

**GOLGI APPARATUS, GERL, AND LYSOSOMES
OF NEURONS IN RAT DORSAL ROOT GANGLIA,
STUDIED BY THICK SECTION AND
THIN SECTION CYTOCHEMISTRY**

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ABSTRACT

New insights into the ultrastructure and phosphatase localizations of Golgi apparatus and GERL, and into the probable origin of lysosomes in the neurons of fetal dorsal root ganglia and the small neurons of adult ganglia have come from studying thick (0.5–1.0 μ) as well as thin (up to 500 Å) sections by conventional electron microscopy. Tilting the thick specimens, by a goniometer stage, has helped to increase our understanding of the three-dimensional aspects of the Golgi apparatus and GERL. One Golgi element, situated at the inner aspect of the Golgi stack, displays thiamine pyrophosphatase and nucleoside diphosphatase activities. This element exhibits regular geometric arrays (hexagons) of interconnected tubules without evidence of a flattened portion (sacculae or cisterna). In contrast, GERL shows acid phosphatase activity and possesses small cisternal portions and anastomosing tubules. Lysosomes appear to bud from GERL. Osmium deposits, following prolonged osmication, are found in the outer Golgi element. Serial 0.5- μ and thin sections of thiamine pyrophosphatase-incubated material demonstrate that, in the neurons studied, the Golgi apparatus is a continuous network coursing through the cytoplasm. Serial thick sections of acid phosphatase-incubated tissue suggest that GERL is also a continuous structure throughout the cytoplasm. Tubules of smooth endoplasmic reticulum, possibly part of GERL, extend into the polygonal compartments of the inner Golgi element. The possible physiological significance of a polygonal arrangement of a phosphatase-rich Golgi element in proximity to smooth ER is considered. A tentative diagram of the Golgi stack and associated endoplasmic reticulum in these neurons has been drawn.

INTRODUCTION

Evidence by Palade and collaborators (1–3) from biochemistry, electron microscopy, and radioautography have established the intracellular pathway taken by secretory proteins in acinar cells of fasted guinea pig pancreas. Following synthesis at the

ribosomes attached to the endoplasmic reticulum (ER), these proteins are transported via the ER cisternae to the Golgi zone where they are concentrated in membrane-bounded condensing vacuoles inside of which the zymogen matures. The

mature zymogen is ultimately released to the acinar lumen by fusion of the vacuole membranes with the plasma membrane.

Electron microscope evidence from many laboratories suggests that in a wide variety of animal and plant cell types condensation of secretory and lysosomal proteins occurs in the saccules (cisternae) of the Golgi apparatus. The proteins are packaged in vesicles or vacuoles whose membranes are derived from Golgi saccule membranes (for reviews see Beams and Kessel [4] and Whaley [5]).

Rapid progress is currently being made in elucidating the functional roles of the Golgi apparatus. Two procedures have been largely responsible for this progress: (a) electron microscope radioautography, and (b) biochemical assay of Golgi-enriched fractions isolated from tissue homogenates. With sulfate- ^{35}S and carbohydrates- ^3H it has been shown that sulfation of mucopolysaccharides and addition of carbohydrate moieties to secretory proteins occur in the Golgi apparatus (or related smooth-membraned structures) (see Beams and Kessel [4], also references 6-9). Three glycosyltransferases involved in glycoprotein synthesis have been found concentrated in Golgi-enriched fractions isolated from liver homogenates (10-13). In the most complete study (13) one of the Golgi-enriched fractions showed eight- to tenfold enrichments over the homogenate (on a protein basis) of the three transferases. This fraction possessed 35-40% of the enzyme activity of the homogenate.

The description of precise structural features of the Golgi apparatus and the relations of ER to this organelle is still lacking in cells generally, including the cells which have provided the bulk of the biochemical and radioautographic evidence. The precise localizations of phosphatases (the functional roles of these phosphatases are currently unknown) in the Golgi apparatus and GERL (14) are also unsettled.

New insights into both structural details and phosphatase localizations have resulted from the study of thick sections (0.5-1.0 μ) as well as the usual thin (up to 500 A) sections. The use of 0.5-1.0- μ sections to reveal stained structures in the conventional electron microscope (100 kv) was introduced by Rambourg (15). Phosphatase cytochemistry is eminently suited for thick section study because the reaction product, lead phosphate, is intensely electron opaque and because

different phosphatases are localized in different cytoplasmic organelles (14, 16). The ability to alter the angle of inclination of the thick sections with respect to the incident beam is a useful means of studying the three-dimensional aspects of the phosphatase-rich structures.

MATERIALS AND METHODS

Enzyme Activities

Dorsal root ganglia from fetal (Sprague-Dawley) and adult (Holtzman) rats were studied. The fetuses were taken from ether-anesthetized females 16 days after they had been placed with males overnight. In each experiment one embryo was placed in warm Hanks' solution. The whole spinal column was rapidly removed and transferred to ice-chilled glutaraldehyde-paraformaldehyde fixative (17). The fixative consists of 2.5% glutaraldehyde (TAAB Laboratories, Emmergreen, Reading, England), 2% formaldehyde (prepared from paraformaldehyde), and 0.025% CaCl_2 in 0.09 M cacodylate buffer, pH 7.4 (all expressed as final concentrations). About 12 ganglia were separated from the spinal cord within 1-3 min, and then placed into fresh ice-chilled fixative. After a total fixation time of 60 min, the ganglia were rinsed in three changes of cold 0.1 M cacodylate buffer, pH 7.4, containing 7.5% sucrose, and kept in the refrigerator overnight. The ganglia were then dissected into small pieces, using a piece of razor blade held in a needle holder. The dissection was performed under a binocular microscope at high magnification. After rinsing in cold 7.5% sucrose, the pieces were incubated as indicated below.

For study of dorsal root ganglia of adult rats (150-250 g each) tissue was usually fixed by perfusion, via the aorta, with cold 3% glutaraldehyde (Ladd Research Industries, Inc., Burlington, Vt.) in 0.1 M cacodylate buffer, pH 7.4 (18), for 20 min. The spinal cord was removed and placed into cold fixative. Then the ganglia were separated while in fixative. The immersion fixation time was approximately 20 min. The ganglia used for serial thin sectioning were perfused for 15 min with cold 1.25% glutaraldehyde-1% paraformaldehyde, 0.025% CaCl_2 , 0.9 M cacodylate, pH 7.4. They were then immersed in fixative for an additional 15 min. The ganglia were rinsed several times with 0.1 M cacodylate buffer, pH 7.4, and generally stored overnight in buffer. They were then sectioned with a Smith-Farquhar tissue sectioner (19) at approximately 40 μ . The sections were collected in 7.5% sucrose and incubated as indicated below. Incubation times were determined by monitoring sections with light microscopy, after ammonium sulfide treatment. Fol-

lowing incubation tissues were rinsed in cold 7.5% sucrose, fixed in cold 1% OsO₄-cacodylate buffer, pH 7.4, dehydrated in ethanols, treated with propylene oxide, and embedded in Epon 812 (20). In each experiment some pieces were treated, before dehydration, with 0.5% uranyl acetate in Veronal-acetate buffer (21) for 60 min at room temperature in the dark.

Thiamine Pyrophosphatase and Nucleoside Diphosphatase

Pieces (fetus) or sections (adult) were incubated at 37°C in the medium of Novikoff and Goldfischer (22), containing 5% sucrose, for thiamine pyrophosphatase (TPPase) and nucleoside diphosphatase (NDPase) activities. The ingredients are 25 mg thiamine pyrophosphate (TPP) (Sigma Chemical Co., St. Louis, Mo.) or inosine diphosphate (IDP) (Sigma Chemical Co.); 7 ml distilled water; 10 ml 0.2 M Tris-maleate buffer, pH 7.2; 5 ml 0.025 M manganese chloride; and 3 ml 1% lead nitrate. The medium is filtered after 5–10 min, and renewed after 30 min, when longer incubation times are used.

Extensive observations were made of TPPase-incubated tissue of both fetal and adult rat ganglia. The results of more limited observations with NDPase localization did not differ in any manner from those with TPPase localizations. For the sake of brevity we will often refer to the two activities as diphosphatase activity. The work of Yamazaki and Hayaishi (23) raises the possibility that both activities are due to a single enzyme, like the highly purified liver microsomal nucleoside diphosphatase (23). If the Golgi apparatus enzyme (or enzymes) has (or have) properties like those of the Yamazaki-Hayaishi diphosphatase, then the incubation procedure employed by us could be markedly suboptimal. We therefore tested the staining of spinal ganglia (also liver and epididymis) at the near optimal pH 8.5, in the absence and presence of adenosine triphosphate (ATP) (which stimulates the TPPase activity of the purified enzyme [23]). Other conditions which were tested include: (a) a three-fold increase in TPP concentration (22); (b) addition of 2.5×10^{-3} M cysteine hydrochloride which stimulates nucleoside phosphatase staining of hepatocyte plasma membranes in liver sections (24); and (c) reduction of lead concentration by 25%. In addition, the fixation time was reduced to 15 min in some experiments. In other experiments glutaraldehyde was omitted entirely and tissues were fixed in 2% paraformaldehyde, 0.025% CaCl₂, 0.1 M cacodylate buffer, pH 7.4, for 15 min, 30 min, and overnight. Although the TPPase staining is markedly increased by several of these procedures, there is no

increase in the number of Golgi elements which show activity.¹

Acid Phosphatase

Pieces (fetus) and sections (adult) were incubated at 37°C in the medium of Gomori (25) with the substitution of 5'-cytidylic acid (CMP) for glycerophosphate as substrate (26), and the addition of 5% sucrose. The ingredients are 25 mg CMP (Sigma Chemical Co.); 12 ml distilled water; 10 ml 0.05 M acetate buffer, pH 5.0; and 3 ml 1% lead nitrate.

Osmium Reduction

Neurons in adult dorsal root ganglia survive the prolonged osmication procedure of Friend (27) but 16-day fetal neurons do not. The procedure consists of immersion fixation in 2% aqueous OsO₄, followed by 48 hr at 40°C, with renewal of the OsO₄ after 16–24 hr. Fetal neurons neither darkened nor remained intact in four experiments, with fetuses from different rats. Only the nuclei were recognizable when the straw-colored ganglia were examined in the electron microscope. The following methods were used in the effort to overcome this disintegration: (a) reducing the time in warm OsO₄ to 16–24 hr which, in adult ganglia, suffices to give results like those after 48 hr; (b) the use of the Friend-Murray procedure (28) which involves initial fixation in buffered OsO₄ or glutaraldehyde before prolonged fixation in warm OsO₄ (the fixation we used was 2% OsO₄ in 0.1 M cacodylate buffer, pH 7.4, for 1 or 2 hr; or 3% glutaraldehyde–0.1 M cacodylate buffer, pH 7.4, for 30 min followed by overnight rinse in buffer); and (c) substituting 4% aqueous OsO₄ for the 2% aqueous OsO₄. None of these procedures was successful. Our description of the osmium-reducing Golgi element is based only on the small neurons of adult ganglia.

