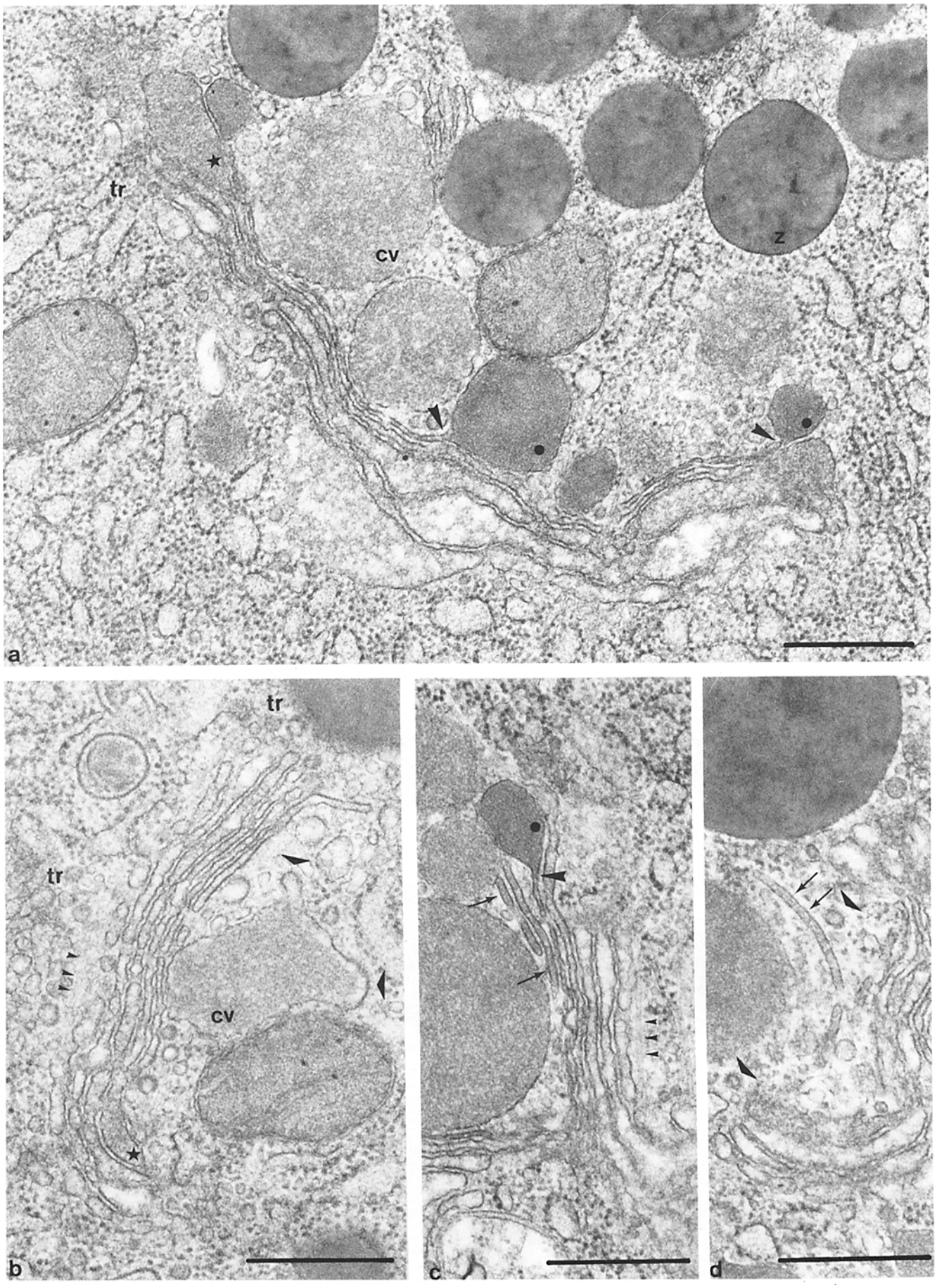


FIGURE 1 (*a–d*) Golgi stacks from pancreatic acinar cells of control animals. Golgi cisternae contain a finely granular material with increasing density from the proximal to the distal face of the stacks. The content of distal cisternae frequently resembles the material in condensing vacuoles (★) or in mature zymogen granules (●). Large arrowheads mark continuities between distal Golgi cisternae and forming condensing vacuoles. Rigid lamellae can be identified by their regular luminal width, and by "mid-line densities" (arrows in c and *d*). *cv*, condensing vacuoles; *z*, mature zymogen granules; ▲, coated areas, coated vesicles; *tr*, transport vesicles. Small arrowheads indicate microtubules. Bars, 0.5 μm. (*a*) × 47,000; (*b*) × 55,000; (*c*) × 52,000; (*d*) × 56,000.

