Golgi Apparatus, GERL, and Secretory Granule Formation within Neurons of the Hypothalamo-Neurohypophysial System of Control and Hyperosmotically Stressed Mice

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ABSTRACT The vasopressin-producing neurons of the hypothalamo-neurohypophysial system are a particularly good model with which to consider the relationship between the Golgi apparatus and GERL and their roles in secretory granule production because these neurons increase their synthesis and secretion of vasopressin in response to hyperosmotic stress. Enzyme cytochemical techniques for acid phosphatase (AcPase) and thiamine pyrophosphatase (TPPase) activities were used to distinguish GERL from the Golgi apparatus in cell bodies of the supraoptic nucleus from normal mice, mice hyperosmotically stressed by drinking 2% salt water, and mice allowed to recover for 5–10 d from hyperosmotic stress. In nonincubated preparations of control supraoptic perikarya, immature secretory granules at the *trans* face of the Golgi apparatus were frequently attached to a narrow, smooth membrane cisterna identified as GERL. Secretory granules were occasionally seen attached to Golgi saccules. TPPase activity was present in one or two of the *trans* Golgi saccules; AcPase activity appeared in GERL and attached immature secretory granules, rarely in the *trans* Golgi saccules, and in secondary lysosomes. As a result of hyperosmotic stress, the Golgi apparatus hypertrophied, and secretory granules formed from all Golgi saccules and GERL. Little or no AcPase activity could be demonstrated in GERL, whereas all Golgi saccules and GERL-like cisternae were TPPase positive. During recovery, AcPase activity in GERL returned to normal; however, the elevated TPPase activity and secretory granule formation seen in GERL-like cisternae and all Golgi saccules during hyperosmotic stress persisted. These results suggest that under normal conditions GERL is the predominant site for secretory granule formation, but during hyperosmotic stress, the Golgi saccules assume increased importance in this function. The observed cytochemical modulations in Golgi saccules and GERL suggest that GERL is structurally and functionally related to the Golgi saccules.

In mammalian exocrine and endocrine cells, the elaboration of secretory protein is believed to follow six successive steps collectively termed the secretory process (46). Secretory proteins are: (*a*) synthesized on ribosomes associated with the endoplasmic reticulum; (*b*) segregated within the cisternal space of the rough endoplasmic reticulum; (*c*) transported to the vicinity of the Golgi apparatus; (*d*) concentrated and further processed in Golgi saccules or condensing vacuoles; (*e*) stored in secretory granules; and (*f*) discharged from secretory granules to the outside of the cell. Although much is known about the secretory process, many specific details remain speculative. Included among the poorly understood aspects of the secretory process are the roles of the Golgi apparatus and/or GERL[1] in the processing and packaging of secretory proteins.

[1] Novikoff (32, 33, 35) has assigned the acronym GERL to an acid-phosphatase reactive, smooth membrane cisterna that lies adjacent to the *trans* side of the Golgi apparatus, is connected to the endoplasmic reticulum, and gives rise to lysosomes.

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Secretory granules may arise from the *trans* Golgi saccule (2), from GERL or a similar acid phosphatase-positive structure (5, 15, 18–20, 22, 35, 37, 40, 47), or by coalescence of Golgi-associated condensing vacuoles (9, 25, 26). Whether or not GERL is morphologically and functionally distinct from the Golgi apparatus (34, 35, 38) or is part of the Golgi apparatus (24, 29, 55) remains unresolved.

The details of the secretory process or its variations in peptidergic neurons have not been thoroughly studied. Among the different classes of peptidergic neurons in the central and peripheral nervous systems, the best known from morphological, functional, and biochemical viewpoints are those affiliated with the hypothalamo-neurohypophysial system (21). The neurosecretory neurons of the hypothalamic supraoptic and paraventricular nuclei produce the peptide hormones vasopressin and oxytocin along with their associated carrier proteins, the neurophysins. Biochemical (16, 50), autoradiographic (30), and immunocytochemical (7) data have combined to conclusively demonstrate that the basic steps of the secretory process are followed within the peptidergic neurons of the hypothalamo-neurohypophysial system; these investigations, however, have not provided evidence for the precise role of either the Golgi apparatus or GERL in the secretory process.

In the present study we have examined GERL, the Golgi apparatus, and their participation in secretory granule production in supraoptic neurons from normal and hyperosmotically stimulated mice. Hyperosmotic stimulation was accomplished by having the animals drink 2% sodium chloride, a physiological stimulus known to increase the synthesis and release of vasopressin (12, 16, 17, 27). Thiamine pyrophosphatase (TPPase) activity was used as a marker for the *trans* Golgi saccules, and acid phosphatase (AcPase) activity was used to identify GERL (35).

MATERIALS AND METHODS

70 young adult, female, NIH (National Institutes of Health) Swiss mice weighing 30–35 g each were used. 25 of these animals received tap water to drink and served as controls. The remaining 45 mice were given 2% salt water to drink for 5–8 d. 20 of these hyperosmotically stressed mice were then rehydrated by giving them normal tap water to drink for 5–10 d. All animals were perfused through the heart with fixative consisting of 1% paraformaldehyde, 1.25% glutaraldehyde (Ladd Research Industries, Inc., Burlington, Vt.), and 0.025% calcium chloride in 0.1–0.2 M cacodylate buffer (pH 7.4). Each brain was removed from the skull, immersed in fixative for 1 h at room temperature, and then transferred to cacodylate buffer at 4°C overnight. Blocks of the hypothalamus, brain stem, and pituitary gland were cut into 50-μm thick sections using a Sorvall TC-2 tissue sectioner (DuPont Instruments, Sorvall, DuPont Co., Newtown, Conn.). Only sections containing the neurohypophysis, supraoptic nucleus, and VII, X, and XII cranial motor nuclei were saved for processing.

To demonstrate TPPase activity, sections were incubated at pH 6.8–7.2 in the Novikoff and Goldfischer (36) medium for 60–90 min at 37°C with thiamine pyrophosphate (Sigma Chemical Co., St. Louis, Mo.) serving as substrate. For demonstration of acid hydrolase activity, sections were incubated for 40–60 min at 37°C in the medium described by Novikoff (31) (pH 5.0) with cytidine 5′-monophosphate as substrate, in the medium described by Barka and Anderson (1) (pH 5.0) with β-glycerophosphate as substrate, or in the medium described by Doty et al. (11) (pH 3.9) with trimetaphosphate as substrate (each from Sigma Chemical Co.). Each incubation medium was replaced with fresh medium at 20–30-min intervals. After incubation, the sections were washed several times in cacodylate buffer and rinsed briefly in a solution of 1% sodium sulfide in cacodylate buffer in order to visualize the reaction product for light microscopy. Sections incubated in medium not containing substrate served as controls.

All sections were postfixed in 1% osmium tetroxide in cacodylate buffer for 2 h. Nonincubated sections and some sections incubated for enzyme cytochemistry were stained *en bloc* with 0.5% aqueous uranyl acetate for 2 h. All sections were dehydrated in ethanol and embedded in Araldite resin (Cy 0655, British Grade, Polysciences, Inc., Warrington, Pa.). 1-μm thick sections of the material incubated for trimetaphosphatase (TMPase) activity were mounted on glass slides and observed under dark-field light microscopy. Ultrathin sections cut from the nonincubated material were poststained with 2% uranyl acetate in 50% ethanol and Reynolds' lead citrate (49). Some ultrathin sections from the incubated tissues were stained lightly with lead citrate.

RESULTS

Emphasis was placed on results obtained from neurons of the supraoptic nucleus in mice under control, salt-stimulated, and rehydrated or recovery conditions. Perikarya from the cranial motor nuclei have been included to indicate that the localizations of, and alterations in, enzyme activities, as well as morphological changes induced by salt treatment, were specific to neurons of the hypothalamo-neurohypophysial system. These alterations appeared in cell bodies throughout the supraoptic nucleus, suggesting that oxytocin-producing perikarya, like those synthesizing vasopressin, responded to hyperosmotic stress.

Control Supraoptic Somata

The Golgi apparatus of the neurosecretory cell was located in the perinuclear cytoplasm. It consisted of several discrete stacks of saccules that, for the most part, had their inner or *trans* face oriented toward the nucleus. At the outer or *cis* face, ribosome-studded or ribosome-free portions of endoplasmic reticulum and small, smooth-surfaced vesicles were plentiful. Each Golgi stack contained three to seven saccules of irregular width and length. The saccules at the *cis* face were consistently greater in width than those at the *trans* face. The *cis* saccules frequently appeared as several short, dilated, and discontinuous segments. The two to four *trans* face saccules were narrow, regular in contour, and rarely dilated. Secretory granules measuring 100–220 nm in diameter and containing an 80-nm wide electron-dense core were scattered throughout the perikaryal cytoplasm. Many of the secretory granules were located in the vicinity of the Golgi apparatus. Similar sized granules in the hypothalamo-neurohypophysial system are immunoreactive with antisera against neurophysin, vasopressin, or oxytocin (7, 28, 51, 56).