Sectioning and Microscopy

Thick sections were cut with either the LKB Ultratome or Porter-Blum MT-1 Ultra-microtome with glass knives, at thickness settings of 0.25, 0.5, and 1 μ . Sections were examined, sometimes after lead citrate staining (29) or carbon coating, in the Philips 300

¹ In hepatocytes the level of TPPase activity in the ER is much enhanced by pH 8.5. In all three tissues the higher pH, and particularly the addition of cysteine, increased the level of nucleolar staining reported earlier (22). The electron microscope shows that the reaction product is mainly in the "nucleolonema," i.e., the apparent strands composed of aggregated granules and fibrils.

electron microscope at 100 kv, or RCA 3H microscope at 100 kv. All the electron micrographs of thick sections in the figures are of 0.5- μ sections (i.e., 10-20 times thicker, when put into the microscope [see Stenn and Bahr (30)], than the usual thin sections). 0.5- μ sections of fetal ganglia incubated for phosphatase activities were tilted in the Philips microscope by a goniometer stage. The sections were tilted $+45^\circ$ and -45° from the original (0°) position.

Thin sections (up to 500 A) were cut on the same microtomes with diamond knives. They were stained with lead citrate or uranyl acetate followed by lead citrate. Sections were examined in the RCA 3H microscope (100 kv) or in the Siemens Elmiskop I at 80 kv.

Consecutive serial thick sections were studied of material incubated for either TPPase or acid phosphatase (AcPase) activity. Serial thin sections have been studied of TPPase-incubated material; studies on serial AcPase thin sections are in progress.

RESULTS

In the interest of economy, observations not directly related to the phosphatase-positive or osmium-stained structures will be described in the figure legends only and will be considered in the Discussion.

Diphosphatase Activity

The diphosphatase-rich element consists of hexagonal arrays of interconnected tubules. The presence of the hexagons is evident at once in thick sections (Figs. 1-7). Tilting the specimen often shows that apparently straight ("saccular") regions are, in fact, hexagonal arrays (Figs. 2-6). In thin sections of incubated tissue, portions of the hexagonal array in the diphosphatase-rich

element are seen only occasionally (Figs. 9, 11, 13-15). It is far more difficult to appreciate their existence in thin sections of unincubated tissue.

Fig. 11 shows the thin section of incubated tissue which best revealed the hexagonal arrays. Most images are like those of Fig. 8, interpretable without difficulty only after studying thick sections. In Fig. 12 (an uncommon image) most of the structures with reaction product would generally be interpreted as vesicles, distinguishable from other vesicle-like structures of the same size because the tissue has been incubated. However, they are most probably transverse sections of the tubules which constitute the hexagonal array, rather than vesicles.

The position of the hexagonal diphosphatase-positive element in the Golgi stack cannot be ascertained from thick sections. However, when in thin sections the plane of section passes vertically through the stack (Fig. 8, right-hand portion of Fig. 12, and Figs. 13-15), the positive element is clearly seen to be the innermost element of the Golgi stack.

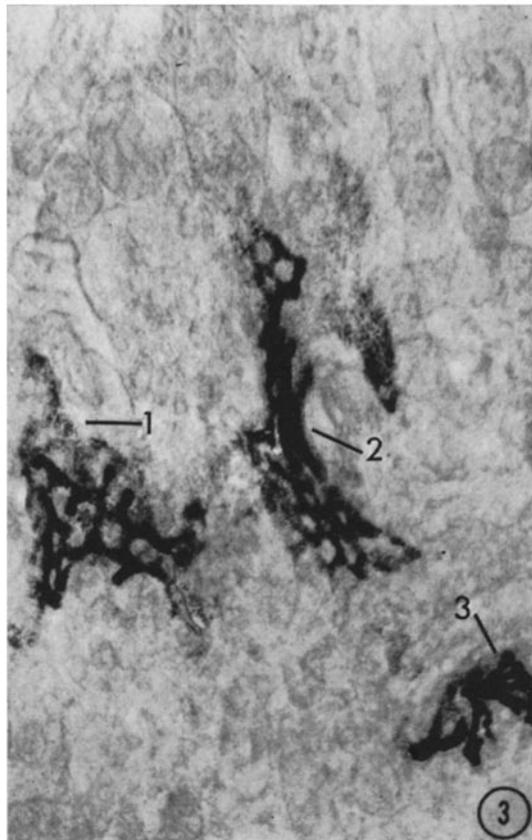
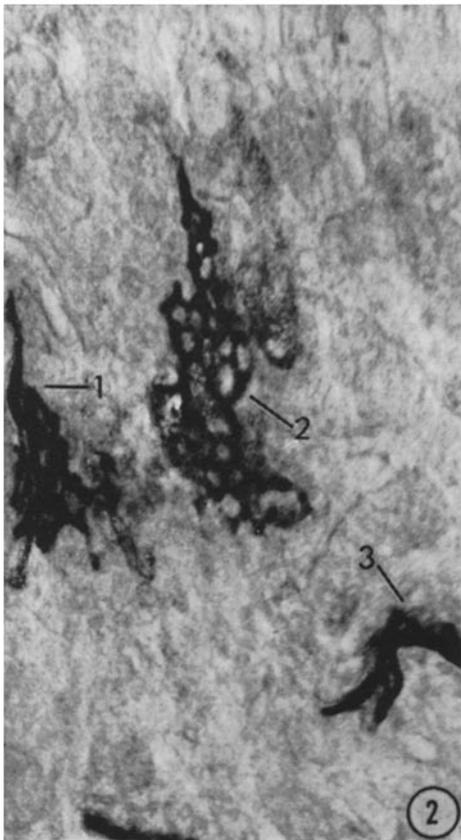
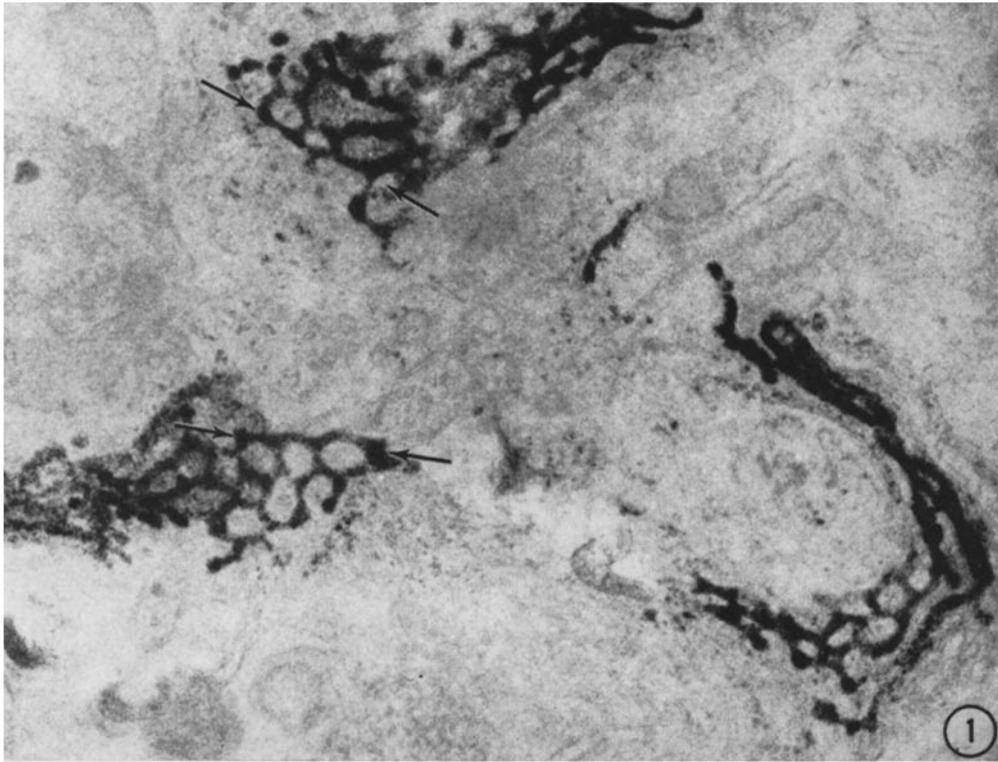
Both thick and thin sections may suggest that there are two diphosphatase-rich elements (Figs. 4-6, 13-15). However, serial sections show these to be twisted portions of a single element (Figs. 13-15). It is possible to encounter in thin sections fairly extensive lengths of the innermost element that appear as one (Fig. 8) or two (Figs. 13 and 14) saccules. The arrows in Fig. 1 indicate how such images may be obtained from a hexagonal array. Lengths of up to 1200 $m\mu$ of apparent saccules are found (cf. Figs. 1, 2, 4, and 8).

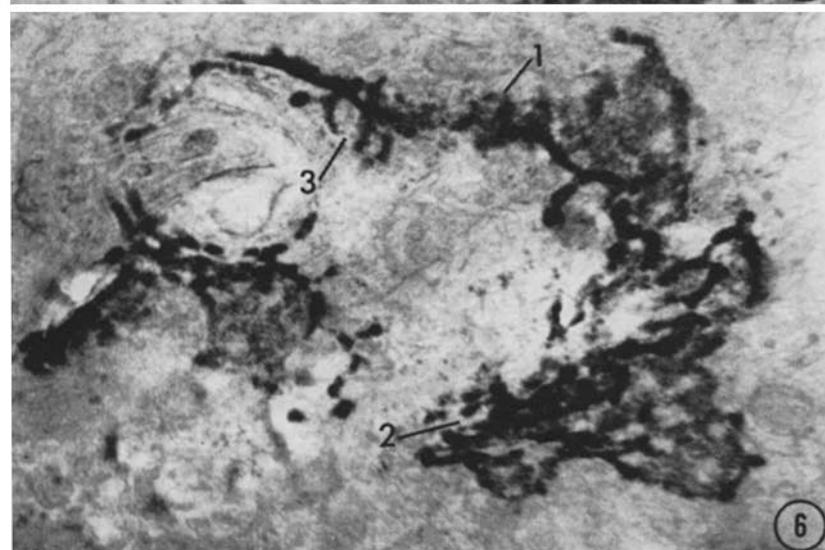
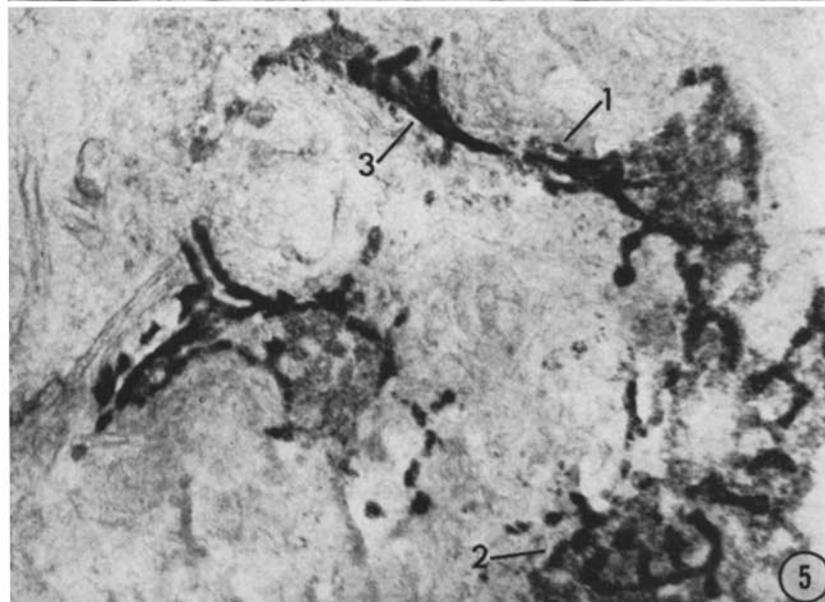
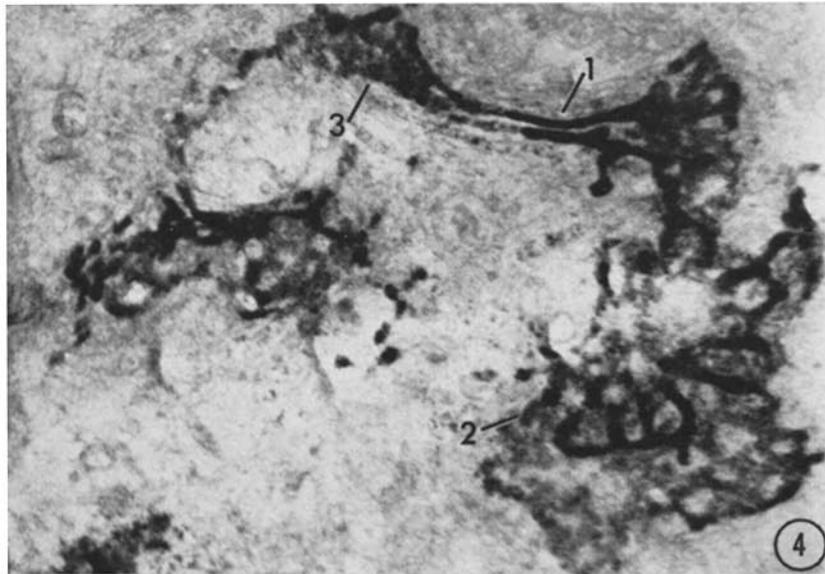
The areas enclosed by the hexagonal arrays will be referred to as *polygonal compartments*, with the