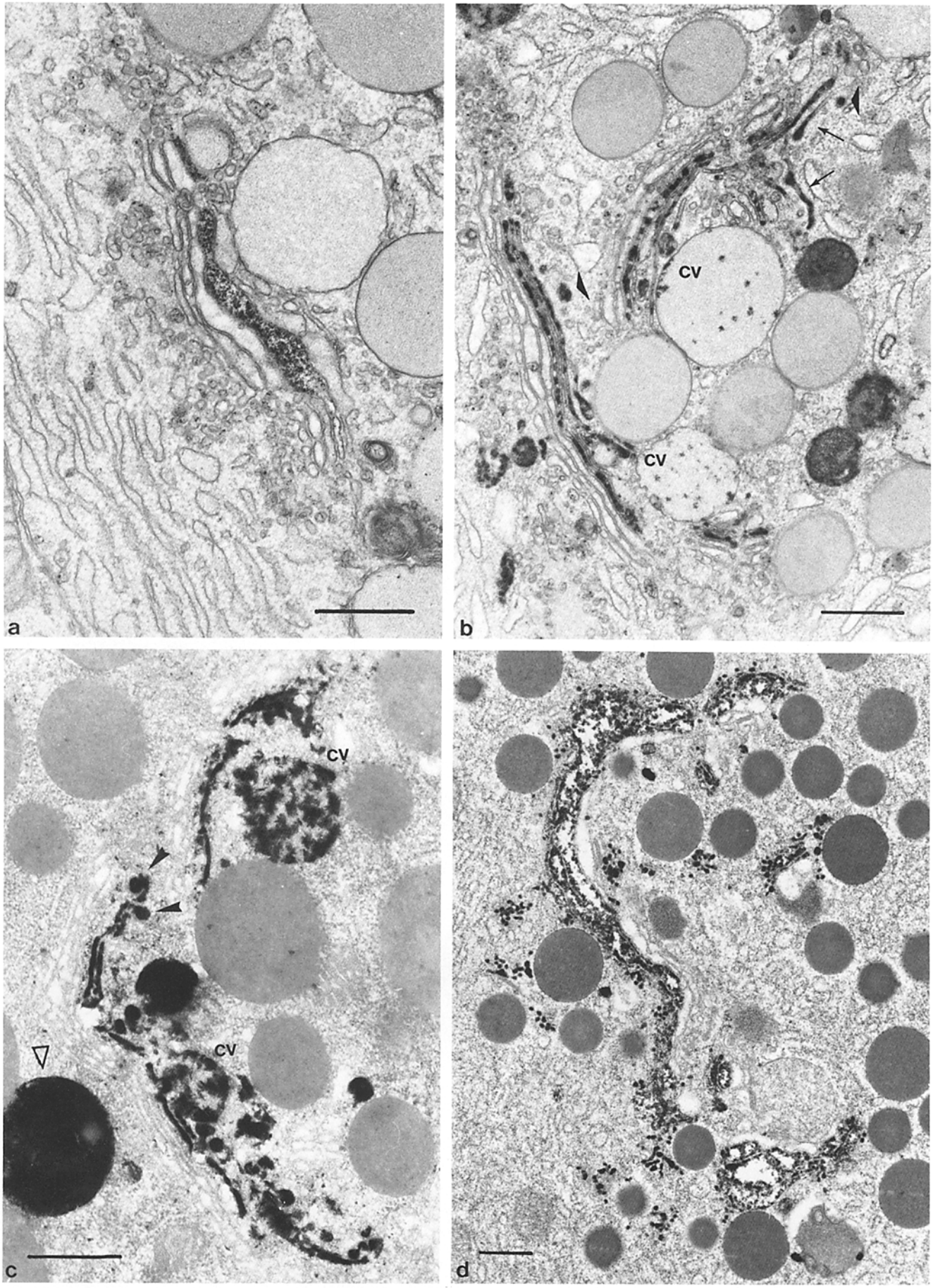


FIGURE 2 Controls and cytochemical results. (*a*) TPPase and (*b*) IDPase. Reaction product is apparent over distal Golgi cisternae, rigid lamellae (arrows in *b*), condensing vacuoles (*cv* in *b*), and over some coated and smooth vesicles (▲ in *b*). (*c*) AcPase. Distal Golgi cisternae, condensing vacuoles (cv), primary (large arrowheads) and secondary (△) lysosomes exhibit intense reaction. (*d*) Prolonged osmification. Osmium deposits are apparent over transport vesicles and over proximal Golgi cisternae; distal cisternae, condensing vacuoles, and mature zymogen granules are devoid of reaction. Bars, 0.5 μm. (*a*) × 37,000; (*b*) × 30,000; (*c*) × 33,500; (*d*) × 17,000.