In control cells immature, dense-core secretory granules were rarely observed to be confluent with Golgi saccules but were frequently seen in continuity with a narrow (30–40 nm wide), smooth-membrane cisterna located either adjacent to the *trans* Golgi saccule or separated from it by a few intervening vesicles (Fig. 1). This smooth membrane cisterna is equivalent to GERL on the basis of its morphology, proximity to the Golgi apparatus, and cytochemical properties (see below). Although cisternae of the rough endoplasmic reticulum were often positioned close to GERL, our preparations have not revealed a direct continuity between these two structures or between GERL and any organelle other than the secretory granule. GERL and the Golgi apparatus in the neurosecretory neuron were similar morphologically to these same organelles in other cell types (3, 13, 14, 19, 20, 23, 24, 42, 44, 45, 47).

ACPASE: Incubation of supraoptic perikarya for AcPase activity yielded reaction product in GERL and in 0.2- to 0.6-μm-wide secondary lysosomes (Fig. 2 *a*). Reaction product was usually absent from Golgi saccules except in a few instances when the *trans* Golgi saccule did contain slight AcPase activity (Fig. 2 *a*). This saccule may be a transition saccule undergoing conversion to GERL. As we previously reported (6), coated 40- to 60-μm-wide vesicles confluent with GERL or located adjacent to secondary lysosomes were also AcPase positive. These reactive vesicles may represent primary lysosomes.

When cut in cross-section, GERL appeared as a thin, dense cisterna sometimes exhibiting one or two 120- to 180-nm-wide dilations (Fig. 2 *a* and *b*). The dilations were AcPase positive and corresponded in morphology to the immature neurosecretory granules forming from the cisterna considered to be GERL in nonincubated preparations. Infrequently, the GERL cisterna would overlap itself, forming a double saccule (Fig. 2 *a*). When portions of GERL were oriented parallel to the plane of section, the peripheral segments of GERL were fenestrated and appeared as a network of anastomosing tubules (Fig. 2 *c*). These tubular or fenestrated segments were continuous with the cisternal portion of GERL.

TPPASE: After incubation for TPPase activity, one or two of the *trans* Golgi saccules contained reaction product (Fig. 3 *a*); GERL appeared consistently unreactive. On a few occasions one or two of the reactive Golgi saccules contained a 120- to 180-nm dilation similar to that associated with the AcPase-positive GERL (Fig. 3 *b*). Such dilations of the Golgi saccules most likely represent immature secretory granules. On very rare occasions an immature secretory granule attached to GERL contained TPPase activity (Fig. 3 *c*). TPPase reaction product was never seen in secretory granules detached from Golgi saccules or GERL. No other organelle contained TPPase reaction product.

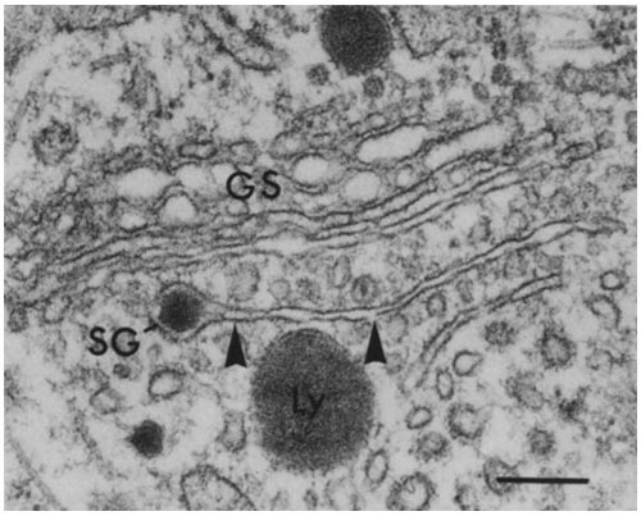

FIGURE 1 Control supraoptic neuron. Formation of secretory granules (*SG*) occurs predominately from GERL (arrowheads). Golgi saccules (*GS*). Lysosome (*Ly*). × 48,000. Bar, 0.25 μm.

Salt-stimulated Supraoptic Somata

Compared with controls, two demonstrable changes in the morphology of the Golgi apparatus resulted from hyperosmotic stimulation. First, when the saccules of the Golgi apparatus and GERL were cut in cross-section, the saccules of the hyperosmotically stimulated samples appeared noticeably longer but not necessarily wider (Fig. 4 *a*). When the Golgi apparatus was cut more or less parallel to the plane of section, it appeared broader and exhibited numerous fenestrations or anastomosing tubules. The second noticeable change was the increased number of immature secretory granules, in the stimulated samples, in continuity with all Golgi saccules (Fig. 4). GERL, likewise, had a greater number of immature secretory granules associated with it. Some of the secretory granules arising from GERL were confluent with coated vesicles measuring 40–60 nm in diameter (Fig. 4 *a* and *c*). These vesicular profiles were never observed in relation to secretory granules forming from Golgi saccules under salt-stimulated conditions and thus aided in the identification of GERL in incubated preparations. The overall number of secretory granules within the cell body was also increased compared with the controls. Even though the concentration of lysosomal dense bodies was elevated in hyperosmotically stressed supraoptic perikarya (6), no autophagic vacuoles or dense bodies were seen attached to GERL.

ACPASE: AcPase activity in GERL from salt-stimulated mice (Fig. 5 *a*) appeared consistently reduced in comparison to that in the controls and to AcPase-positive GERL in cranial

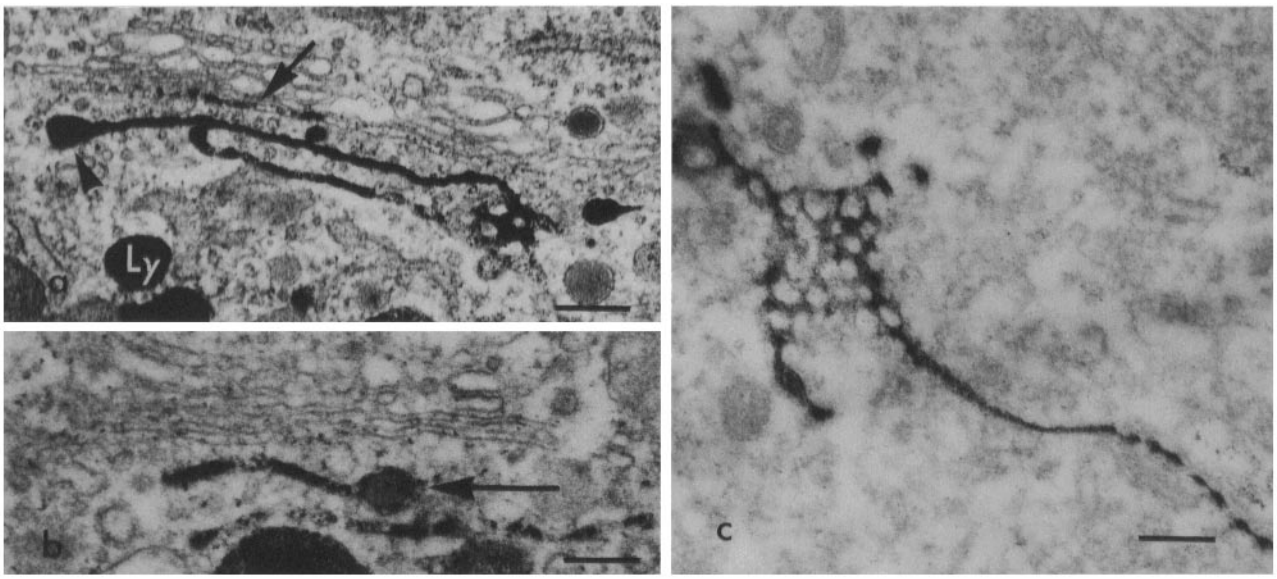

FIGURE 2 Control supraoptic neuron; AcPase. (*a*) Reaction product is restricted to GERL, an immature secretory granule (arrowhead) forming from GERL, and secondary lysosomes (*Ly*). Reaction product is rarely seen in the *trans* Golgi saccule (arrow). This saccule may represent a transition saccule in its conversion to GERL (see Discussion). × 40,000. (*b*) The dense core of a secretory granule forming from GERL is rimmed by reaction product (arrow). × 38,000. (*c*) Fenestrated portions of GERL are particularly evident at the ends of the cisterna. × 40,000. Bar, 0.25 μm.

motor perikarya from hyperosmotically stressed mice. Secretory granules attached to GERL were rarely reactive for AcPase activity (Fig. 5 *b*).