FIGURES 1-6 TPPase preparation (60 min incubation) of fetal ganglion; 0.5- μ sections, not stained with lead.

FIGURE 1 Three portions of the Golgi apparatus of one neuron are seen. The hexagonal arrays of the Golgi element are evident. In two of the three portions, arrows indicate planes through which a section might pass and thus create the impression of a straight (saccular) structure. The distance between the two lower arrows is 530 $m\mu$. In the third portion the straight regions probably do, in fact, pass in such planes. Tilting the specimen (see Figs. 2-6) would probably reveal the tubular nature of the straight regions. $\times 38,000$.

FIGURE 2 and 3 A portion of another neuron photographed in Fig. 2 at a tilt of -45° , and in Fig. 3 at a tilt of $+45^\circ$ from the initial 0° position. Region 1 shows the hexagonal array of tubules more clearly in Fig. 3 than in Fig. 2. The situation is reversed in region 2: the hexagonal array is seen better in Fig. 2; in Fig. 3 it appears straight (saccular) for about 650 $m\mu$. The tubular nature of region 3 is more evident in Fig. 3. Going up from region 2 in Fig. 2 a straight line may be drawn, as between the arrows in Fig. 1, for about 950 $m\mu$. $\times 23,000$.





recognition that they are enclosed only in the planes which show the hexagonal arrays. In thin sections tubular structures are seen within the polygonal compartments (Figs. 8–11, 13–15). They are delimited by smooth membranes, whose structural relations are still being studied in our laboratory. They appear to arise from smooth ER, possibly part of GERL (Fig. 15). They may fill most of the polygonal compartment (Figs. 11 and 13) but often they appear collapsed, as if poorly preserved.

The sides of each hexagon measure about $85\text{ m}\mu$ in length. The distance across the polygonal compartment is approximately $150\text{ m}\mu$. The diameter of the nucleoside-positive tubule which forms the hexagons is about $40\text{ m}\mu$. Thus, the "space" far exceeds the membranous part of the Golgi element. Therefore, the term "fenestrated," is inapplicable to this element since fenestrae imply relatively small openings in a larger sheet of membrane.

Serial thick sections suggest that in both fetus and adult the Golgi apparatus, as judged by its diphosphatase-rich element, forms a continuous network. Gaps between separate sections are bridged by the superimposition of $0.5\text{-}\mu$ sections. Serial thin sections establish such continuity (Figs. 13–15).

AcPase Activity

The images seen in thick sections incubated for AcPase activity differ strikingly from those seen in TPPase- or NDPase-incubated ganglia. Dense body-type lysosomes, with much reaction product, dominate the scene (Fig. 17). Neither the diphosphatase-rich element nor other elements of the Golgi stack gives evidence of AcPase activity (Figs. 17, 24; compare with thin sections [Figs. 30, 32]). GERL, which is negative for both TPPase and NDPase activities, is strongly AcPase-positive.

GERL lies at the inner aspect of the stack and consists of many flattened portions (cisternae) with innumerable tubules connected to each

cisterna. The cisternae show best when seen in face view in thick sections (Figs. 17–20, 27–29). When not directly seen, the presence of cisternae is often revealed when the specimen is tilted (Figs. 21–23, 24–26). Fenestrae or discontinuities are not evident in the cisternae.

Thick sections of both fetal and adult ganglia also dramatize the numerous AcPase-rich tubules of GERL. They are connected with the cisternal portions (Figs. 17–29). Many regions that appear flat reveal their tubular character when tilted (Figs. 21–26). The tubules are considered to be smooth ER because they are connected to rough ER (Fig. 39) (also see Figs. 50, 52–54 in reference 14).

In neurons of fetal ganglia the tubules of GERL do not form geometric arrays such as those found in the diphosphatase-rich Golgi element. However, many interconnections do exist among the tubules (Figs. 18, 20–23). Occasionally the tubules form rough squares or polygons, but generally only irregular anastomoses are seen.

In the few neurons examined in thick sections of adult ganglia the GERL tubules are more regularly arranged, sometimes approaching a regular polygonal arrangement (Figs. 28, 29).

Thick sections strongly suggest that dense bodies arise by separation of dilated areas from GERL. These dense bodies are characterized by fine electron-opaque grains, but occasionally myelin-like lamellae and other membranous structures are present, in fetal as well as in adult ganglia. Most dense bodies appear to arise from the tubular portions of GERL (Figs. 17–19) but some seem to form directly from the cisternae (Fig. 27). It is possible that twisted and sausage-like images such as are seen in Fig. 20 may represent sections of two or more lysosomes separating from a cisternal portion, as in Fig. 27. Images consistent with origins from both tubules and cisternae are encountered, when sought, in thin sections (Figs. 31, 34).

FIGURES 4–6 A portion of another neuron, photographed at a tilt of -45° in Fig. 4, untilted (0°) in Fig. 5, and at a tilt of $+45^\circ$ in Fig. 6. Region 1 appears straight in Fig. 4, for about $880\text{ m}\mu$, but in Figs. 5 and 6 its interconnecting nature is seen. The hexagonal arrays, evident at region 2 in Fig. 4 and less well in Fig. 5, are blurred in Fig. 6. The lower part in region 3 appears straight in Fig. 5 and tubular in Fig. 6; the upper part is clearly tubular in Figs. 4 and 5 but not in Fig. 6. In Figs. 4 and 6 the lower right portion of the diphosphatase-rich element is seen to be twisted upon itself (cf. Figs. 4–6 and Fig. 7). $\times 34,000$.

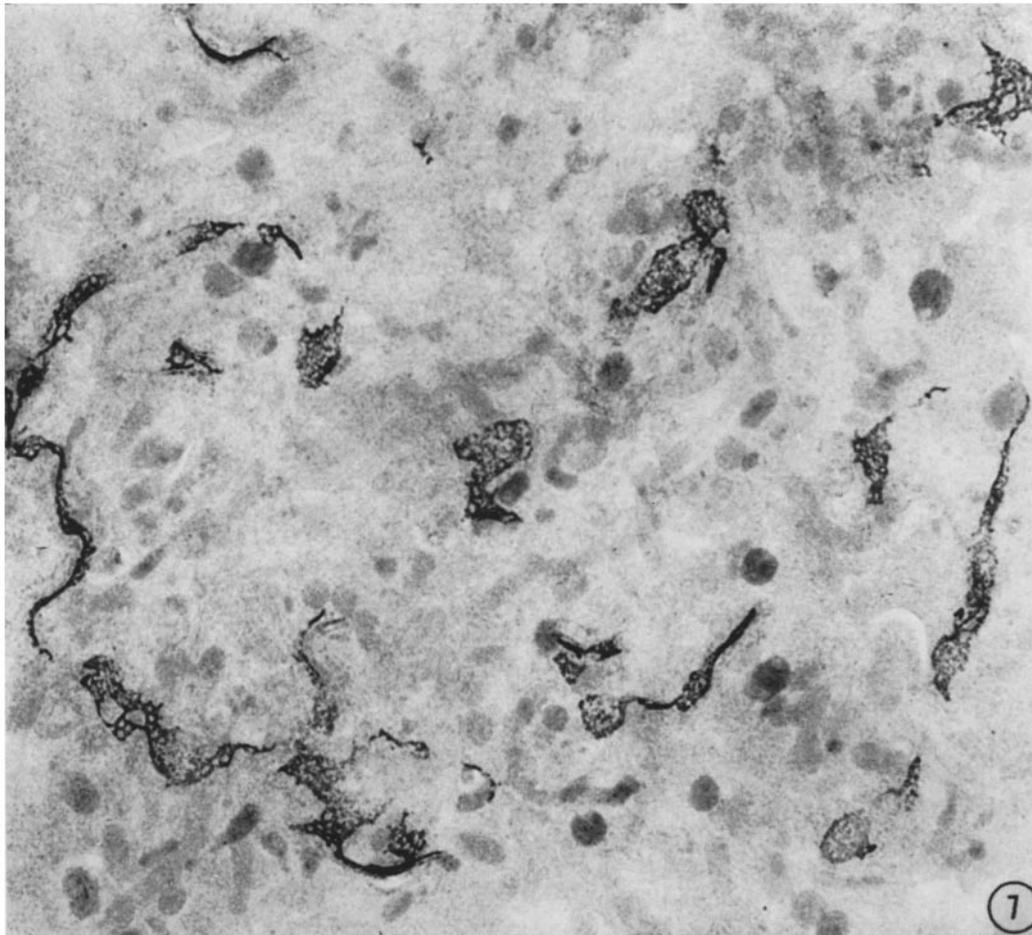


FIGURE 7 TPPase preparation (60 min incubation) of adult ganglion; 0.5μ section, stained with lead. A small neuron was sectioned in a plane of the cytoplasm that shows the extensive Golgi apparatus and its numerous twists and turns. The round bodies are dense bodies, unreactive but with sufficient electron opacity after lead staining to be evident. $\times 13,000$.