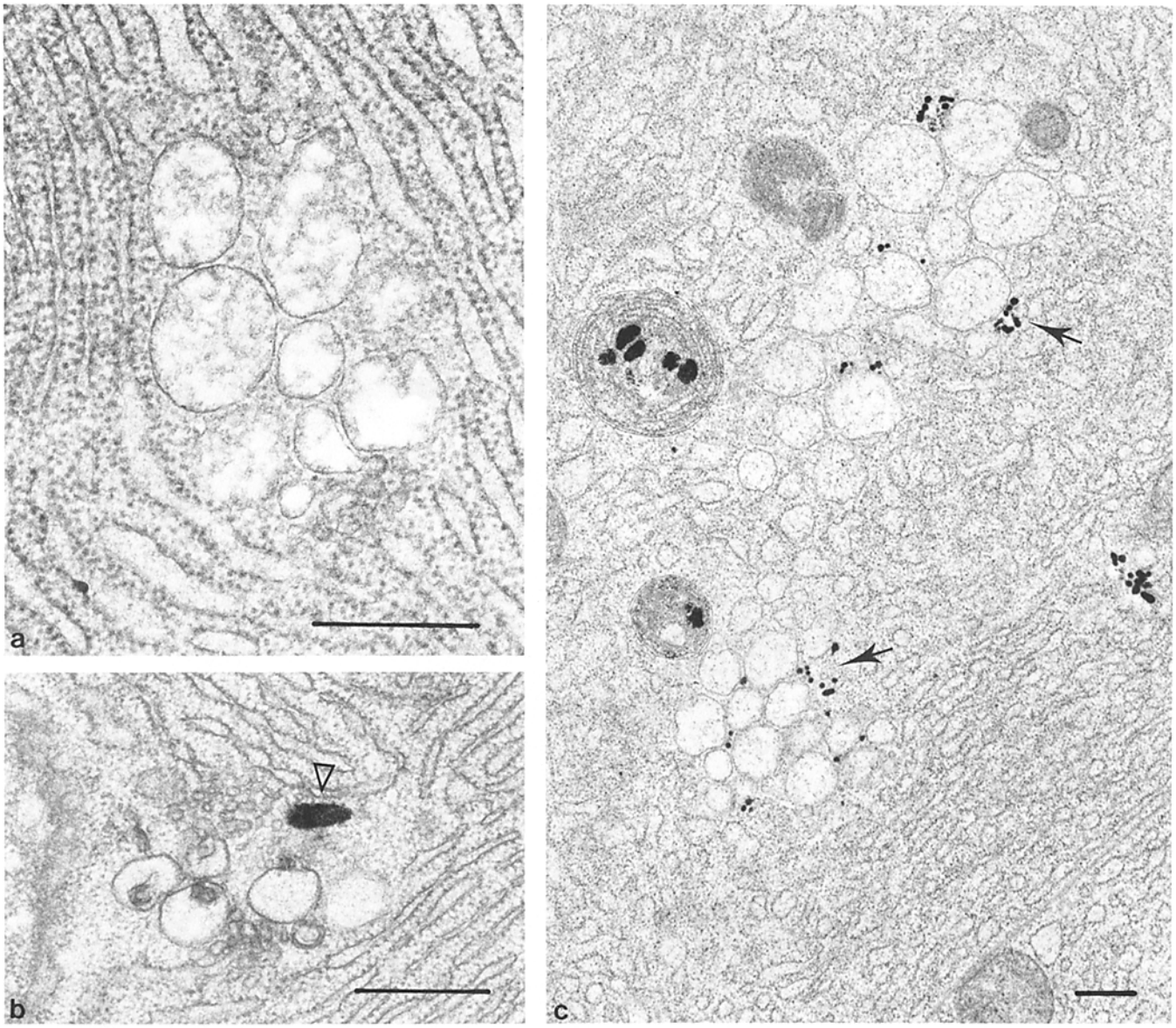


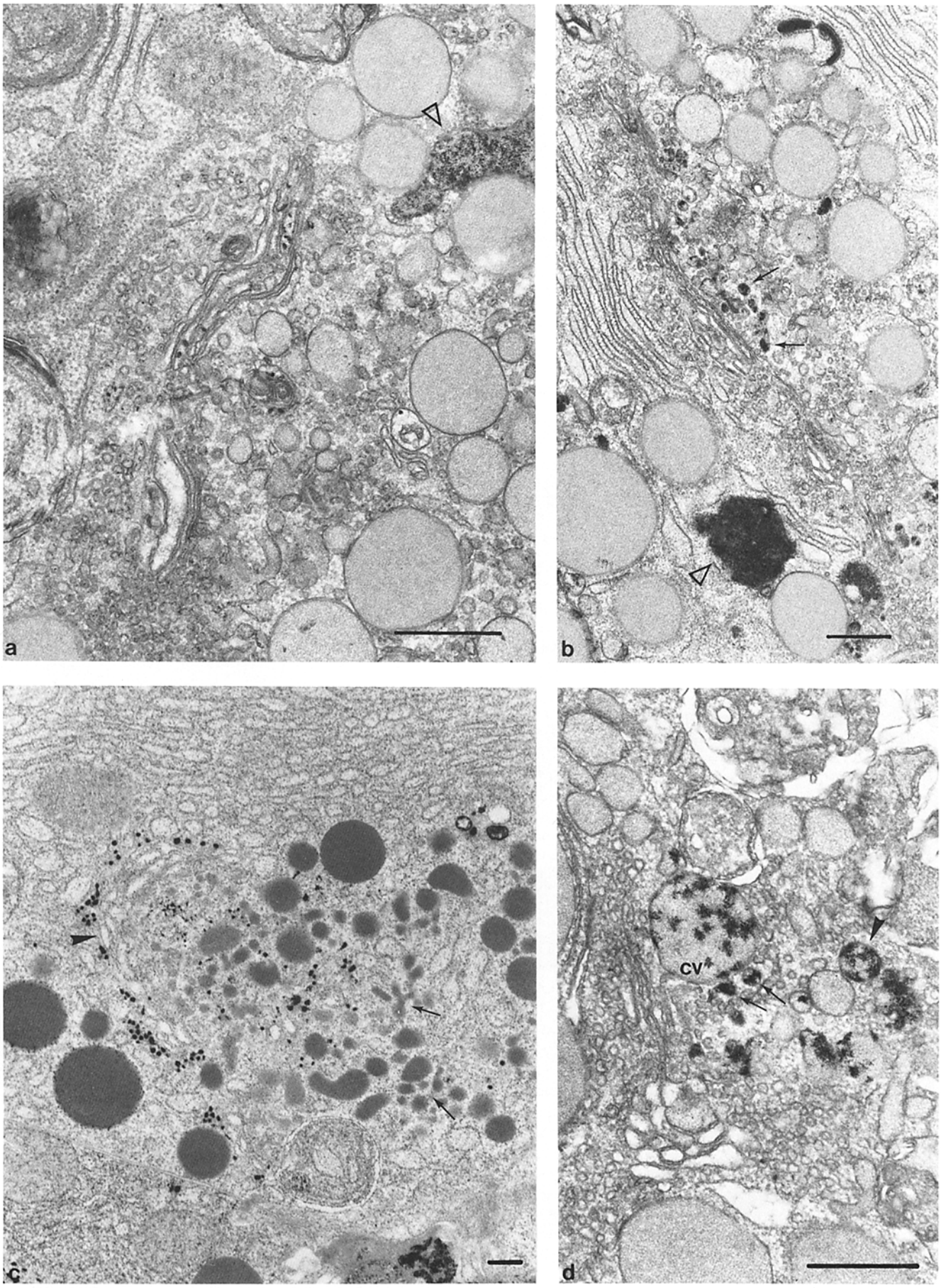
FIGURE 3 Colchicine treatment for 20 min: (*a*) morphology, (*b*) AcPase, (*c*) prolonged osmification. Groups of large vacuoles accompanied by small vesicles are located in distinct fields between cisternae of the rough endoplasmic reticulum. The small vesicles exhibit osmium deposits (*c*, arrows); vesicles as well as vacuoles are devoid of AcPase (*b*). ▷, secondary lysosome. Bars, 0.5 μm. (*a*) × 51,000; (*b*) × 40,000; (*c*) × 18,000.

lacked TPPase, IDPase, and AcPase (Fig. 3*b*). The vesicles exhibited osmium deposits after prolonged osmium treatment (Fig. 3*c*) and, thus, could be identified as transport vesicles.