TPPASE: As a result of hyperosmotic stress, TPPase activity in the Golgi apparatus was demonstrably increased and appeared in nearly all saccules of all the Golgi stacks in supraoptic perikarya (Fig. 6). The Golgi apparatus was hypertrophied with abundant fenestrations and anastomosing tubules among the Golgi saccules (Fig. 7). GERL cisternae and secretory granules attached to all Golgi saccules or GERL were TPPase reactive as well (Figs. 6 and 7). In some of the preparations a network of anastomosing tubules appeared to interconnect the *trans* Golgi saccule and the GERL cisterna (Fig. 7 *b*). On occasion, GERL was confluent with a secretory granule that had a 40- to 60-nm vesicle attached to it (Fig. 7 *b*). These vesicles presumably correspond to the coated vesicles seen in nonincubated preparations. Profiles of the agranular reticulum and secretory granules in the cell body, neurohypophysial axons, and Herring bodies were unreactive for TPPase.

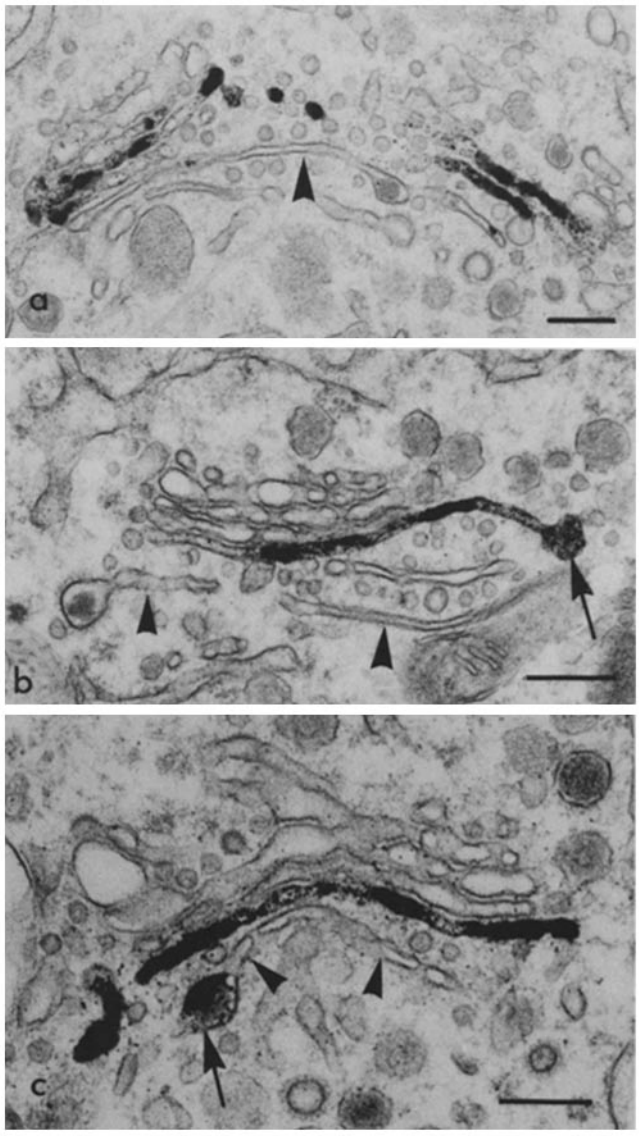

FIGURE 3 Control supraoptic neuron; TPPase. (*a* and *b*) Reaction product is normally present in one or two *trans* Golgi complex saccules and associated forming secretory granules (arrow) but is usually absent from GERL (arrowheads). Note the coat around the secretory granule forming from GERL in *b*. *a*, × 36,000; *b*, × 50,000. (*c*) Infrequently, a secretory granule (arrow) associated with GERL (arrowheads) does exhibit TPPase activity. × 52,000. Bar, 0.25 μm.

Supraoptic Somata from Rehydrated Mice

Supraoptic perikarya from mice hyperosmotically stressed and subsequently given tap water to drink for 5–10 d presented morphological and enzyme cytochemical alterations intermediate between control and salt-stimulated conditions. Neither the Golgi apparatus nor GERL appeared as extensive as in salt-stimulated perikarya. The elevated concentration of immature secretory granules in the cell body during salt treatment was sustained during the recovery period. GERL and Golgi saccules remained active in the production of secretory granules. Coated vesicles 40–60 nm in diameter were attached to secretory granules arising from GERL. The most striking characteristic of supraoptic cell bodies in the recovery state was the cytoplasmic accumulation of lipid droplets measuring up to 5 μm in diameter (Fig. 8). These droplets were never seen in control or salt-stimulated cell bodies. Dark-field, light microscopic examination of sections incubated for TMPase activity revealed that the elevated concentration of perikaryal secondary lysosomes resulting from salt stress (4, 6) was reduced in recovery to a level paralleling that in the controls.

ACPASE: The AcPase activity in GERL, which appeared reduced during hyperosmotic stress, was returned in recovery nearly to the level of that in the control state (Fig. 9 *a*). Secretory granules attached to GERL were likewise reactive.

TPPASE: The increased TPPase activity seen in most Golgi saccules, GERL, and secretory granules forming from these structures in salt-stressed supraoptic somata persisted through the 10-d recovery phase (Fig. 9 *b*). Vesicles in continuity with reactive secretory granules forming from GERL also exhibited TPPase reaction product.

DISCUSSION

Our structural and enzyme cytochemical studies of supraoptic cell bodies in the mouse brain, summarized in Fig. 10, have focused on GERL, the Golgi apparatus, and the packaging of secretory proteins by these organelles. Two important observations have been made: first, a major function of GERL in supraoptic neurons is the production of secretory granules; second, GERL does not appear to be functionally separate from the Golgi apparatus. The peptidergic neurons of the hypothalamo-neurohypophysial system serve as an excellent model in which to study the relationship of GERL to the Golgi apparatus and their participation in the secretory process. Because the biochemical and physiological activities of the secretory process in the supraoptic neuron are accelerated by hyperosmotic stress (16, 17, 27, 50), associated morphological events, that under normal conditions appear subtle in static electron micrographs, are dynamically expressed.

The morphological and enzyme cytochemical alterations described in this study appeared in most somata of the supraoptic nucleus and were not observed in a select population of perikarya. Immunocytochemical studies indicate that, in the supraoptic nucleus of the rat, vasopressin and oxytocin are synthesized in different neurons (54); the ratio of vasopressin-producing neurons to oxytocin-producing neurons is about 5:3 (53). Supraoptic cell bodies responding to salt stimulation and salt stimulation/recovery were so prevalent in our material that