Serial thick sections suggest that GERL, like the Golgi apparatus, is continuous, with the tubules connecting individual cisternae.

AcPase-rich structures too small to be identifiable in thick sections, but readily seen in thin sections, include at least two types of lysosomes, as defined by morphological criteria (31, 26): coated vesicles and autophagic vacuoles. Coated vesicles are numerous in both fetal and adult neurons. They are often encountered near GERL (Fig. 33) and occasionally in continuity with GERL in a manner suggesting their origin from GERL (Fig. 35). There appear to be two types of autophagic vacuoles, the second of which (Figs. 30, 34) is

frequently seen in both fetal and adult ganglia and appears to arise from GERL, as discussed in the legend to Fig. 30.

The smooth tubules leading into the polygonal compartments have not yet been identified in AcPase preparations.

Osmium Reduction

In regions of many small neurons of adult ganglia the ER is strongly stained by osmium following prolonged osmication. These observations will be presented in a separate publication, together with ER as well as Golgi apparatus

staining in a variety of other cell types (hepatocytes, melanomas, thyroid epithelium).

It is the outer Golgi element which reduces osmium (Figs. 37–39) and thus creates the classical Golgi apparatus staining seen by light microscopy. A 0.5 μ Epon section is shown in Fig. 36. This is consistent with the drawings of much thicker sections in the historic papers of Camillo Golgi dealing with spinal ganglia of other species (32, 33).

The outer element is an anastomosing system of highly irregular tubules or small saccules (Figs. 37, 38). This element lacks the regular geometry of the diphosphatase-rich inner Golgi element. The ratio of space to membranous portions is much smaller in the outer element than in the inner element.

The other Golgi elements are not stained by osmium. GERL is unstained, even in areas where the endoplasmic reticulum elsewhere stains. Also unstained are the dense bodies and coated vesicles.

DISCUSSION

The Golgi Apparatus and GERL in these Neurons

Incubation for nucleoside diphosphatase activity shows the Golgi apparatus, in neurons of 16-day fetal rat dorsal root ganglia (where size difference is not evident) and in the small neurons of adult dorsal root ganglia, to be a long, much-twisted organelle coursing through the cytoplasm (for other neurons see references 22, 34, 16, and 14). In the fetal ganglia, where the nuclei are eccentrically situated in the cytoplasm, the Golgi apparatus is found in the major cytoplasmic mass when viewed by light microscopy. In the adult the organelle covers a broad area, roughly concentric to the nucleus. This is clearly shown in Golgi's 1898–1899 publications (Fig. 1 in reference 32 of an adult dog ganglion and Figs. 1–3, 7, and 8 in reference 33 of adult ganglia of horse, rabbit, and dog respectively; cf. Figs. 4 and 5 in reference 33 of fetal cow ganglia).

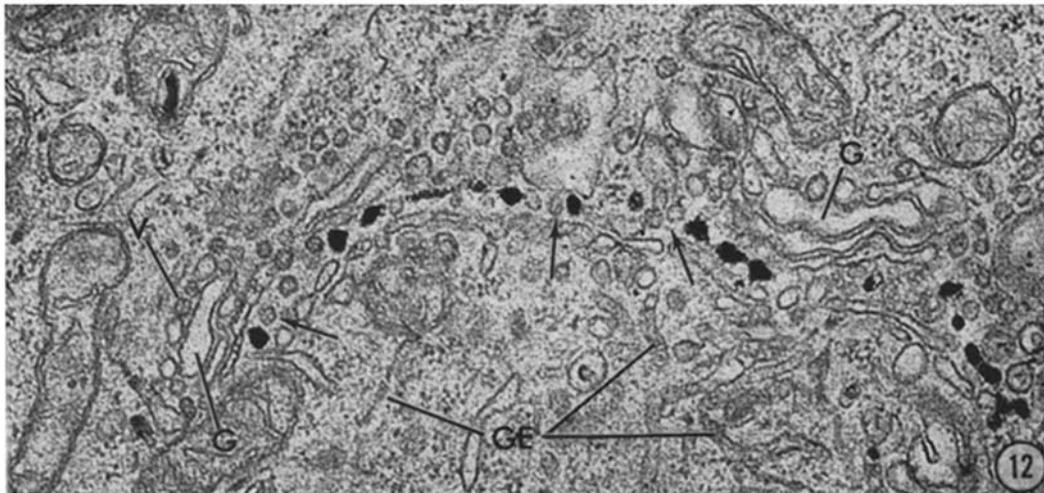
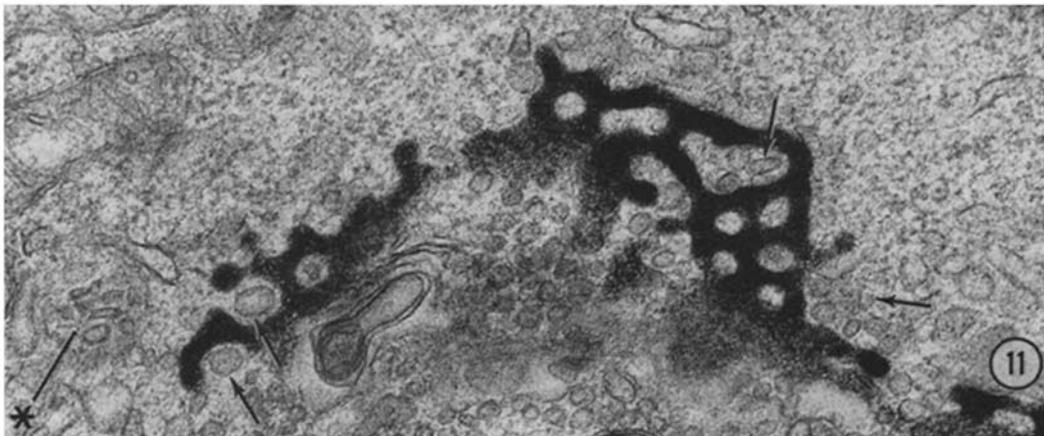
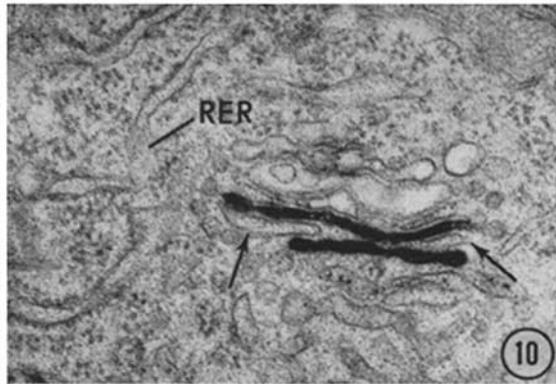
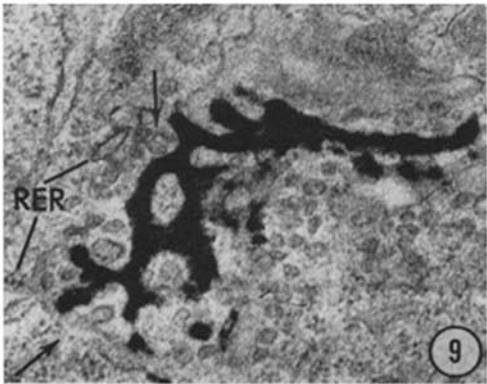
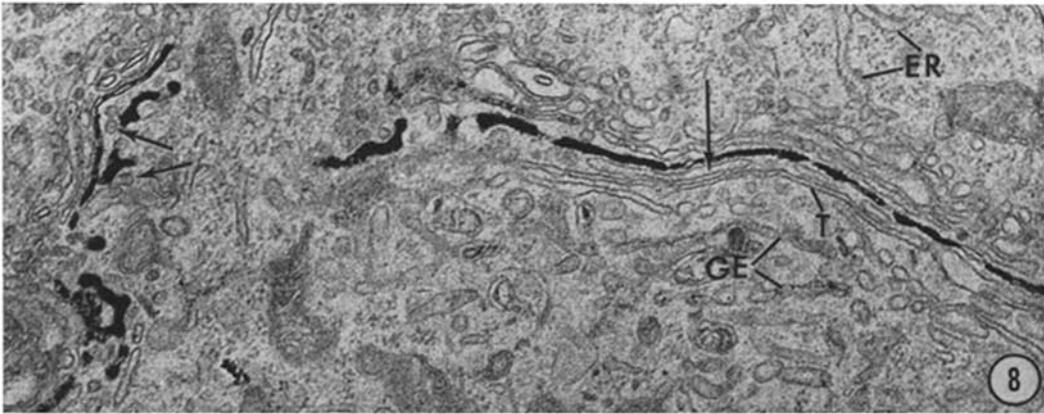
It is notable that in the neurons we have studied, as in other cell types that we have studied by electron microscopy after prolonged osmication, only one element—the outer one—is stained. Incubation for nucleoside phosphatase activity stains one element—the inner one (in other cell types it often appears as if two inner elements show activity but these have not yet been studied by thick sections or serial sectioning). Because both outer and

inner elements are parallel portions of the same stack and since both deposit sufficient metal or reaction product, essentially the same image is seen at the level of light microscopy.

Light microscopy of AcPase preparations of the neurons in the dorsal root ganglion of the fetal rat (unpublished) and in the adult (Figs. 44–48 in reference 14) does not show the classical Golgi appearance. Three factors probably contribute to this: (a) the elements of the Golgi stack have no demonstrable AcPase activity in these cells; (b) although AcPase-positive GERL is always close to the inner surface of the Golgi stack it does not follow its contours exactly; and (c) the staining of adjacent lysosomes is superimposed upon that of GERL when viewed by light microscopy. In other cell types, only a very small number show AcPase activity in Golgi elements sufficient to produce the classical picture of the Golgi apparatus when viewed by light microscopy. In our laboratory we have encountered this only in epithelial cells in the rat glomerulus (36) and in some cells of rat testis and pituitary (22).

The apparent continuity of the Golgi apparatus when viewed by light microscopy is now extended to the level of electron microscopy. As Rambourg and Chrétien (35) showed with the outer Golgi element of Gasserian ganglion neurons stained by osmium (28), superimposition of micrographs of consecutive 0.5- μ sections of fetal and adult dorsal root ganglia incubated for nucleoside diphosphatase activity closes the discontinuities seen in individual sections. Apparent discontinuities often disappear when thick sections are tilted in the microscope. Naturally, small gaps could escape detection in thick sections. These have been eliminated, for the innermost element at least, by studying serial thin sections of adult ganglia incubated for nucleoside diphosphatase activity. The Golgi apparatus is indeed a continuous organelle at the electron microscope level as in the light microscope. The continuous twisting inner element, entirely hexagonal without saccular portions, is represented diagrammatically in Fig. 40.

Serial thick sections of AcPase-incubated material show that the cisternal portions of GERL are spatially separated. They suggest that the GERL tubules connect neighboring cisternal portions to form a continuous network, as drawn diagrammatically in Fig. 40. Serial thin sections are currently being studied for more unequivocal evidence for such continuity and to ascertain



FIGURES 8-12 Portions of neurons seen in thin sections of fetal ganglia (60 min incubation for TPPase or NDPase activity). The tissues were treated with uranyl acetate before embedding; and the section were stained with lead.