At 45 min, similar with the results obtained after 20 to 30 min, the Golgi apparatus may appear morphologically unchanged, exhibiting cytochemical alterations solely. In several stacks initial morphological changes became apparent: the cisternae were dilated and shortened; transport vesicles were accumulated, and small, polymorphic zymogen granules occurred at the distal Golgi face. Groups of large vacuoles and transport vesicles became more pronounced; they were dominant structures in cellular areas outside the Golgi region.

1–6 H OF TREATMENT (FIGS. 4–8): Several Golgi stacks may still appear without prominent morphological alterations but will exhibit cytochemical changes. Most of the stacks showed diminished TPPase, IDPase, and AcPase, or lacked these enzymes (Fig. 4, *a* and *b*), as well as osmium deposits. Most stacks, however, were conspicuously altered. The changes concerned proximal and distal Golgi components including those structures that have been defined as GERL: condensing vacuoles and rigid lamellae. Numerous transport vesicles exhibiting osmium deposits after prolonged osmification were accumulated (Figs. 5, 7, and 8). They accompanied stacks that appeared to be fragmented into smaller units (Figs. 5 and 7); the cisternae were shortened and dilated; they lacked TPPase (Fig. 8*a*), IDPase, AcPase (Fig. 4*d*), and osmium deposits (Fig. 8*b*).

At the distal Golgi face, condensing vacuoles were diminished; they were substituted by small, polymorphic zymogen granules that appeared to be formed by distal Golgi cisternae and by rigid lamellae (Figs. 4*c*, 5, 6, *b–d*, and 7). Distal Golgi cisternae and the small zymogen granules exhibited a dense content resembling that of mature zymogen granules in controls. Unusually extended coated areas, contrasting with the


a
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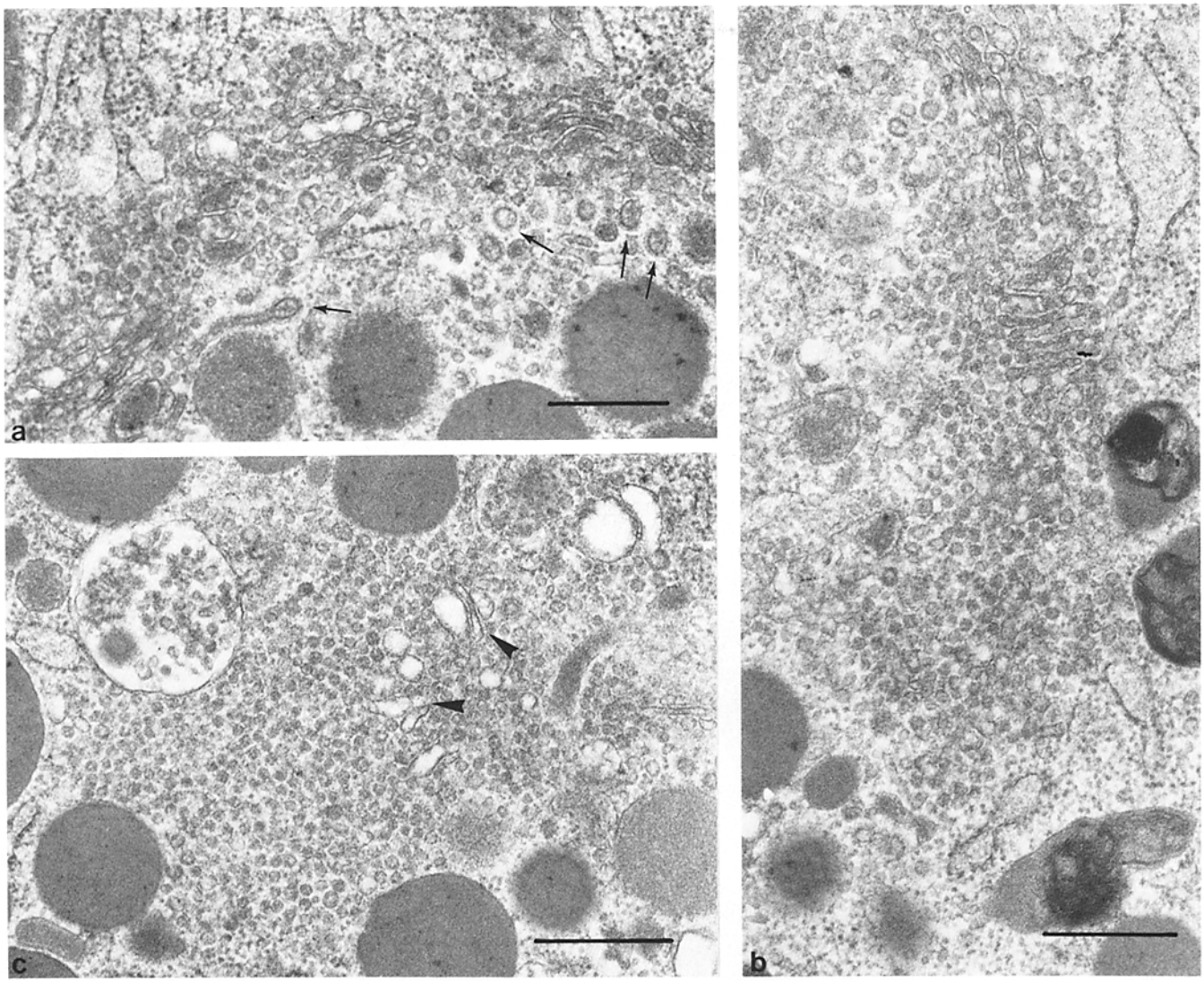

FIGURE 5 Colchicine treatment for 6 h. *a–c* demonstrates different stages of the colchicine-induced alterations of Golgi stacks. In *a* and *b* the cisternae, although shortened and partially dilated, are still in stacked organization; the stacks, however, appear to be fragmented into smaller units. Small, polymorphic zymogen granules, which frequently are covered with extensive coated regions (arrows), are formed at the distal Golgi side. In *b* the short stacks are accompanied by numerous transport vesicles. In c accumulated transport vesicles occupy a large cellular area which, in addition, includes some irregularly shaped vacuoles (large arrowheads); cisternae in stacked organization are not demonstrable. Bars, 0.5 μm. (*a*) × 37,000; (*b* and c) × 40,000.

more patchy appearance of the coats in the controls, covered condensing vacuoles, rigid lamellae, and the polymorphic zymogen granules (Figs. 5–7).