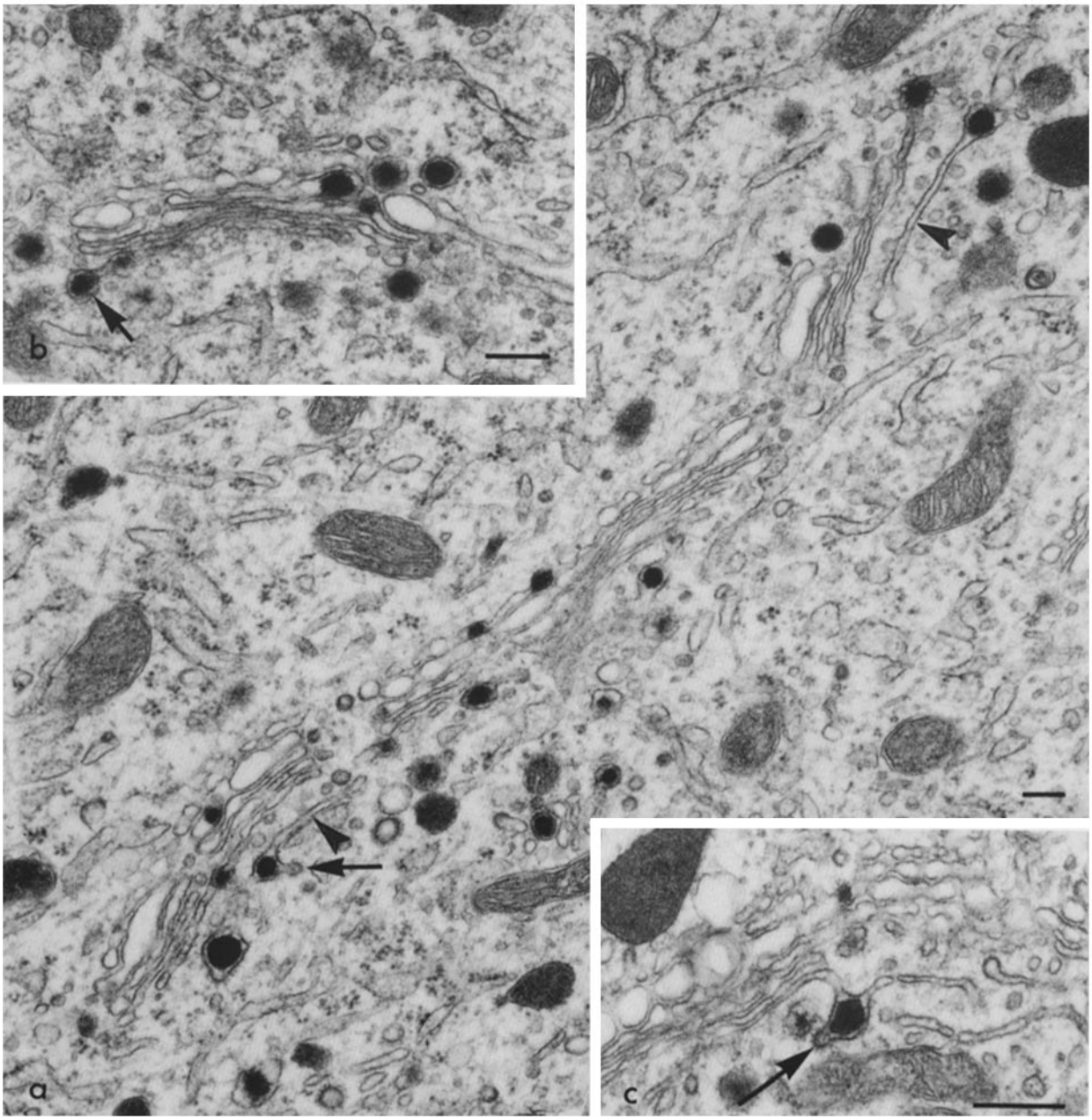

FIGURE 4 Hyperosmotically stressed supraoptic neuron. (*a*) Compared with controls, the length of the Golgi saccules and GERL (arrowheads) appears increased, and secretory granule production occurs from all Golgi saccules and GERL. Coated vesicle (arrow). × 30,000. (*b*) Secretory granules are seen forming from the *cis* face of the Golgi apparatus; one secretory granule (arrow) is forming from GERL. × 40,000. (*c*) Coated vesicles (arrow, also *a*) are frequently attached to secretory granules forming from GERL. × 56,000. Bar, 0.25 μm.

oxytocin-producing cells, as well as those synthesizing vasopressin, may have been involved. This interpretation is supported by experimental data suggesting that, with prolonged osmotic stress, vasopressin and oxytocin synthesis are increased (16), while the neurohypophysis becomes depleted of both hormones (12, 17).

In contrast to the resting state, the Golgi apparatus in the hyperosmotically stimulated neurosecretory cell assumes greater importance in the production of secretory granules. The Golgi apparatus appears to have hypertrophied, and secretory granule production occurs from all Golgi saccules and GERL. TPPase, an enzyme thought active in glycosylation (10, 19, 34), parallels the increased production of secretory granules. Recent biochemical data suggest that the precursor molecule for vasopressin is glycosylated (8). The differences, if any, that distinguish the neurosecretory granules arising from the *cis* Golgi saccules from those forming from GERL remain to be determined. A similar hypertrophy of the Golgi apparatus has been observed in rat hepatocytes hyperstimulated by hepatotoxic drugs (52).

Picard et al. (48) have proposed the concept of a functionally bipolar Golgi complex in neurosecretory cells. They believe, in opposition to the view expressed here, that secretory granules form normally from the *cis* Golgi saccules and that GERL is concerned solely with the production of lysosomes. Picard et al. (48) have based their proposal primarily on the evidence

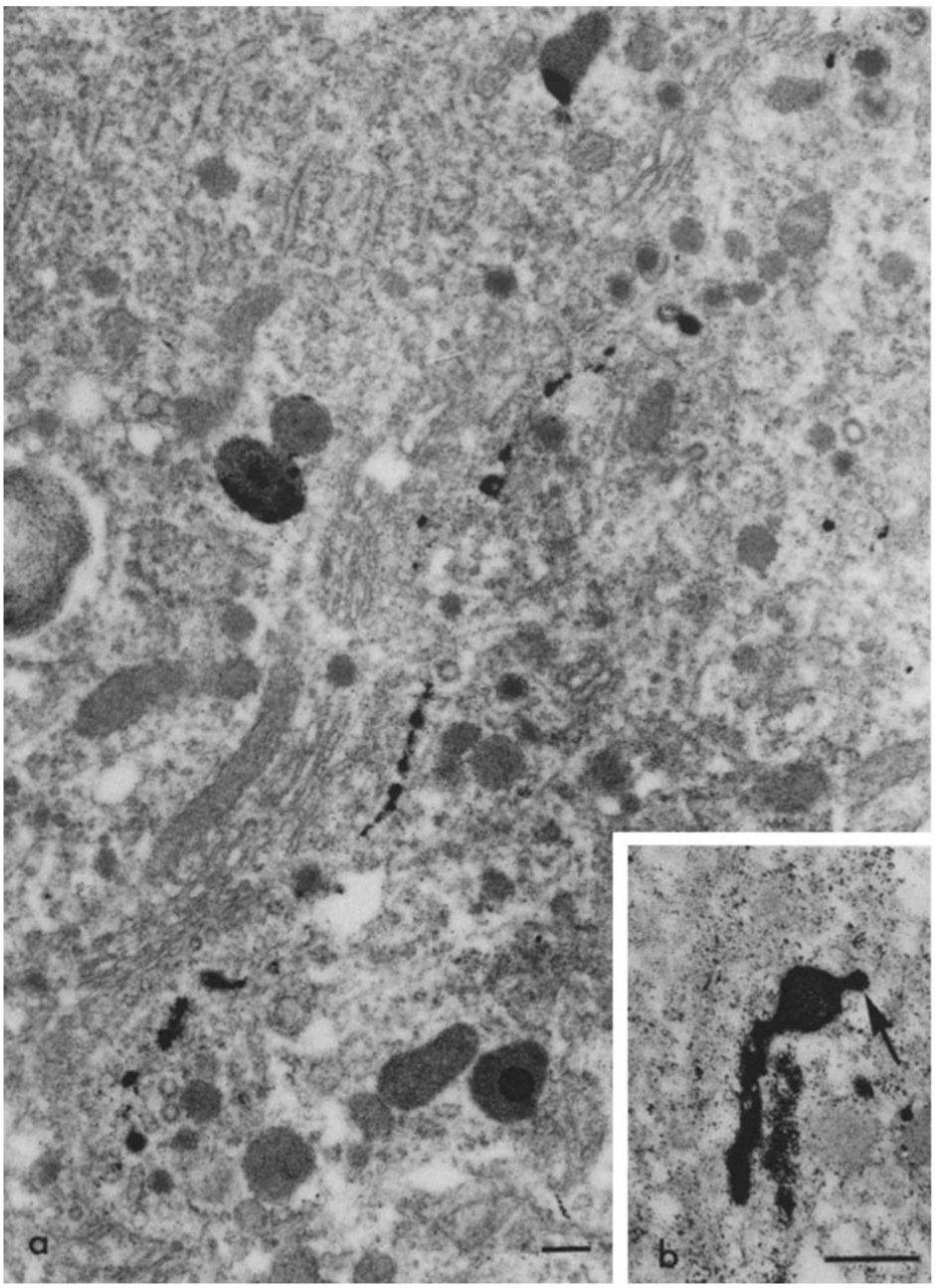

FIGURE 5 Hyperosmotically stimulated supraoptic neuron; AcPase. (*a*) Acid phosphatase activity in GERL is demonstrably reduced during stimulation. × 26,000. (*b*) Reactive secretory granules forming from GERL are rare. A coated vesicle (arrow) is confluent with the secretory granule. × 50,000. Bar, 0.25 μm.

reported by Novikoff and co-workers (37–42) that GERL is separate from the Golgi apparatus and is connected directly to the rough endoplasmic reticulum. Novikoff et al. (37–42), however, have repeatedly shown that GERL is involved in the packaging and processing of secretory material. We have never observed direct continuities between GERL and the rough endoplasmic reticulum in supraoptic neurons. Other investigators studying additional types of secretory cells have been equally unsuccessful in confirming this structural association (19, 20, 24).