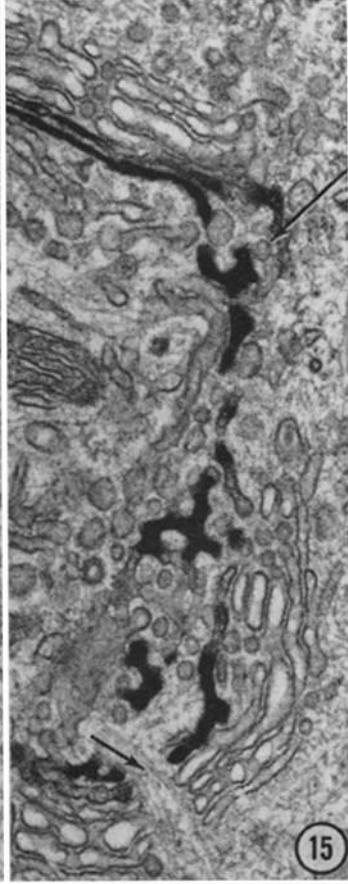
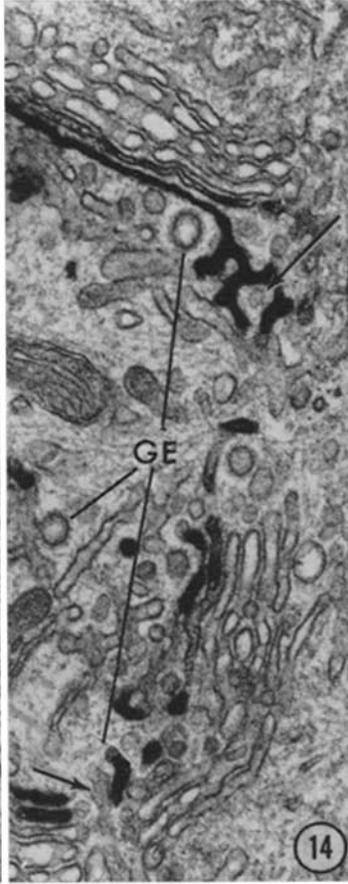
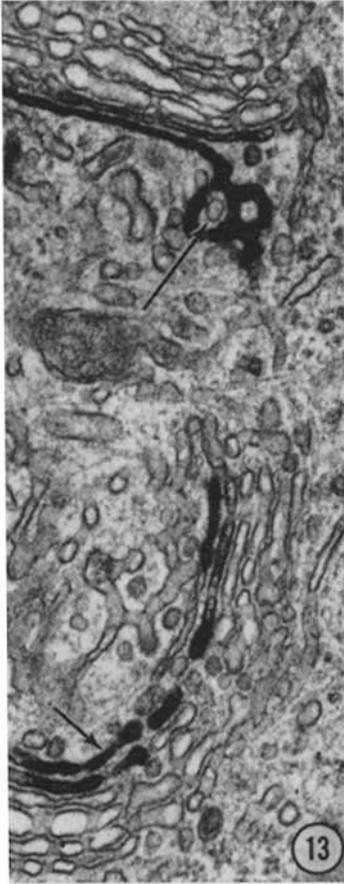
FIGURE 8 TPPase preparation. The polygonal nature of the nucleoside diphosphatase-rich element is suggested only by its appearance at the left of the figure. In the apparently continuous portion of the element in the right half of the figure, small breaks may be seen. If the structure just to the left of the head of the long arrow is considered a break, the straight portion would extend for about 580 $m\mu$. If, instead, the structure is considered a slightly disrupted area of the phosphatase-rich element, then it would continue, as an arc, for another 580 $m\mu$. Figs. 1 and 2 show how saccular lengths of up to 950 $m\mu$ may be obtained; in Fig. 11 (right side), a straight line could be drawn for 800 $m\mu$. In other micrographs, lengths of up to 1200 $m\mu$ have been seen. Proceeding downward towards GERL (*GE*) from the rough ER at the top right (*ER*), the following elements are seen (a) three elongate smooth-membraned transitional sheets (see Fig. 40); (b) the outer Golgi element—it is an interrupted smooth membrane structure which osmium staining (Figs. 37, 38) shows to be an irregularly branched element; (c) two smooth-membraned Golgi elements, the first swollen and the second flat with some interruptions that show better in Figs. 13-15; (d) the innermost Golgi element, filled with TPPase reaction product. The short arrows at the left are directed towards smooth-membraned tubules within the polygonal compartments (incompletely shown in the figure). The upper tubule appears circular and the lower one more elongate. The lower one lies close to another elongated vesicle which is close to, possibly continuous with, the long tubule of smooth ER or GERL (see Fig. 15). The long arrow is directed to a long smooth tubule, from which branches probably arise in other planes, to enter the polygonal compartments (cf. Figs. 10, 13-15). The thin tubule (*T*) and the extensive, wider, smooth-membraned tubules (*GE*) are part of GERL. $\times 24,000$.

FIGURE 9 TPPase preparation; a portion of the diphosphatase-positive element which shows some of its hexagonal nature. Note the tubules, sectioned roughly perpendicularly, within the polygonal compartments. Arrows indicate regions suggestive of continuities with other tubules or smooth ER. Portions of rough ER are seen at *RER*. $\times 48,000$.

FIGURE 10 TPPase preparation. There would appear to be two diphosphatase-positive structures (saccules). However, the cumulative evidence from micrographs like Figs. 1-9 and from the study of serial thick sections and thin sections (Figs. 13-15) makes it highly probable that these are sections through the single hexagonal Golgi element. Including the portion that is arched rather than straight, the continuous length measures 820 $m\mu$. Arrows indicate two smooth-membraned structures, part of a tubule which dips in and out of the plane of section. Like the tubule seen at the long arrow in Fig. 8, it is smooth ER, probably GERL. *RER* indicates a region of smooth ER that is continuous with rough ER. $\times 39,000$.

FIGURE 11 NDPase preparation. Because of the unusually favorable plane of section, the hexagonal nature of the diphosphatase-positive element is more evident than in any of the numerous thin sections studied. At the right, the hexagons are more rounded than usual, perhaps due to overincubation. A straight line through the upper part of the element would measure 820 $m\mu$. The two arrows at the left indicate roughly transverse sections of the tubules within the polygonal compartments. The two arrows at the right (also the upper arrow in Fig. 14) suggest that these tubules branch, probably from GERL (Figs. 13-15). The asterisk is directed towards a structure frequently encountered in these neurons and in the small neurons of adult ganglia. In this section, its delimiting membrane is seen only in some regions (cf. Fig. 11 and serial sections in a neuron of an adult ganglion, Figs. 53, 54 in reference 14). This body is considered a type of autophagic vacuole (see legend to Fig. 30). $\times 44,000$.

FIGURE 12 TPPase preparation. The small diphosphatase-positive structures which appear like vesicles or short tubules are interpreted as transverse or slightly oblique sections of the tubules which constitute the diphosphatase-positive element. Note the absence of reaction product in the other Golgi elements (*G*), GERL (*GE*), and numerous small vesicles. The Golgi stack apparently dips in and out of the section. Many of the negative small vesicles (*V*) are near the outer aspect of the Golgi stack and may be transitional vesicles (see Fig. 40). Some of the negative vesicles (arrows) among the diphosphatase-positive tubules are probably transverse sections of tubules within the polygonal compartments. $\times 48,000$.



whether all interconnecting tubules show AcPase activity.

The Golgi Stack in these Neurons

Fig. 40 summarizes: (a) our present knowledge of the Golgi stack of the neurons we have studied; and (b) the probable relations of ER to the outer and inner aspects of the stack.

Various mechanisms have been suggested by which materials may be transported from the ER to the outer aspect of the stack or to other structures in the Golgi zone. In fasted guinea pig pancreas, Palade and colleagues (1-3) have described small "peripheral vesicles" that ferry secretory materials from the "transitional elements" (with part rough and part smooth membranes) of the rough ER to "condensing vacuoles." From the absence of radioautographic grains over the flattened Golgi elements, Jamieson and Palade (3) suggest that these elements are not involved in transporting and concentrating the secretory proteins carried by the vesicles. These roles, in this cell, are played by condensing vacuoles. The origin of the vacuoles has not been described in guinea pig pancreas, but in rat and mouse pancreas the secretory vacuoles appear to derive from the innermost of the "piled Golgi cisternae" (3). Perhaps the direct movement from ER to condensing

vacuoles represents a special feature of these cells under these specific conditions.

Many investigators have described small vesicles as carrying ER products to the outer element of the Golgi stack (e.g., references 17, 37-39). In our laboratory we have stressed another possible mechanism, a "membrane flow" to the outer Golgi element that involves larger sheetlike derivatives of the ER (40-42). These sheets often show ribosomes on the outer surface but not on the surface adjacent to the Golgi stack. Such sheets have been observed in cells of adrenal medulla (43), Reuber H-35 hepatoma (41), and thyroid epithelium (unpublished). A similar interpretation may be placed on images seen by Flickinger (44) in neonatal rat epididymis cells, by Maul (45) in cultured human melanoma cells, and by Holtzman (46) in neurons of cultured mouse dorsal root ganglia.

We propose to use the terms "transitional vesicles" and "transitional sheets" for the structures transporting materials from ER to the outer Golgi element. Both are found in the neurons of dorsal root ganglia (Figs. 8, 12). Whether sheets or vesicles contribute more membrane to the outer Golgi element is not evident; nor is it known that the same materials are carried by vesicles and

FIGURES 13-16 Thin sections of adult ganglia incubated for TPPase activity, 75 min. The tissue was rinsed in uranyl acetate before embedding. The thin sections were stained first with uranium and then lead. FIGURES 13-15 Portions of three consecutive sections of a small neuron. Reaction product marks the diphosphatase-rich element. Its hexagonal nature is suggested in regions. Apparent discontinuities, too small to be resolved in thick sections, are present in one section but these discontinuities disappear in other sections of the series. For example, the region at the lower arrow is interrupted in Fig. 15 but not in Fig. 13. The long arrows above indicate transverse sections of tubules within adjacent polygonal compartments. In Fig. 15 the tubule lies close to a tubular element of GERL. The latter is seen to anastomose with other GERL tubules in Fig. 14. The short arrows at the bottom indicate where a smooth ER tubule (Fig. 14), probably part of GERL, is located within a depression in the Golgi stack; note the neurofilaments in the depression (Fig. 15). The extensive area covered by GERL is indicated in Fig. 14 (GE); note its numerous coated vesicles. Note the discontinuities in the three Golgi elements adjacent to the diphosphatase-rich elements (the clear areas are sections through the interior of the element). $\times 43,000$.