All changes in Golgi components were apparent 1 h after administering colchicine. After 4 to 6 h, accumulated transport vesicles occupied extensive cellular areas; these regions included a few short cisternal fragments and some vacuoles, but cisternae in stacked organization were not demonstrable (Figs. 5 *c*, 7, and 8). The groups of large vacuoles and transport vesicles that were demonstrable at 20 min after administering colchicine were dominant in all cellular areas after 1 to 6 hs of treatment as well (Figs. 7 and 8 *b*). Fig. 9 summarizes the main colchicine-induced alterations of the Golgi complex in pancreatic acinar cells.

FIGURE 4 Colchicine treatment for 6 h; (a) TPPase, (*b*) IDPase, (*c*) prolonged osmification, (*d*) AcPase. (*a* and *b*) Morphological alterations of these Golgi stacks are minor, but reaction after localization of TPPase (*a*) and IDPase (*b*) is very weak or absent. Primary (arrows) and secondary lysosomes (▷) are stained intensely. (*c*) Osmium deposits after prolonged osmification are restricted to transport vesicles, whereas proximal Golgi cisternae (large arrowhead), which are usually reactive in controls, are unstained. Numerous small, polymorphic zyomgen granules characterize the morphological picture at the distal Golgi face (arrows). (*d*) Reaction product of AcPase is attached to condensing vacuoles (cv), primary lysosomes (arrows), and some small zymogen granules (large arrowhead). Golgi cisternae and accumulated vesicles lack deposits of reaction product. Bars, 0.5 μm. (*a*) × 40,000; (*b*) × 23,000; (c) × 14,000; (*d*) × 42,000.

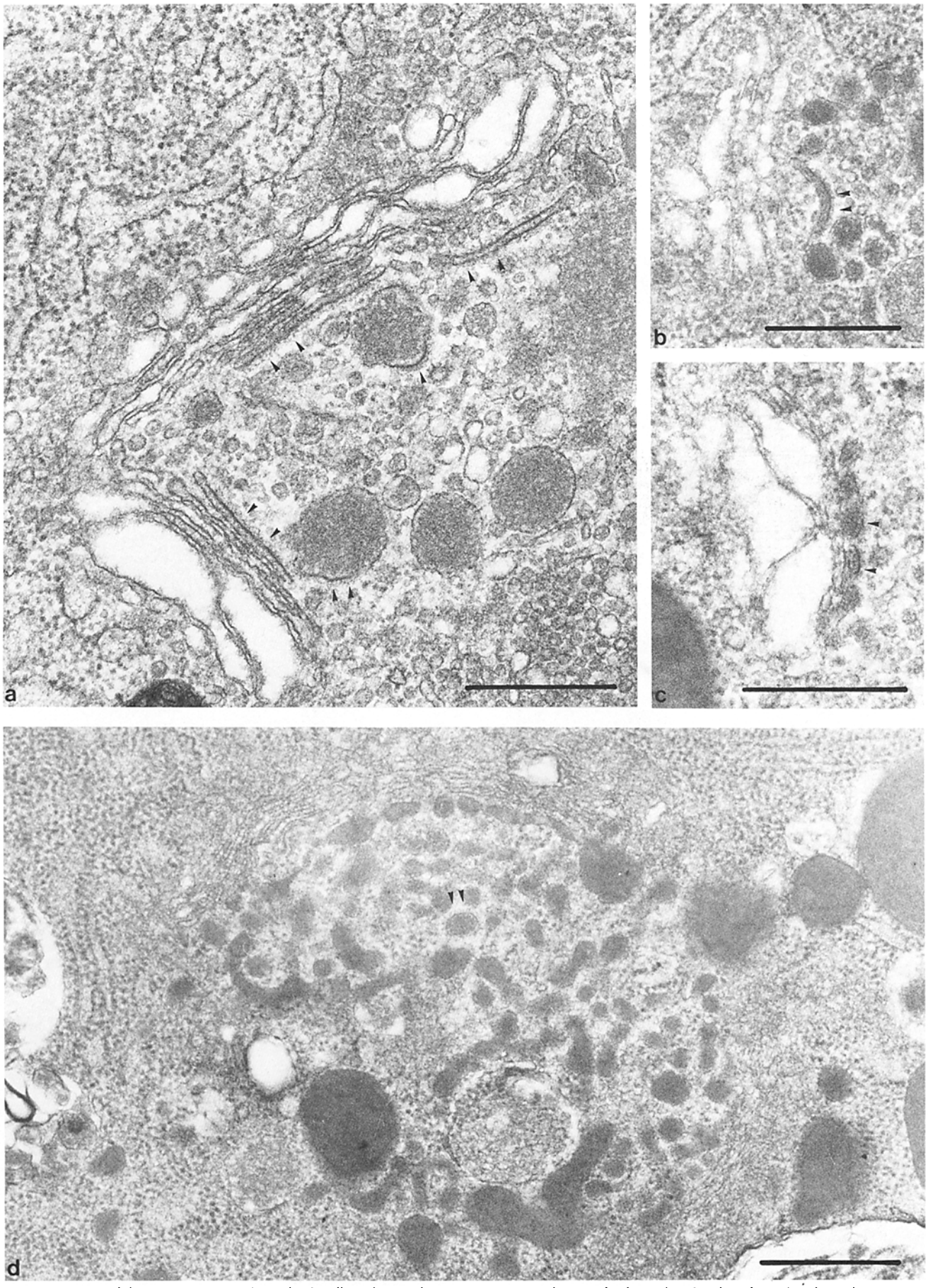

FIGURE 6 Colchicine treatment for 6 h. Small, polymorphic zymogen granules are built at the distal Golgi side; their dense content resembles that of mature zymogen granules in controls. Partially, they are coated (small arrowheads); extended coated areas, in addition, cover condensing vacuoles and rigid lamellae (small arrowheads in *a* and *b*). Bars, 0.5 μm. (*a*) × 57,000; (*b*) × 51,000; (*c*) × 62,000; (*d*) 52,000.