The precise relationship of GERL to the lysosomal system of organelles in the supraoptic neuron is difficult to evaluate conclusively. GERL in this cell appears to be more involved in the production of secretory granules than with lysosomes. The 120- to 180-nm-wide, dilated portions of the GERL cisterna in nonincubated and incubated preparations of supraoptic cell bodies should not necessarily be considered prospective lysosomes as believed by Picard et al. (48). The assertion that these dilations in GERL represent immature secretory granules is supported by the following observations: in nonincubated material the dilations contain a dense core, not present in lysosomes, that is seen in secretory granules detached from GERL; in individual Golgi saccules, similar dilations with dense cores increase in number during salt stimulation and never contain AcPase activity; neither dense bodies nor AcPase-positive lysosomes in the size range of secretory granules (100–220 nm) have been observed in the supraoptic perikaryon; no form of lysosome, other than perhaps the 40- to 60-nm-wide vesicles,

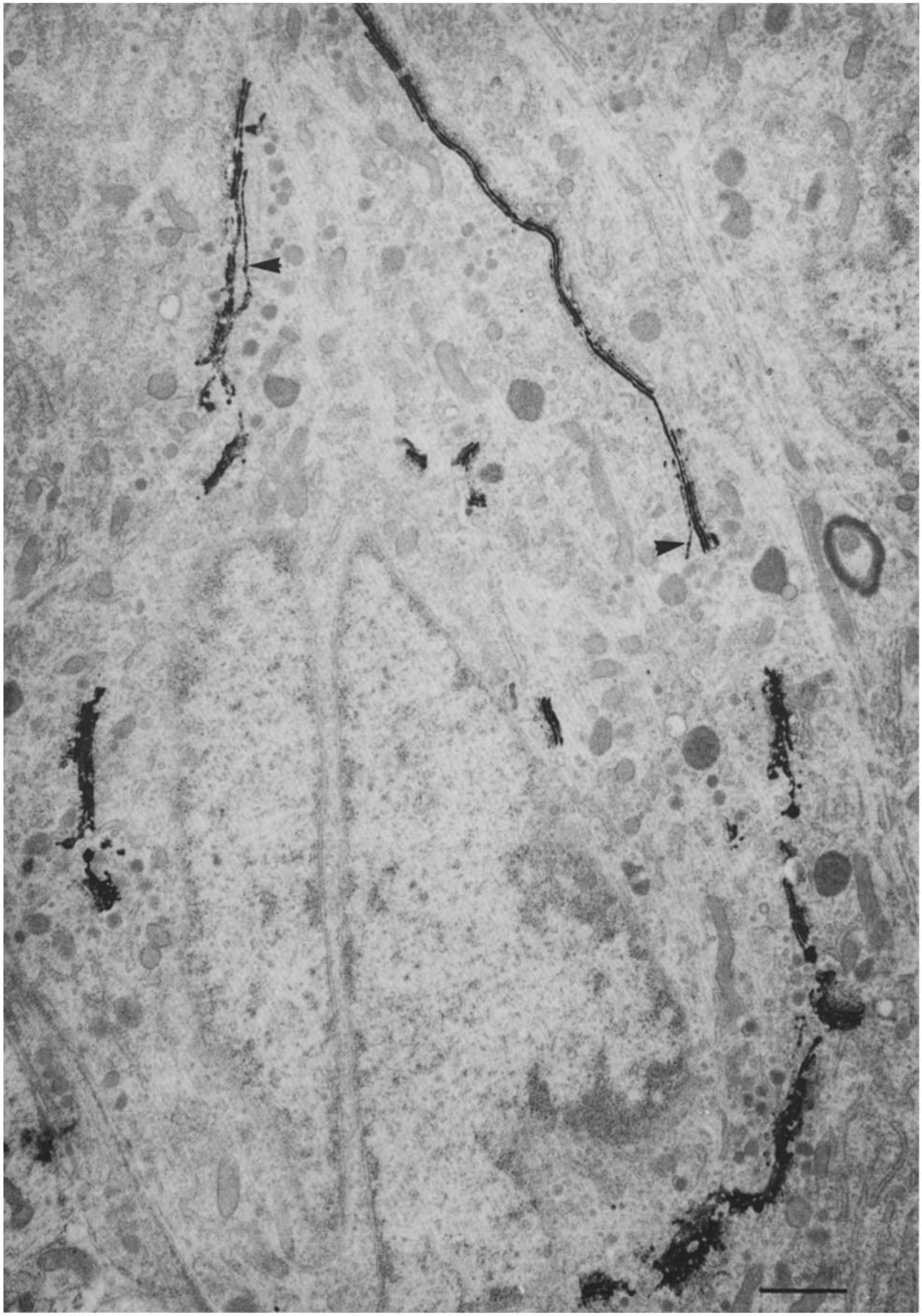

FIGURE 6 Hyperosmotically stimulated supraoptic neuron; TPPase. Reaction product is present in the Golgi saccules and in GERL-like cisternae (arrowheads) throughout the cell body. × 16,000. Bar, 1 μm.

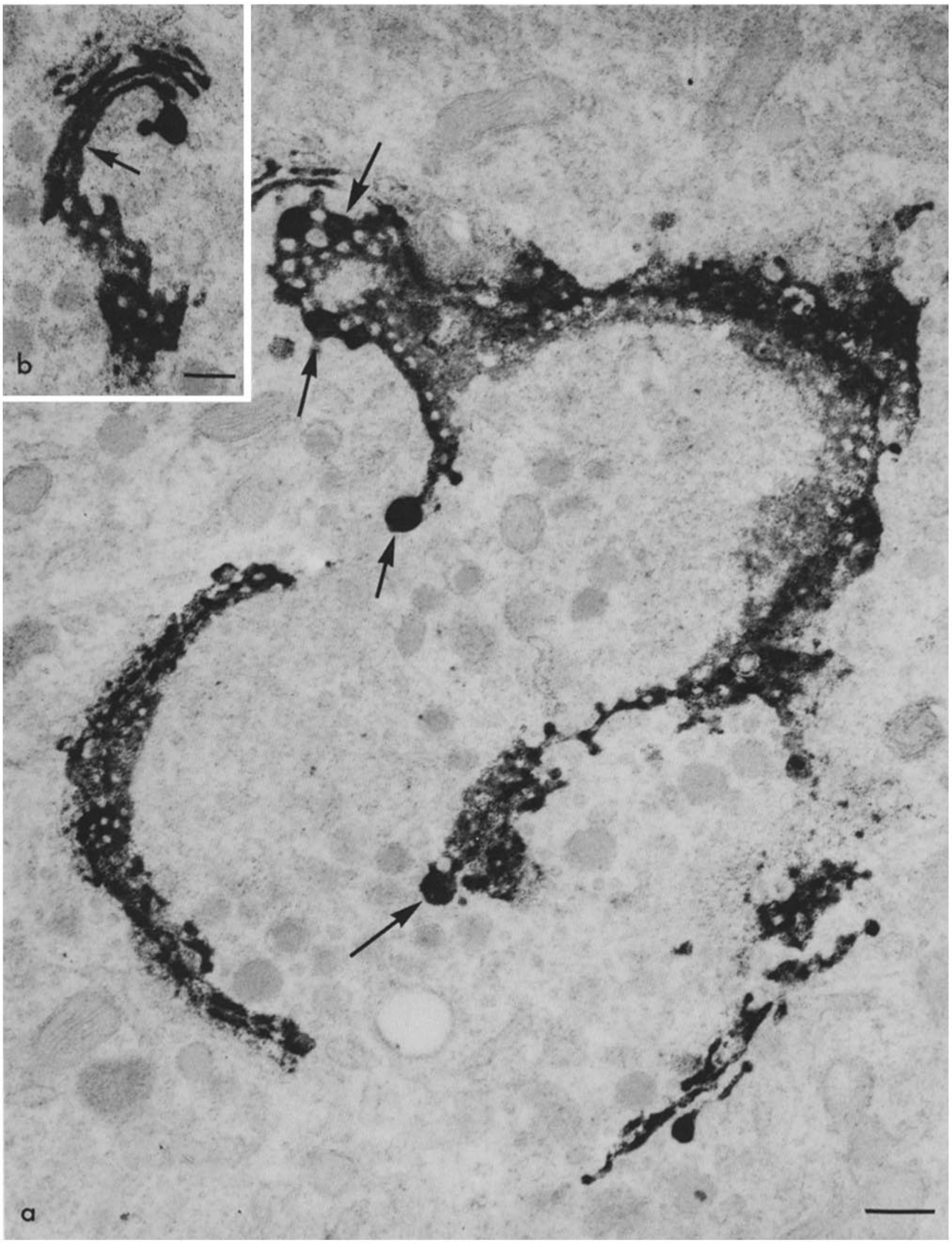

FIGURE 7 Hyperosmotically stimulated supraoptic neuron; TPPase. (*a*) The Golgi apparatus is hypertrophied and highly fenestrated. All saccules and forming secretory granules are positive for TPPase activity. Forming secretory granules (arrows) are especially numerous in fenestrated regions. × 48,000. (*b*) TPPase-positive secretory granule with associated coated vesicles is attached to a GERL cisterna. This cisterna may be continuous with the Golgi saccules at the fenestrated region (arrow). × 36,000. Bar, 0.25 μm.