FIGURE 16 A portion of another small neuron which, in this region, shows no reaction product, presumably because the TPPase activity was inhibited by the glutaraldehyde-containing fixative. Note that the smooth ER region is continuous with rough ER of the Nissl body (at upper right). The smooth ER (long arrow) is directed roughly perpendicular to the Golgi stack. As it approaches the stack the ER is roughly angular. Nearby are small vesicles, possibly transitional vesicles (see Fig. 40). Short arrows indicate membranes where ribosomes are absent from the side of the membrane facing the Golgi stack; these may be transitional sheets (see Fig. 40). A probable transitional vesicle (Fig. 40) is seen at V. AV indicates a body interpreted as a later stage in development of the type of autophagic vacuole seen in Fig. 11 (cf. Figs. 30, 34). $\times 45,000$.

sheets into the tubules or small cisternae of the irregularly anastomosing Golgi element.

It is only the outer element of the Golgi stack which is stained by osmium upon prolonged osmication. As noted earlier, in these neurons, as in other cell types studied, the endoplasmic reticulum is often also stained by osmium. It may be that the osmium staining of the outer element is due to an increase in concentration of a particular liquid or other constituent of the ER membranes which occurs in this Golgi element.

The structure of the outer Golgi element varies considerably from one cell type to another. In the neurons of the Gasserian ganglion it is composed of regular polygonal arrays (35). In other cell types it is fenestrated and there is more membrane than fenestrae. It is sometimes difficult to distinguish the outer Golgi element from ER structurally (see references 41–43). Claude (47) speaks of an “intermediate type of Golgi profile” in rat hepatocytes which retains its continuities with smooth ER. When the Reuber H-35 hepatoma was studied in our laboratory an image was obtained with the outer Golgi element still continuous with rough ER; the element was smooth on its inner aspect but still had ribosomes on its outer surface (Fig. 40 in reference 42). In that study (41) we stressed the likelihood of “modulation” in different states of activity in the dynamic relation of ER to the outer Golgi element. If ER were rapidly transforming into the outer Golgi element some ribosomes might still be left on its surface. This may also account for the images shown by Flickinger

(48) of the outer Golgi element continuous with RER when the Golgi apparatus of enucleated amoebae rapidly enlarges following implantation of nuclei. Claude (47) has recently described the coalescence of smooth ER tubules which carry the small VLDL (very low density lipoprotein) particles to form an “intermediate fenestrated plate.”

Little can be said about the next two to four elements lower down in the Golgi stack except that they appear to be fenestrated (Fig. 40). Since these elements neither react with osmium nor display any of the phosphatase activities studied, it is not possible to describe the geometrical forms of these elements at this time.

The innermost element of the Golgi stack in these neurons has the most dramatic structure and the most intimate relationship to ER. It is composed solely of hexagonal arrays of tubules (Fig. 40). In each polygonal compartment a smooth-surfaced tubule is present. These tubules appear to be extensions of smooth ER tubules. Studies are in progress to determine whether these ER tubules come directly from rough ER or are part of GERL, or whether both situations exist. Serial thin sectioning of ganglia incubated for AcPase are expected to show whether the ER tubules and the tubules inside the polygonal compartments show AcPase activity.

The intimate spatial relation of smooth ER to a hexagonal array of tubules of the Golgi apparatus provides a large area of contact between the two organelles. The area for molecular interchange

FIGURES 17–27 Portions of neurons in AcPase preparations (40 min incubation) of fetal ganglia; 0.5- μ sections, not stained with lead.

FIGURE 17 The extent of GERL is emphasized in this micrograph. The numerous dense bodies (lysosomes) are intensely electron opaque (*L*). The elongate forms are interpreted as dense bodies in formation—by budding from swollen areas of GERL. Numerous sections through GERL are seen. The marked area shows a face view of a cisternal region; it is enlarged in Fig. 20. The asterisk (upper right corner) indicates a cisternal region seen on end. Because it lacks AcPase activity, the Golgi stack is barely evident; a small portion is seen at *G. C*, cisternae; *T*, tubules. $\times 20,000$.

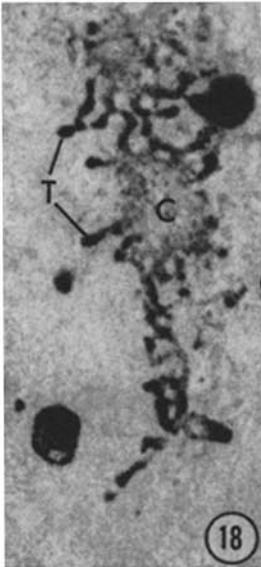
FIGURE 18 This demonstrates continuity of tubules (*T*) with a cisternal portion (*C*) of GERL. The two roughly circular bodies are dense bodies. $\times 26,000$.

FIGURE 19 Another portion of the neuron from which Fig. 18 was taken. A cisternal portion (*C*) of GERL and tubules (*T*) connected with it are seen. The arrow indicates a region interpreted as a dense body arising from a swollen area of a GERL tubule. $\times 26,000$.

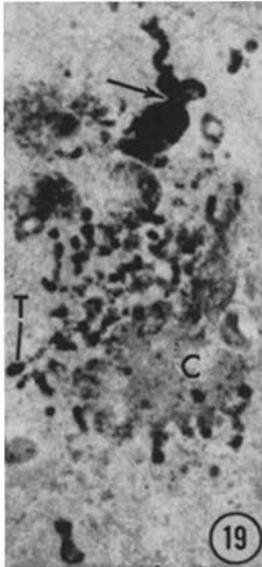
FIGURE 20 An enlargement of the area within the square of Fig. 17. A cisternal portion (*C*) and connecting tubules (*T*) of GERL are evident. The continuities among the dense bodies seen at the top of the figure are interpreted as dense bodies in formation, perhaps from a cisternal portion (see Fig. 27). $\times 41,000$.



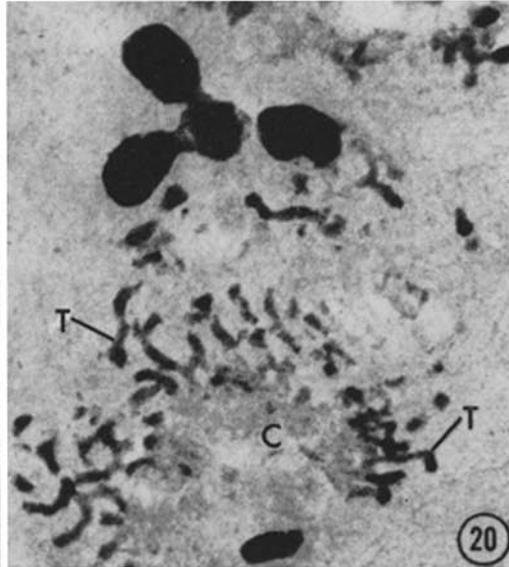
17



18



19



20

between ER and Golgi element is much larger with a hexagonal tubular arrangement than is possible with a flattened saccular Golgi element. Modulation in the extent and nature of polygonal arrays may be expected within the same cell under varied physiological states of experimental conditions. Wide variations among different cell types are evident from the literature and from unpublished observations in our laboratory. Among the most dramatic changes in Golgi apparatus structures that have been reported are those following testosterone treatment of castrated mice (35), during the growth of fetal epididymis (44), and in mitosis of cultured cells (see, for example, reference 49).

The hexagonal arrays in the innermost Golgi element of the neurons studied are reminiscent of the "reticulate system of tubes" described in meristematic cells of *Anthoceros* by Manton (50), the "honeycomb or lattice-type arrangement" seen in the test cells of *Styela* ovary by Kessel and Beams (51), the "anastomosing network" described by Cunningham et al. (52) and Mollenhauer and Morr  (53) in plant cells *in situ* and in negatively stained structures isolated from plant homogenates, and the innermost Golgi "cisterna" of epididymis with many "fenestrae," some suggesting "a hexagonal pattern," described by Flickinger (54).

Of greatest potential physiological significance is the presence of NDPase and TPPase activities in the hexagonally arranged tubules of the innermost element of the neurons described in this study. Two of the known glycosyltransferases considered to be localized in the Golgi apparatus of hepatocytes and several other cell types (see Introduction) yield uridine diphosphate (UDP) as a reaction product (13). NDPase is capable of rapidly hydrolyzing UDP, and increased availability and activity of NDPase would be expected to drive the reaction in the direction of glycoprotein synthesis. Presently no cytochemical method is

available for observing glycosyltransferase activities and thus their localizations in the Golgi stack are unknown.

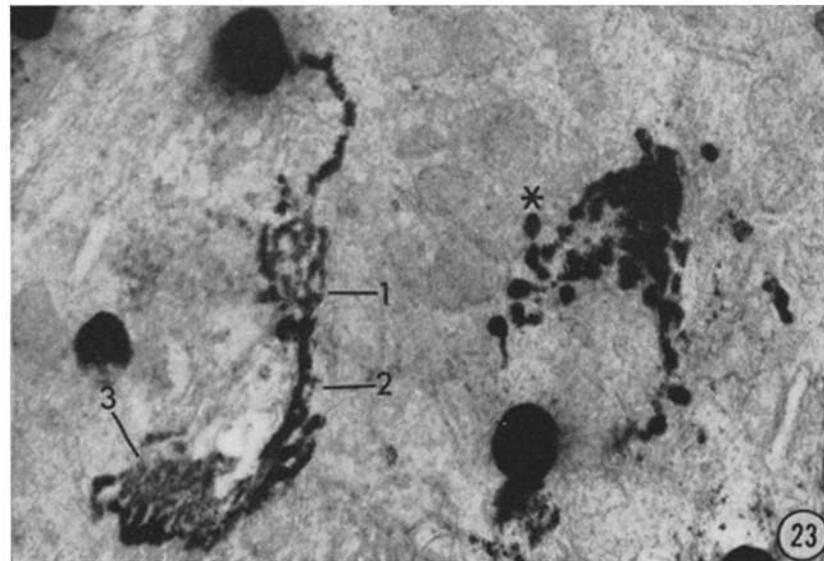
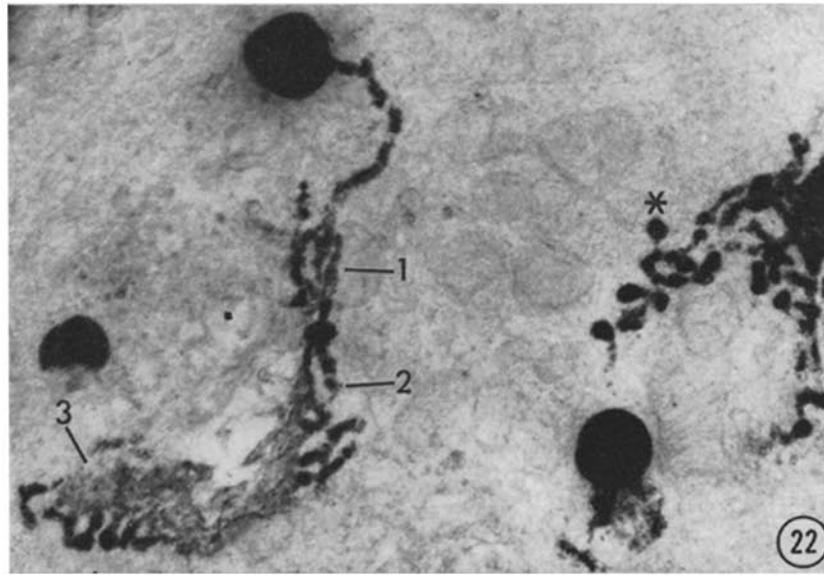
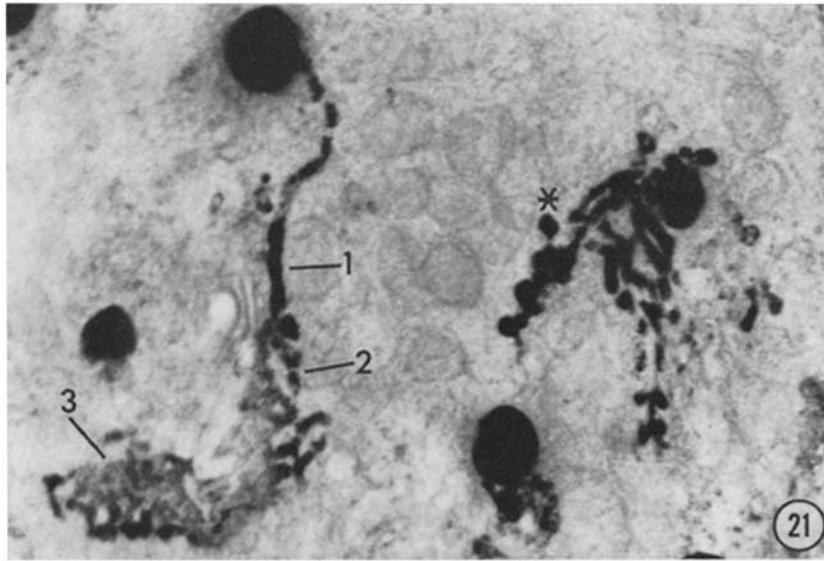
By short incubations for diphosphatase activity or by use of tissue fixed in glutaraldehyde-containing fixative for too long a period (Fig. 16), it is possible to see reaction product in the membranes and not in the interior of the Golgi element. With higher resolution the triple-layer membrane is seen and reaction product is present along its inner surface. Continued incubation fills in the cavity of the Golgi element but does not spill outside the element. However, the possibility has not been excluded that specific seeding sites for lead phosphate precipitation exist along the inner surface of the membrane. Thus it is presently prudent not to assert unequivocally that the diphosphatase sites are indeed along the membrane surface facing the cavity of the tubules composing the innermost Golgi element. Nonetheless, the association of the NDPase with the tubules of the Golgi element seems clearly established.