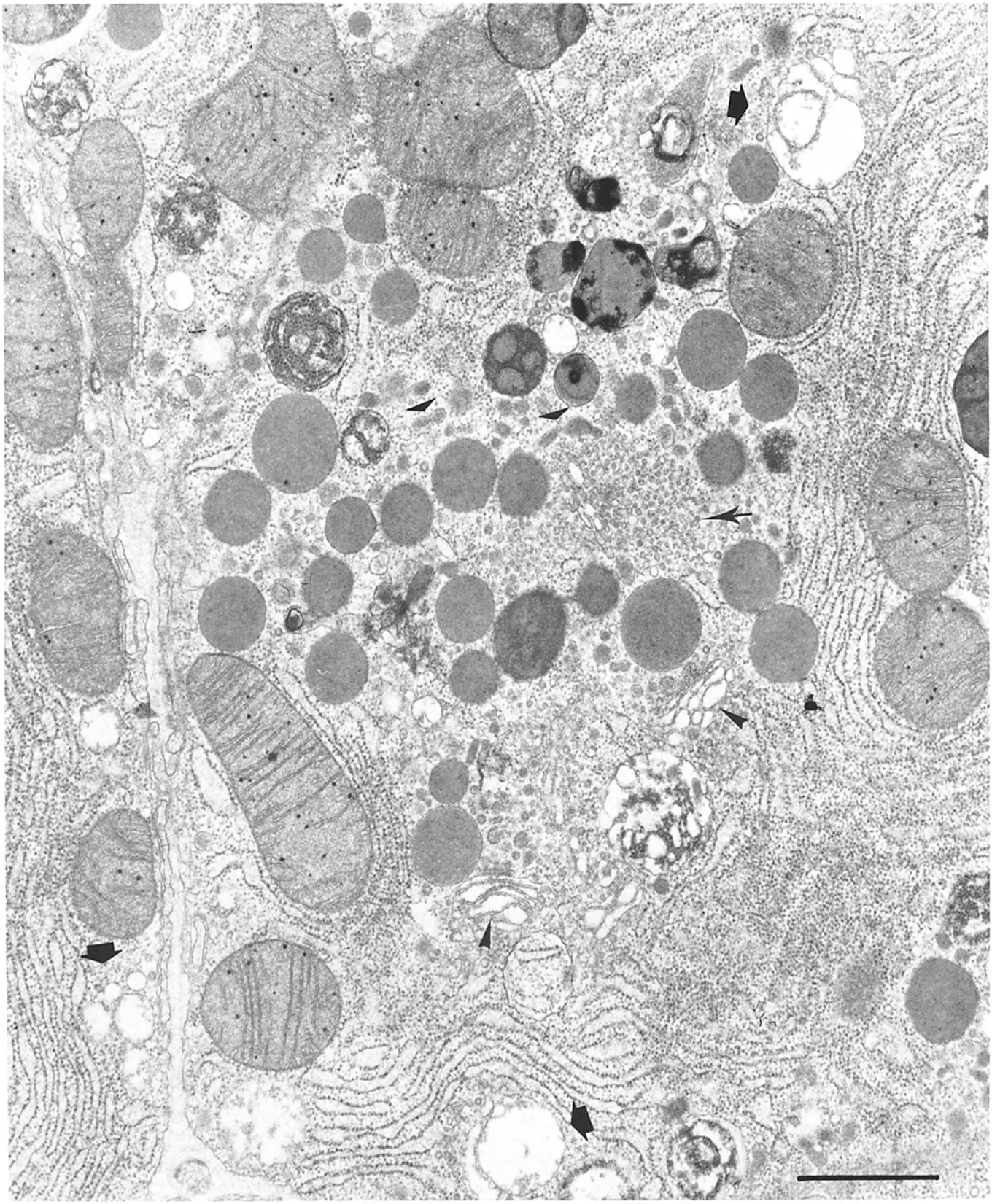

FIGURE 7 Colchicine treatment for 6 h. Survey micrograph showing the main colchicine-induced alterations of the Golgi complex. The arrowheads indicate Golgi stacks with short, partially dilated cisternae; the thin arrow points to the accumulation of transport vesicles; ▲, polymorphic zymogen granules; thick arrows point to groups of large vacuoles accompanied by transport vesicles, which are apparent near as well as outside the Golgi region. Bar, 1 μm. × 24,000.