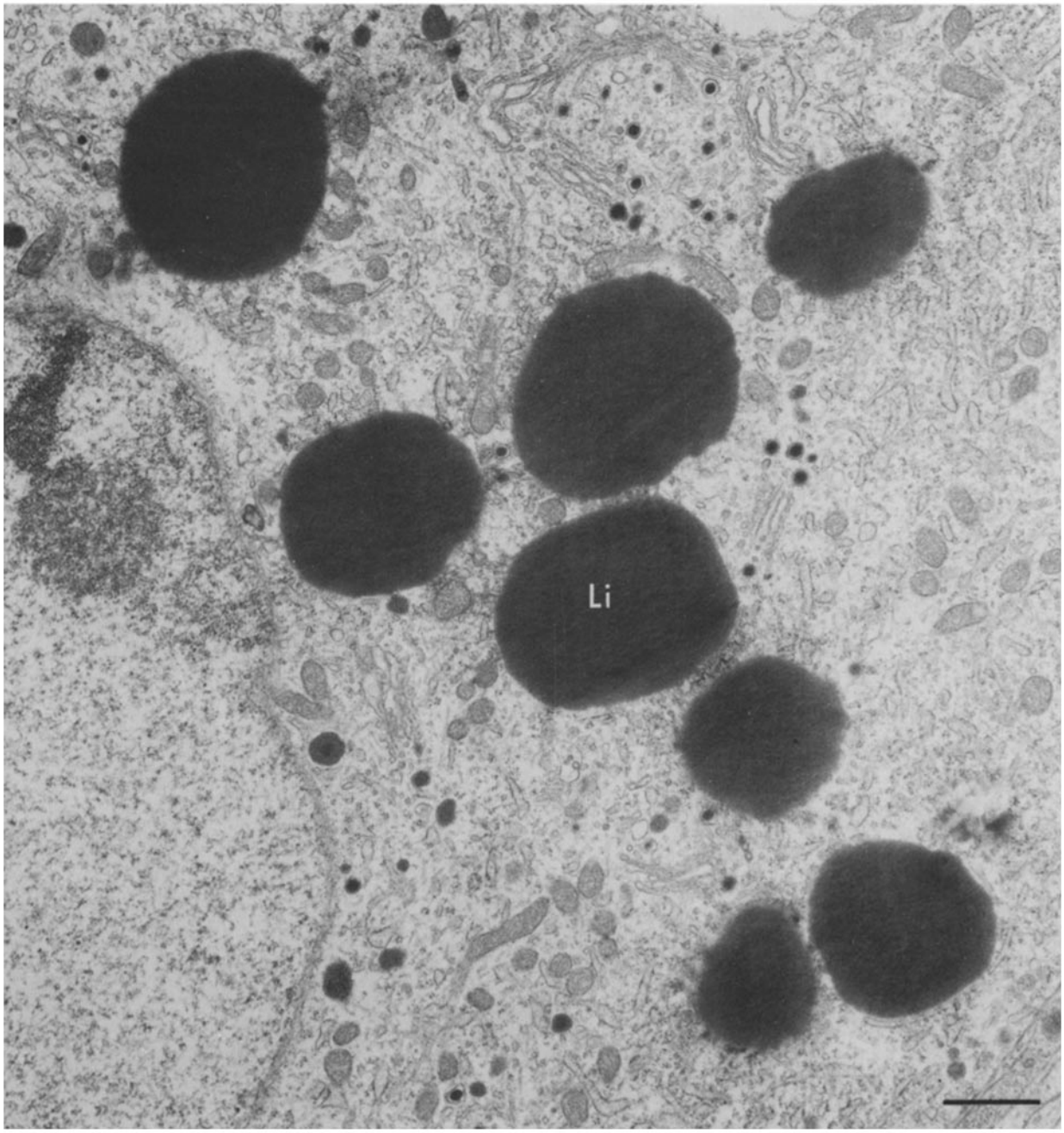

FIGURE 8 10-d recovery from salt stimulation. During recovery, large lipid droplets (*Li*) accumulate in the cytoplasm. The Golgi apparatus is still somewhat enlarged and numerous secretory granules are associated with both faces. × 15,000. Bar, 1 μm.

was seen forming from GERL; and, lastly, GERL and its dilations contain TPPase activity during hyperosmotic stress. We (6) and others (13, 14, 23, 34, 41, 45) have observed AcPase-positive 40- to 60-nm-wide, coated or smooth-surfaced vesicles attached to the ends of the GERL cisterna, but whether or not these vesicular profiles are indeed primary lysosomes remains unclear. Because GERL consists of a system of anastomosing tubules at the ends of its cisterna, the vesicular profiles may represent sections cut through the GERL tubules.

The modulations in AcPase and TPPase activities between GERL and the Golgi apparatus in supraoptic perikarya from control, hyperosmotically stressed, and rehydrated mice (see Fig. 10) strongly suggest that, in this cell type, GERL and the Golgi apparatus are structurally and functionally interrelated. The occasional localization of AcPase activity in the *trans* Golgi saccule and of TPPase activity in secretory granules forming from GERL in the resting state lends support to this proposal. Published reports detailing similar enzyme cytochemical alterations between GERL and Golgi saccules are scarce. In cells of the guinea pig corpus luteum during progesterone secretion, AcPase activity is low or absent in GERL but is intense, along with TPPase activity, in all Golgi cisternae (44). Only when these cells degenerate does GERL display demonstrable AcPase activity (45). Enzyme cytochemical changes, not unlike those reported here, have been documented in rat parotid acinar cells recovering from ethionine intoxication (43). In this instance as well, the morphological and cytochemical alterations were related to increased secretory granule production. Studies such as these provide evidence for a functional relationship between GERL and the Golgi apparatus and

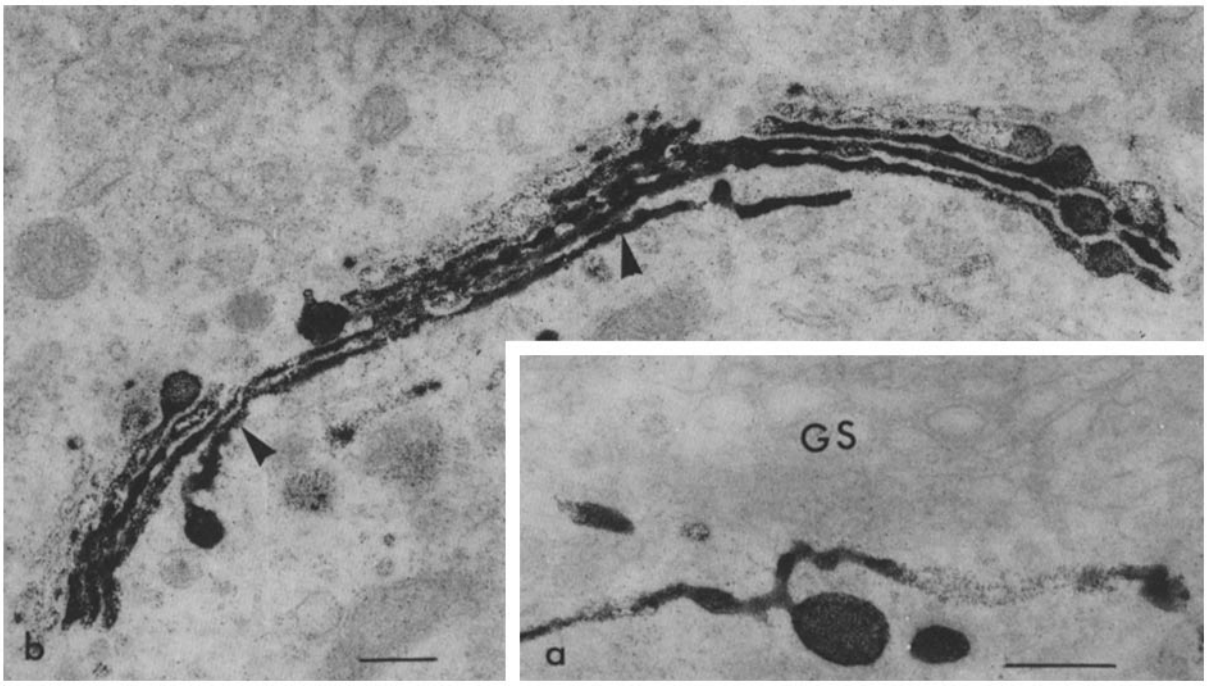

FIGURE 9 10-d recovery from salt stimulation. (*a*) Compared with salt-stimulated conditions, AcPase activity in GERL has returned to normal. GERL and a forming secretory granule both contain reaction product. Golgi saccules (*GS*). × 52,000. Bar, 0.25 μm. (*b*) Most of the Golgi saccules possess TPPase activity. The *cis* saccules contain only sparse reaction product, whereas the *trans* saccules, GERL (arrowheads), and forming secretory granules are filled with reaction product. Secretory granule formation persists from all Golgi saccules. × 42,000. Bar, 1 μm.