It is of interest that in our laboratory CMP is routinely used as the substrate for demonstrating AcPase activity. Is it a coincidence that one of the three glycosyltransferases found concentrated in isolated "Golgi-enriched fractions," sialyltransferase, yields CMP as substrate? Future studies may provide the answer.

Why GERL is Presently Excluded from the Golgi Stack

It may be a matter of personal preference whether to include GERL with the Golgi apparatus. Whaley et al. (55) would like to think of GERL as part of the Golgi stack. We prefer that GERL still be considered as a distinct structure. There are six reasons for our preference: (a) As yet, no evidence has established a functional relationship between GERL and the innermost Golgi element. If, in the neurons we have studied,

FIGURES 21-23 The same area is photographed in Fig. 21 at a tilt of -45° , in Fig. 22 untilted (0°), and in Fig. 23 at a tilt of $+45^\circ$. Region 1 of GERL appears as a single straight structure in Fig. 21, but in Figs. 22 and 23 its anastomosing tubular character is evident. Region 2 shows its tubular character better in Figs. 21 and 22 than in Fig. 23. Region 3 shows the cisternal portion of GERL most clearly in Fig. 22. A vesicular structure whose appearance remains essentially unaltered when tilted is seen at the asterisks. Its continuity with GERL is suggested most clearly in Fig. 22. The large, approximately circular structures are dense bodies. $\times 32,000$.



the tubules within the polygonal compartments prove to be part of GERL, the relationship of GERL to the innermost Golgi element would be vastly more complex than that of adjacent elements in the Golgi stack of this and other cell types. (b) Frequently, apparent vesicles and, occasionally, tubules (Fig. 8) are seen between GERL and the innermost Golgi element. Membranous structures are not generally thought to be present between Golgi elements. (c) Direct membrane continuities of GERL with RER are readily found, particularly with serial sectioning. (d) A typical Golgi apparatus is not seen when AcPase preparations are observed by light microscopy; but see Discussion. (e) We consider it desirable to focus the attention of other laboratories on the uniqueness of this structure, distinct from the Golgi apparatus (if GERL is considered a distinct organelle) or from the other Golgi elements (if GERL is considered as part of the Golgi stack). (f) If GERL is included in the stack, conceiving a membrane flow from outer aspect to inner aspect of the Golgi stack becomes very difficult. The present study of GERL reveals (in this cell type) cisternal portions and numerous tubules continuous with the cisternae and apparently connecting adjacent cisternal portions. The tubules undoubtedly correspond to what our laboratory has considered a specialized system of smooth ER with continuities with rough ER, in neurons (56, 14, 57; also see Holtzman [58] and

[46]), in adrenal medulla (43), mouse melanomas (59), rat and human liver (42, 60, 61), and rat thyroid (62). It is easy to conceive that some materials, presumably synthesized on membrane-attached polyribosomes, are transported from rough ER via smooth ER to tubules and cisternal portions of GERL, in both of which concentration and packaging of materials may occur. The other alternative is considerably more difficult to suggest, namely that the adjacent innermost Golgi element with its hexagonal arrays, absence of cisternal portion, and presence of TPPase and NDPase activities (under the conditions of fixation and incubation employed), "flows" down the stack, transforming into GERL with major differences in structural and enzymatic properties that characterize this structure. Membrane flow from ER to the outer Golgi element seems inescapable in almost all cell types that have been adequately studied. Yet, membrane flow beyond this, from the top to the bottom of the stack, may well be proven not to occur. Evidence in the literature clearly established "polarity" (i.e., differences among elements of the Golgi stack). This is true for function, i.e. packaging of secretory materials, lysosomal hydrolases, and other enzymes into granules (e.g., references 63, 37, 64), membrane thickness (65), and enzyme activities (e.g. references 66-70, 61). All such differences might, however, exist without membrane flow down the entire Golgi stack. Extreme as it may

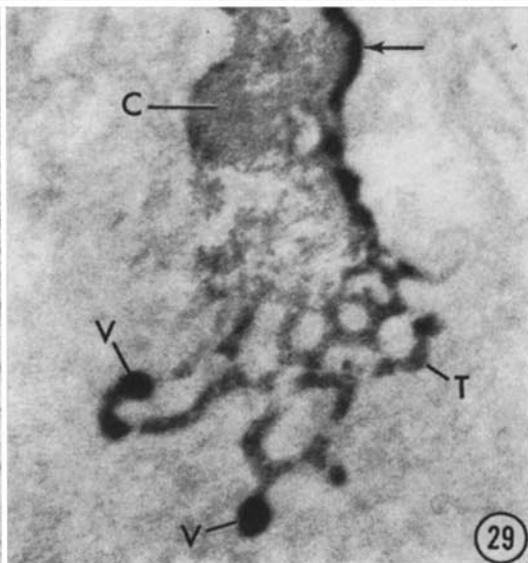
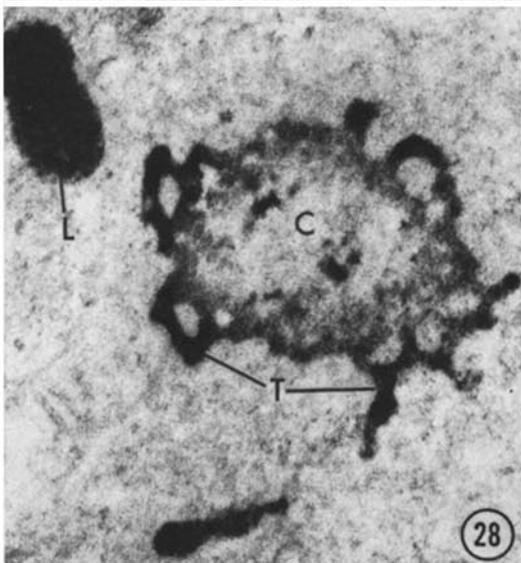
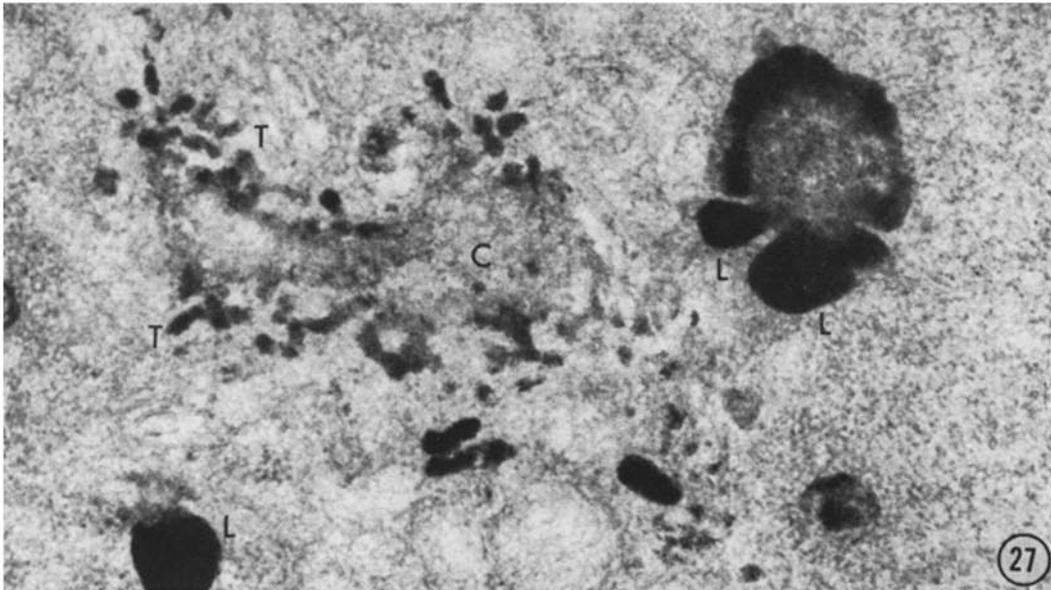
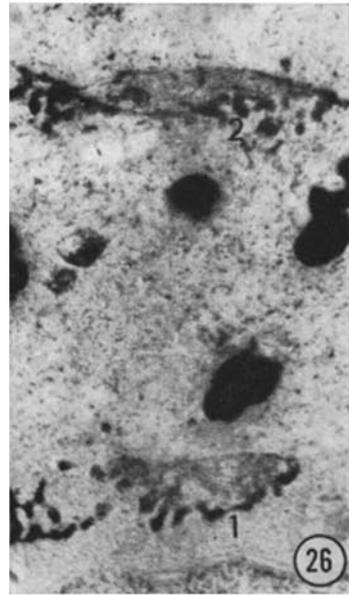
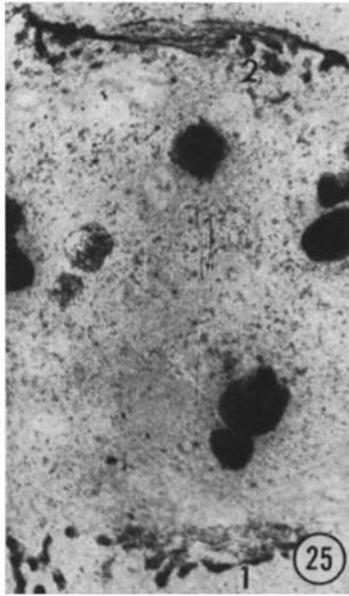
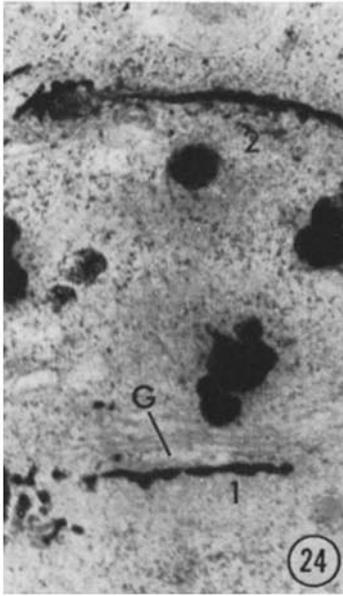
FIGURES 24-26 The same area of a neuron is photographed in Fig. 24 at a tilt of -45° , in Fig. 25 untilted (0°), and in Fig. 26 at a tilt of $+45^\circ$. Region 1 of GERL appears straight in Fig. 24; in Fig. 25 its cisternal and tubular components are seen; and in Fig. 26 these become more evident. Region 2 shows the same progression, although less dramatically. In Fig. 24, a portion of the Golgi stack is seen at G; it lacks reaction product. $\times 27,000$.