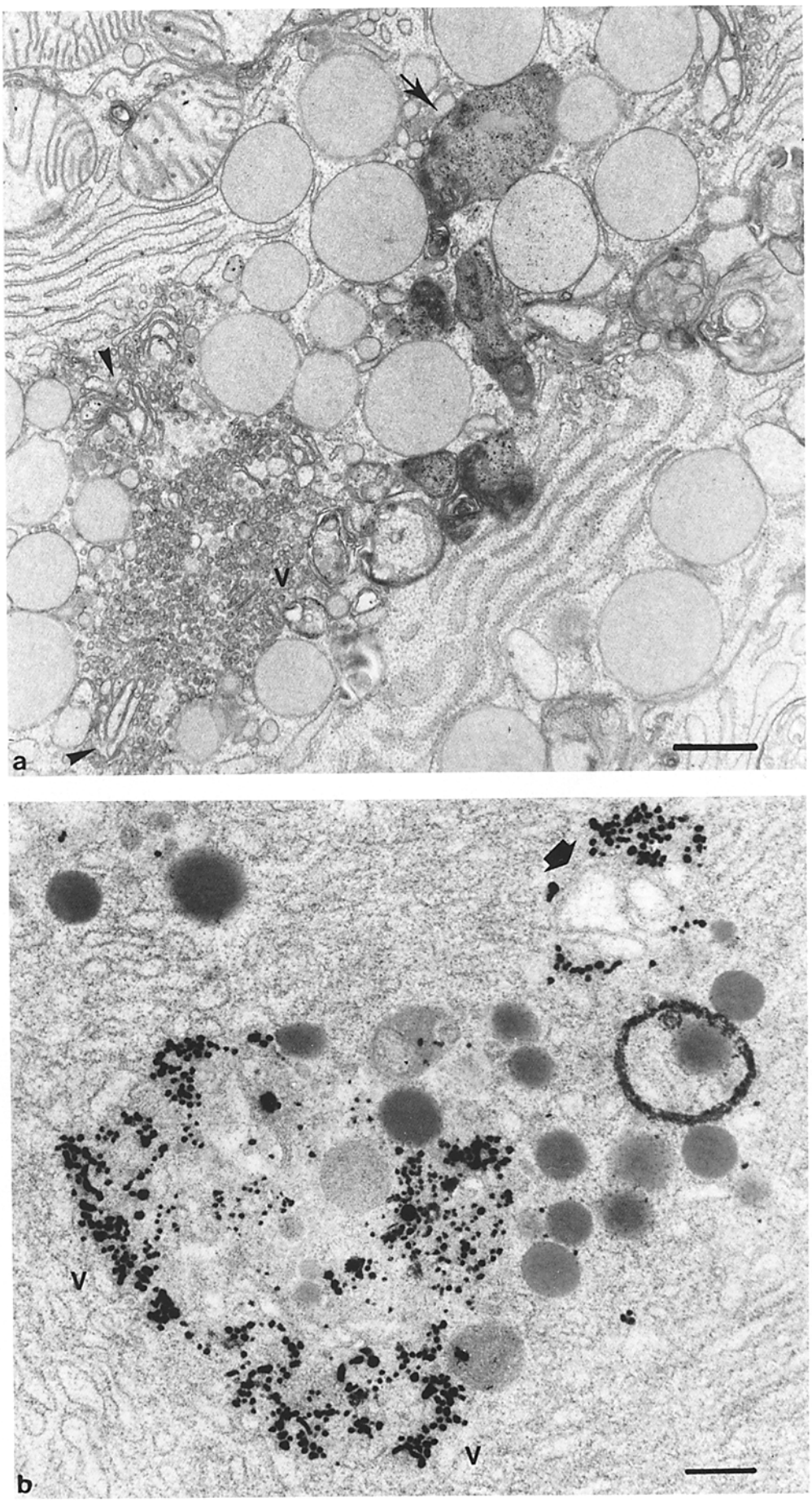

FIGURE 8 Colchicine treatment for 6 h. (a) TPPase. Reaction product is localized over secondary lysosomes (thin arrow) but is absent from accumulated vesicles (*v*). Arrowheads indicate Golgi stacks with short, partially dilated cisternae which exhibit slight deposits or are devoid of reaction. (*b*) Prolonged osmification. Osmium deposits are apparent over the accumulated transport vesicles (*v*). The thick arrow labels a group of vacuoles accompanied by small osmium-reactive vesicles. Bars, 0.5 μm. (*a*) × 26,000. (*b*) × 23,000.

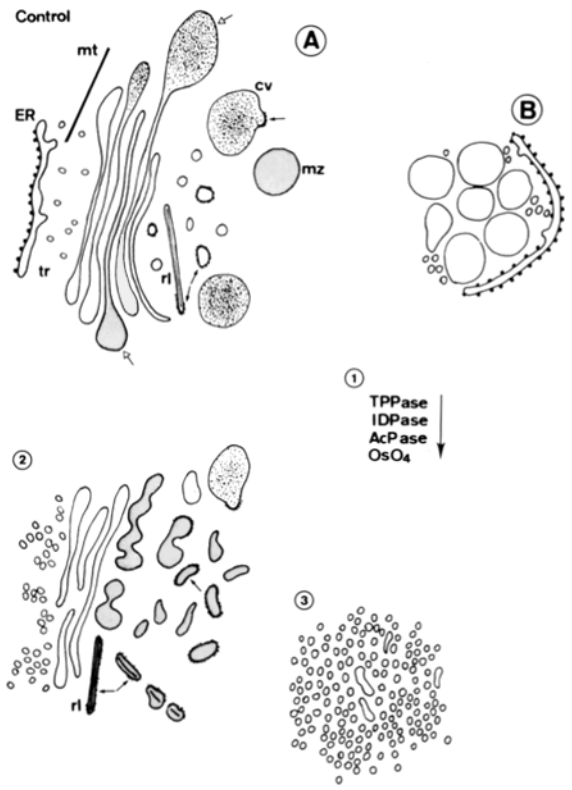

FIGURE 9 Summary diagram of the main colchicine-induced alterations of the Golgi complex. (*A*) Alterations of Golgi stacks. (*1*) The stacks may appear morphologically unchanged but exhibit cytochemical alterations: they show reduced TPPase, IDPase, and AcPase, or lack these enzymes as well as osmium deposits. (*2*) Morphological changes: the stacks appear to be fragmented into smaller units; the cisternae are shortened and partially dilated; the number of transport vesicles is increased; condensing vacuoles are diminished and substituted by small, polymorphic zymogen granules; extended coated regions cover rigid lamellae, condensing vacuoles, and the small polymorphic zymogen granules. (*3*) After 4 to 6 h of treatment, accumulations of transport vesicles occupy large cellular areas; cisternae in stacked organization are not demonstrable in these regions. (*B*) Groups of large vacuoles accompanied by transport vesicles predominate in cellular areas outside the Golgi region. *ER*, endoplasmic reticulum; *tr*, transport vesicles; *mt*, microtubule; *rl*, rigid lamella; *cv*, condensing vacuole; *mz*, mature zymogen granule; ⊣▷, forming condensing vacuoles, → coated regions.