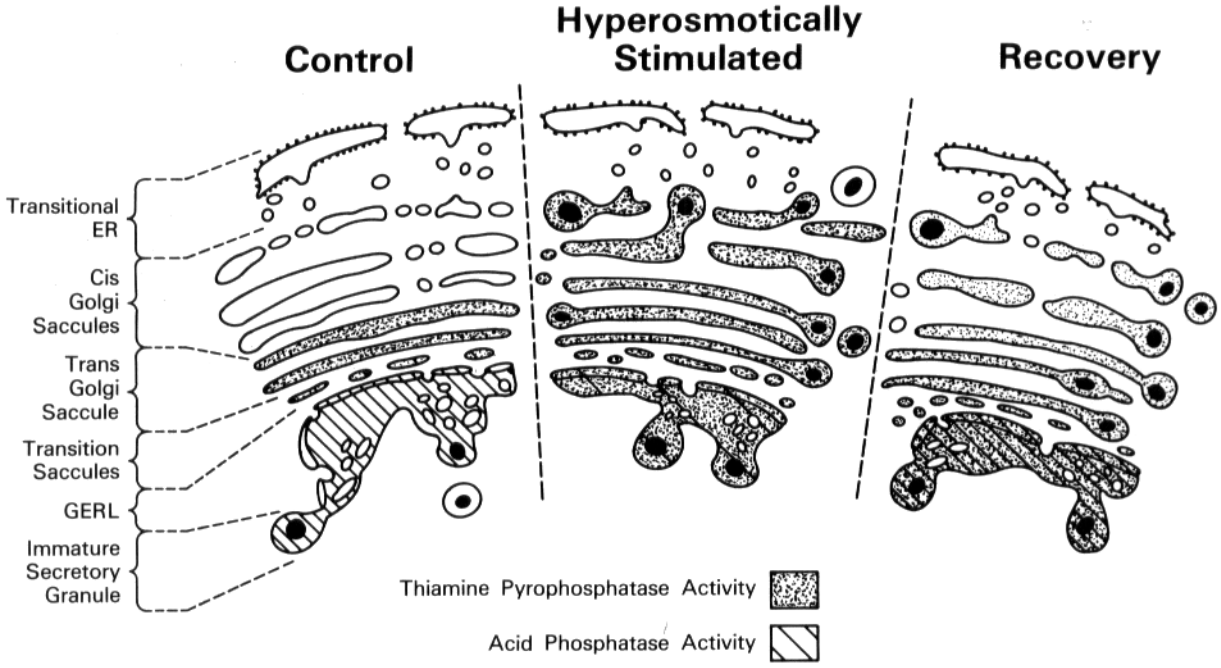

FIGURE 10 In nonincubated preparations of resting supraoptic cell bodies, immature secretory granules 120–180 nm wide possess a dense core and appear as expanded cisternal portions of GERL. GERL, along with its forming secretory granules, is AcPase positive. The *trans* Golgi saccule rarely contains AcPase activity. This saccule may represent a transition saccule. In contrast to GERL, secretory granule production from the Golgi saccules is minimal under normal conditions. The few secretory granules in the process of forming from the Golgi saccules exhibit a size and appearance similar to those associated with GERL. TPPase activity is present in one or two of the *trans* Golgi but normally not in GERL; in some instances, a secretory granule forming from GERL does contain TPPase activity. With salt stimulation, the Golgi apparatus becomes hypertrophied, and secretory granule production is increased from all saccules. TPPase activity is present in all Golgi saccules, GERL, and secretory granules arising from these structures. The AcPase activity in GERL is demonstrably reduced compared to controls. When hyperosmotically stressed mice are given normal tap water to drink for 5–10 d, secretory granule production from Golgi saccules and GERL remains elevated. TPPase activity is present in all saccules in these structures. The most striking enzyme cytochemical change in supraoptic cells during the recovery period is the return of AcPase activity to GERL.

further strengthen the suggestion that in secretory cells GERL may be derived from the *trans* Golgi saccule (19, 20). This saccule may normally lose its TPPase activity and acquire AcPase activity (see Fig. 2*a*). Through the accelerated production of secretory granules, the conversion of the *trans* Golgi saccule to GERL may occur at a rate too rapid for normal enzyme modulation to take place.

An alternative possibility suggested in our experimental material is that GERL and the *trans* Golgi saccule may be connected by anastomosing channels (Fig. 7*b*). The intimate structural relationship of the Golgi apparatus, GERL, and forming secretory granules suggests that a thorough examination of the function of AcPase in secretory cells may be warranted. The association of AcPase with forming secretory granules, the ability to modulate enzyme activity and secretory granule production, as well as the lack of a clear relationship between GERL and lysosome formation, indicate that the AcPase in secretory cells may be primarily involved in the processing of exportable proteins. Additional investigations of hyperstimulated secretory cells may provide the necessary documentation to support a possible structural interrelationship between GERL and the Golgi apparatus and to define the role of AcPase in these cells.

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REFERENCES

1. Barka, T., and P. Anderson. 1962. Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. *J. Histochem. Cytochem.* 10:741–753.
2. Bainton, D., and M. Farquhar. 1966. Origin of granules in polymorphonuclear leukocytes. Two types derived from opposite faces of the Golgi complex in developing granulocytes. *J. Cell Biol.* 39:229–317.
3. Boutry, J. M., and A. B. Novikoff. 1975. Cytochemical studies on Golgi apparatus, GERL, and lysosomes in neurons of dorsal root ganglia in mice. *Proc. Natl. Acad. Sci. U. S. A.* 72: 508–512.
4. Broadwell, R. D. 1980. Cytochemical localization of acid hydrolases in neurons of the mammalian central nervous system. *J. Histochem. Cytochem.* 28:87–89.
5. Broadwell, R. D., and C. Oliver. 1980. Localization of intravenously injected horseradish peroxidase (HRP) within cells of the anterior pituitary gland. *J. Cell Biol.* 87:206a.
6. Broadwell, R. D., C. Oliver, and M. W. Brightman. 1980. Neuronal transport of acid hydrolases and peroxidase within the lysosomal system of organelles: the involvement of agranular reticulum-like cisterns. *J. Comp. Neurol.* 190:519–532.
7. Broadwell, R. D., C. Oliver, and M. W. Brightman. 1979. Localization of neurophysin within organelles associated with protein synthesis and packaging in the hypothalamo-neurohypophysial system: an immunocytochemical study. *Proc. Natl. Acad. Sci. U. S. A.* 76:5999–6003.
8. Brownstein, M. J., J. T. Russell, and H. Gainer. 1979. Synthesis, transport, and release of posterior pituitary hormones. *Science (Wash. D. C.).* 207:373–378.
9. Caro, L. G., and G. E. Palade. 1964. Protein synthesis, storage and discharge in the pancreatic exocrine cell. An autoradiographic study. *J. Cell Biol.* 20:473–495.
10. Dauwalter, N., W. G. Whaley, and J. F. Kephart. 1969. Phosphatases and the differentiation of the Golgi apparatus. *J. Cell Sci.* 4:455–498.
11. Doty, S. B., C. E. Smith, A. R. Hand, and C. Oliver. 1977. Inorganic trimetaphosphatase as a histochemical marker for lysosomes in light and electron microscopy. *J. Histochem. Cytochem.* 25:1381–1384.
12. Dyball, R. E. J. 1971. Oxytoxin and ADH secretion in relation to electrical activity in antidromically identified supraoptic and paraventricular units. *J. Physiol. (Lond.).* 214: 245–256.
13. Essner, E., and H. Haimes. 1977. Ultrastructural study of GERL in beige mouse alveolar macrophages. *J. Cell Biol.* 75:381–387.
14. Essner, E., and C. Oliver. 1974. Lysosomes formation in hepatocytes of mice with Chediak-Higashi syndrome. *Lab. Invest.* 30:596–607.
15. Farquhar, M. G. 1971. Processing of secretory products by cells of the anterior pituitary gland. *Mem. Soc. Endocrinol.* 19:79–124.
16. Gainer, H., Y. Sarne, and M. Brownstein. 1977. Biosynthesis and axonal transport of rat neurohypophyseal proteins and peptides. *J. Cell Biol.* 73:366–381.
17. George, J. 1976. Vasopressin and oxytocin are depleted from rat hypothalamic nuclei after oral hypertonic saline. *Science (Wash. D. C.).* 193:146–148.
18. Hand, , A. R. 1971. Morphology and cytochemistry of the Golgi apparatus of rat salivary gland acinar cells. *Am. J. Anat.* 130:141–158.
19. Hand, A. R., and C. Oliver. 1977a. Cytochemical studies of GERL and its role in secretory granule formation in exocrine cells. *Histochem. J.* 9:375–392.
20. Hand, A. R., and C. Oliver. 1977b. Relationship between the Golgi apparatus, GERL, and secretory granules in acinar cells of the rat exorbital lacrimal gland. *J. Cell Biol.* 74: 399–413.
21. Handbook of Physiology, Endocrinology, Sect. 7, Vol. IV. 1974. W. H. Sawyer and E. Knobil, editors. American Physiological Society, Washington, D. C.
22. Holtzman, E., and R. Dominitz. 1968. Cytochemical studies of lysosomes, Golgi apparatus, and endoplasmic reticulum in secretion and protein uptake by adrenal medulla cells of the rat. *J. Histochem. Cytochem.* 16:320–336.
23. Holtzman, E., A. B. Novikoff, and H. Villaverde. 1967. Lysosomes and GERL in normal and chromatolytic neurons of the rat ganglion nodosum. *J. Cell Biol.* 33:419–435.
24. Inoué, K., and K. Kurosumi. 1977. Cytochemical and three-dimensional studies of Golgi apparatus and GERL of rat anterior pituitary cells by transmission electron microscopy. *Cell Struct. Funct.* 2:171–186.
25. Jamieson, J. D., and G. E. Palade. 1967. Intracellular transport of secretory proteins in the pancreatic exocrine cell. I. Role of the peripheral elements of the Golgi complex. *J. Cell Biol.* 34:577–596.
26. Jamieson, J. D., and G. E. Palade. 1967. Intracellular transport of secretory proteins in the pancreatic exocrine cell. II. Transport of condensing vacuoles and zymogen granules. *J. Cell Biol.* 34:597–615.
27. Jones, C. W., and B. T. Pickering. 1969. Comparison of the effects of water deprivation and sodium chloride inhibition on the hormone content of the neurohypophysis of the rat. *J. Physiol. (Lond.).* 203:449–458.
28. Kozlowski, G. P., S. Frenk, and M. S. Brownfield. 1977. Localization of neurophysis in the rat supraoptic nucleus. I. Ultrastructural immunocytochemistry using the post-embedding technique. *Cell Tissue Res.* 179:467–473.
29. Morré, D. J., T. W. Keenan, and C. M. Huang. 1974. Membrane flow and differentiation: origin of Golgi apparatus membranes from endoplasmic reticulum. *Adv. Cytopharmacol.* 2:107–125.
30. Nishioka, R. S., D. Zambrano, and H. A. Bern. 1970. Electron microscope radioautography of amino acid incorporation by supraoptic neurons of the rat. *Gen. Comp. Endocrinol.* 15: 477–495.
31. Novikoff, A. B. 1963. Lysosomes in the physiology and pathology of cells: contributions of staining methods. *In* Ciba Foundation Symposium on Lysosomes. A. V. S. de Reuck and M. P. Cameron, editors. Little, Brown & Co., Boston. 35–77.
32. Novikoff, A. B. 1964. GERL, its form and function in neurons of rat spinal ganglia. *Biol. Bull. (Woods Hole).* 127:358.
33. Novikoff, A. B. 1967. Enzyme localization and ultrastructure of neurons. *In* The Neuron. H. Hyden, editor. Elsvier, Amsterdam. 255–318.
34. Novikoff, A. B. 1973. Lysosomes: a personal account. *In* Lysosomes and Storage Diseases. H. G. Hers and F. VanHoff, editors. Academic Press, Inc. New York. 1–41.
35. Novikoff, A. B. 1976. The endoplasmic reticulum: a cytochemist's view (a review). *Proc. Natl. Acad. Sci. U. S. A.* 73:2781–2787.
36. Novikoff, A. B., and S. Goldfischer. 1961. Nucleosidediphosphatase activity in the Golgi apparatus and its usefulness for cytological studies. *Proc. Natl. Acad. Sci. U. S. A.* 47:802–810.
37. Novikoff, A. B., M. Mori, N. Quintana, and A. Yam. 1977. Studies of the secretory process in the mammalian exocrine pancreas. I. The condensing vacuoles. *J. Cell Biol.* 75:148–165.
38. Novikoff, A. B., and P. M. Novikoff. 1977. Cytochemical contributions to differentiating GERL from the Golgi apparatus. *Histochem. J.* 9:1–27.
39. Novikoff, A. B., P. M. Novikoff, M. Ma, W. Shin, and N. Quintana. 1974. Cytochemical studies of secretory and other granules associated with the endoplasmic reticulum in rat thyroid epithelial cells. *Adv. Cytopharmacol.* 2:349–368.
40. Novikoff, A. B., A. Yam, and P. M. Novikoff. 1975. Cytochemical study of secretory process in transplantable insulinoma of Syrian golden hamster. *Proc. Natl. Acad. Acad. Sci. U. S. A.* 72:4501–4505.
41. Novikoff, P. M., A. B. Novikoff, N. Quintana, and J.-J. Hauw. 1971. Golgi apparatus, GERL, and lysosomes of neurons in rat dorsal root ganglia, studied by thick section and thin section cytochemistry. *J. Cell Biol.* 50:859–886.
42. Novikoff, P. M., and A. Yam. 1978. The cytochemical demonstration of GERL in rat hepatocytes during lipoprotein mobilization. *J. Histochem. Cytochem.* 26:1–13.
43. Oliver, C., R. E. Auth, and A. R. Hand. 1980. Morphological and cytochemical alterations of the Golgi apparatus and GERL in rat parotid acinar cells during ethionine intoxication and recovery. *Am. J. Anat.* 158:275–284.
44. Paavola, L. 1978. The corpus luteum of the guinea pig. II. Cytochemical studies of the Golgi complex, GERL, and lysosomes in luteal cells during maximum progesterone secretion. *J. Cell Biol.* 79:45–48.
45. Paavola, L. 1978. The corpus luteum of the guinea pig. III. Cytochemical studies of the Golgi complex and GERL during normal postpartum regression of luteal cells, emphasizing origin of lysosomes and autophagic vacuoles. *J. Cell Biol.* 79:59–73.
46. Palade, G. 1975. Intracellular aspects of the process of protein synthesis. *Science (Wash. D. C.).* 189:347–358.
47. Pelletier, G., and A. B. Novikoff. 1972. Localization of phosphatase activities in the rat anterior pituitary gland. *J. Histochem. Cytochem.* 20:1–12.
48. Picard, D., M. Michel-Bechet, A. M. Athouel, and S. Rua. 1972. Granules Neurosecretories, Lysosomes et Complexe GRL dans de Noyau Supra-Optique du Rat. Bipolarite des Complexes Golgiens. *Exp. Brain Res.* 14:331–335.
49. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17:208–212.
50. Sachs, H., P. Fawcett, Y. Takabatake, and R. Portanova. 1969. Biosynthesis and release of vasopressin and neurophysin. *Recent Prog. Horm. Res.* 25:447–484.
51. Silverman, A. J. 1976. Ultrastructural studies on the localization of neurohypophysical hormones and their carrier proteins. *J. Histochem. Cytochem.* 24:816–828.
52. Sturgess, J. M., and M. A. Moscarello. 1976. Alterations in the Golgi complex and glycoprotein biosynthesis in normal and discarded tissues. *Pathol. Annu.* 6:1–29.
53. Swaab, D. F., F. Nijveldt, and C. W. Pool. 1975. Distribution of oxytocin and vasopressin in the rat supraoptic and paraventricular nucleus. *J. Endocrinol.* 67:461–462.
54. Vandesande, F., and K. Dierickx. 1975. Identification of the vasopressin-producing and of the oxytocin-producing neurons in the hypothalamic magnocellular neurosecretory system of the rat. *Cell Tissue Res.* 164:153–162.
55. Whaley, W. G., N. Dauwalter, and J. E. Kephart. 1972. Assembly, continuity, and exchanges in certain cytoplasmic membrane systems. *In* Origin and Continuity of Cell Organelles. J. Reinert and H. Ursprung, editors. Springer-Verlag, Heidelberg, Germany. 1–45.
56. Zimmerman, E. A. 1976. Localization of hypothalamic hormones by immunocytochemical techniques. *In* Frontiers in Neuroendocrinology. L. Martini and W. F. Ganong, editors. Raven Press, New York 4:25–62.