DISCUSSION

Morphological alterations of the Golgi apparatus induced by treatment with antimicrotubular agents have been described in exocrine pancreatic cells (1–4, 18) and in several other cell types (5–9, 15–17). Our own observations on pancreatic acinar cells showed that colchicine treatment effected (*a*) alterations of the Golgi complex: accumulation of transport vesicles, formation of small polymorphic secretion granules, and alterations in the cytochemical localization of enzymes and reaction product after osmification; and (*b*) formation of large vacuoles which, arranged into groups, predominate in cytoplasmic areas outside the Golgi region.

The augmentation of transport vesicles coincident with the duration of treatment was paralleled by a progressive disorganization of the stacks of cisternae. In those cellular fields that were occupied by extensive vesicle accumulations stacked Golgi cisternae were not demonstrable. Few short and dilated cisternal fragments and some vacuoles may represent the remnants of Golgi stacks. These findings indicate that fusion of transport vesicles, formation of cisternae, and their organization into integral stacks are impaired after colchicine treatment. It is interesting that depletion of cytoplasmic calcium results in similar accumulations of transport vesicles (37) as seen after administration of colchicine.

Cytochemically, the disorganization of Golgi stacks coincided with a reduction of TPPsae, IDPase, and AcPase over Golgi components. Extremely altered Golgi stacks were devoid of these enzymes, as well as of osmium deposits. TPPase, nucleoside diphosphatase, and AcPase are considered to be concerned with the removal of uridine diphosphate and other nucleoside phosphates originating during the glycosylation processes (21, 22, 38). The reduction or absence of these enzymes from the disorganized Golgi stacks may reflect a functional deficiency of the altered Golgi components. The cytochemical alterations can be caused by the impairment in the formation of integral Golgi stacks; in addition, a direct effect of colchicine on the activity of enzymes as has been reported for liver Golgi membranes (39) must be considered.

The main colchicine-induced morphological changes at the distal Golgi face are appearance of a dense content in distal Golgi cisternae, a diminished number of condensing vacuoles, and the formation of small polymorphic zymogen granules. These changes strongly resemble the picture obtained after stimulation of pancreatic acinar cells (Figs. 7 and 11 in reference 40). The dense material in distal Golgi cisternae indicates a translocation of condensing mechanisms from condensing vacuoles in controls to more proximal regions of Golgi stacks, as has also been described as occurring after stimulation (40).

Extensive coated areas on rigid lamellae, condensing vacuoles, and on the polymorphic zymogen granules after colchicine treatment contrast with the more patchy appearance of the coats in controls. Besides their role in receptor-mediated endocytosis (41), coated regions and vesicles are assumed to be involved in the intracellular transport and the partitioning of lysosomal and secretory material (42–44). Recently, it has been shown that colchicine influences the distribution of coated pits on the cell surface of macrophages, causing an accumulation of these structures at one pole of the cells (45). A similar effect of colchicine on the distribution of coated regions on components of the distal Golgi face could be responsible for the occurrence of the extensive coated areas on rigid lamellae, condensing vacuoles, and on the small, polymorphic zymogen granules. It remains to be clarified whether the alterations of distal Golgi components including the cytochemical changes are the result of processes occurring at more proximal sites in the stacks, or whether colchicine is effective at more than one point within the Golgi region.

Membrane continuities between endoplasmic reticulum and elements of GERL providing a direct route for secretory components from the endoplasmic reticulum to the distal Golgi face have been postulated (25). We could not find indications for such a pathway. We were not able to demonstrate membrane continuities between endoplasmic reticulum and condensing vacuoles or rigid lamellae; condensing vacuoles were clearly demonstrated to form from distal Golgi cisternae. Administration of colchicine affected both Golgi components and those structures that are defined as GERL. A cytochemical distinction between Golgi components and GERL was possible neither in controls nor after colchicine treatment.

20 min after administration of colchicine, conspicuous groups of large vacuoles accompanied by transport vesicles became apparent predominating in cellular areas outside the Golgi region. Similar "Golgi apparatus—like formations" induced by colchicine have been reported to occur in secreting cells of the lacrimal gland (17). Since these groups of vacuoles and vesicles became demonstrable at early time intervals after administration of colchicine (20–30 min), it is unlikely that they were derived from pre-existent Golgi stacks, which appeared morphologically unaltered after these periods of treatment. The findings at short time intervals after colchicine application rather suggest the new formation of the vacuoles.

In summary, the results provide evidence that the formation of stacked Golgi cisternae is disturbed after treatment with colchicine; rudimentary Golgi formations are built that are dominant in cellular regions outside the Golgi area. The regulative mechanisms that direct membranes and secretory material via transport vesicles from the endoplasmic reticulum to the Golgi region seem to be impaired after administration of colchicine.

From the antimicrotubular effect of colchicine it may be deduced that microtubules function in the traffic between endoplasmic reticulum and the Golgi complex. In addition, direct colchicine-membrane interactions (46) must, at least partially, be considered as the cause of the colchicine—induced alterations. The inhibitory effect of colchicine on the protein synthesis is not apparent before 1 h of treatment (47) and, thus, can be excluded as being responsible for the changes.

The occurrence of rudimentary Golgi formations already after 20 min of treatment indicates that the traffic from the endoplasmic reticulum to the Golgi apparatus is disorganized before alterations in the Golgi area are detectable. The results are in concert with the radiochemical and radioautographical data showing the colchicine-induced delay of the intracellular transport of secretory components preceding morphological changes in the Golgi stacks (2, 17).

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