FIGURE 27 This section is from tissue rinsed in uranyl acetate before dehydration and embedding. Cisternal (C) and tubular (T) portions of GERL are evident. One dense body is seen at L in the lower left corner. In the upper right area two (or more) dense bodies (L) are interpreted as separating from a cisternal portion of GERL. $\times 44,000$.

FIGURES 28-29 Portions of neurons in an AcPase preparation (17 min incubation) in thymidylic acid at pH 5 (see reference 14) of an adult ganglion; 0.5μ section, not stained with lead.

FIGURE 28 Reaction product is present in a lysosome (L) and in GERL. A cisternal portion of GERL is seen in face view (C). The interconnecting tubules (T) form a more regular pattern than in the neurons of fetal ganglia. $\times 50,000$.

FIGURE 29 A cisternal portion of GERL is seen at C and anastomosing tubules forming a regular pattern at T. V indicates vesicles or sections of dense bodies attached to tubules. The arrow indicates a portion of GERL seen in end view. $\times 55,000$.



seem at the moment, different portions, or even individual elements, of the Golgi stack may prove to have their own structural relation to the ER, as GERL apparently has. The elements may play different functional roles while held in relatively fixed positions, perhaps by mechanisms like the "intercisternal structure" described by Mollenhauer (71).

We have noted that the anastomosing GERL tubules are more regularly arranged in the adult than in the fetal rat. Both cisternal and tubule portions may be expected to change in size and distribution under different conditions. Indeed, in GERL, as in Golgi elements, an anastomosing tubular structure may be more readily responsive to changed cell function than a flattened sheet of membrane.

GERL as a Concentrating and Packaging System; Origin of Lysosomes

Observations have been accumulating in our laboratory that suggest that GERL is a region where not only AcPase (and presumably other lysosomal hydrolases) but other enzymes and non-enzymatic proteins are concentrated and packaged into a variety of cytoplasmic granules. In two mouse melanomas that we have studied in our laboratory (59), apparently tyrosinase as well as AcPase is concentrated and packaged into pre-melanosomes by GERL. In cultured human melanoma cells and normal melanocytes of fowl, Maul and colleagues (72, 73) also find tyrosinase in GERL but these authors consider that the tyrosinase is packaged into coated vesicles in these cells. Holtzman and Dominitz (43) suggest that

FIGURES 30-35 Portions of neurons in *thin* sections of fetal ganglia in AcPase preparations (40 min incubation). The tissue was rinsed in uranyl acetate before dehydration and embedding. The thin sections were stained with lead.

FIGURE 30 No reaction product is seen in the Golgi stack (*G*). Reaction product is evident in (*a*) a small part of GERL (*GE*), including small portions of tubules (*T*); (*b*) an early autophagic vacuole at *AV*, within which membranes, probably artifactually produced, are seen; and (*c*) another type of autophagic vacuole indicated by the arrow. A discussion of the likely origin of the first type of autophagic vacuole (see Fig. 32) from "compaction" of ER is found in references 75 and 76; also see Figs. 1, 17, and 18 in reference 76 and Fig. II-53 in reference 77. The second type of autophagic vacuole (see Fig. 34) is described and discussed in relation to multivesicular bodies in references 75, 57, 41 and 46; also see Fig. 10 in reference 76, Figs. 28-30 and 38-40 in reference 57, Figs. 23 and 44 in reference 41, and Fig. 28 in reference 42. Autophagic vacuoles of the second type are numerous in neurons of both fetal and adult ganglia. The vacuoles appear to arise by enlargement and twisting of coated regions of GERL. As the vacuoles enlarge, their membranes apparently internalize to form the characteristic inner tubules that often appear as vesicles when cut transversely (see Fig. 40). In the process of membrane internalization, bits of cytoplasm are incorporated into the autophagic vacuoles. $\times 41,000$.

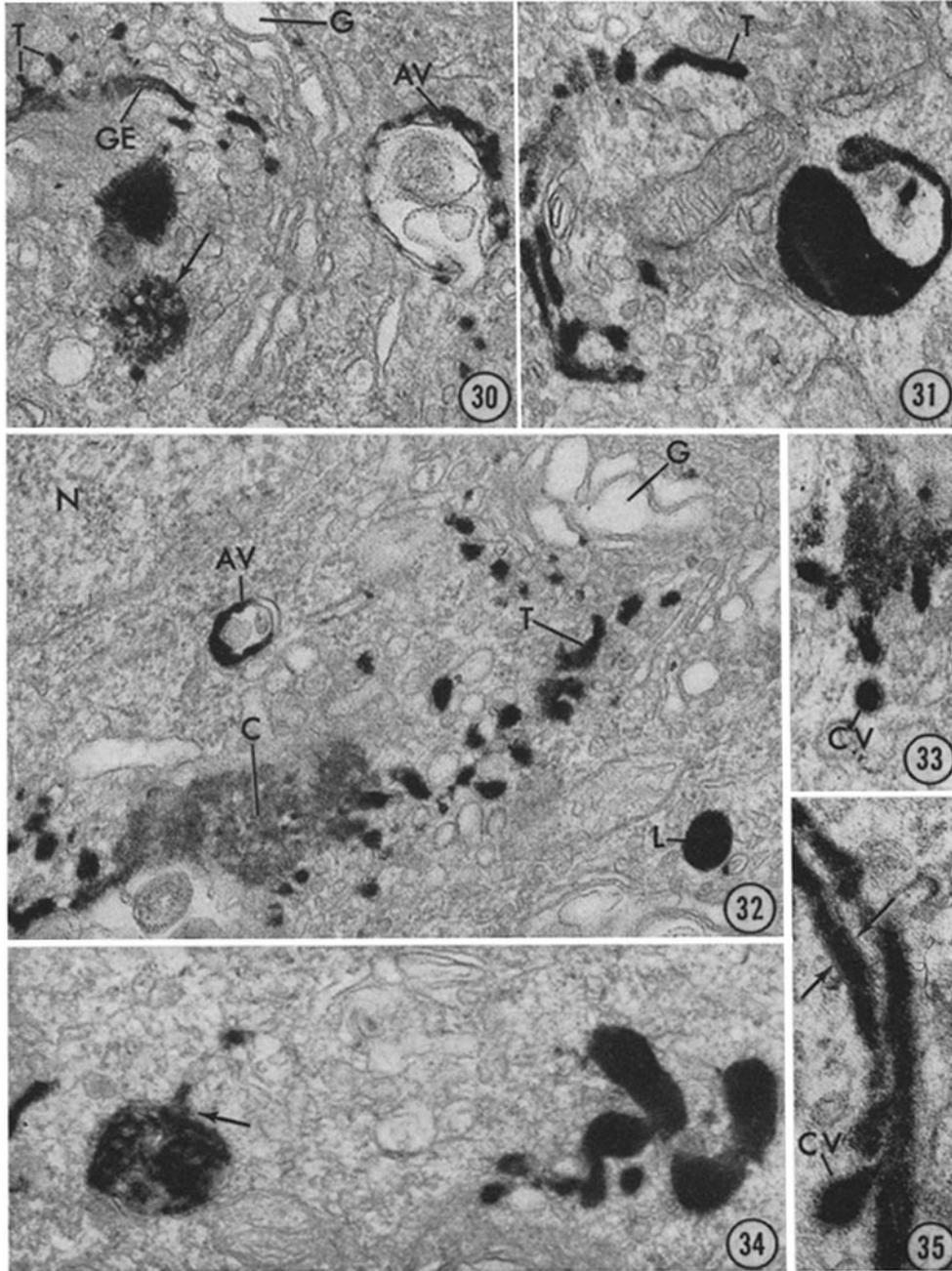
FIGURE 31 In this micrograph, tubular portions (*T*) of GERL are more evident and a suggestion of an anastomosis is seen at the lower left. At the right, the structure with reaction product is interpreted as a dense body (possibly two) separating from a GERL tubule. $\times 41,000$.

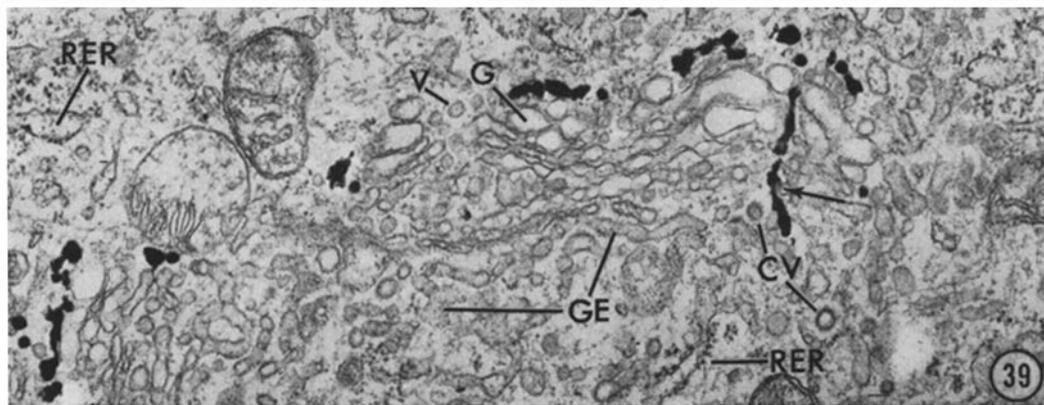
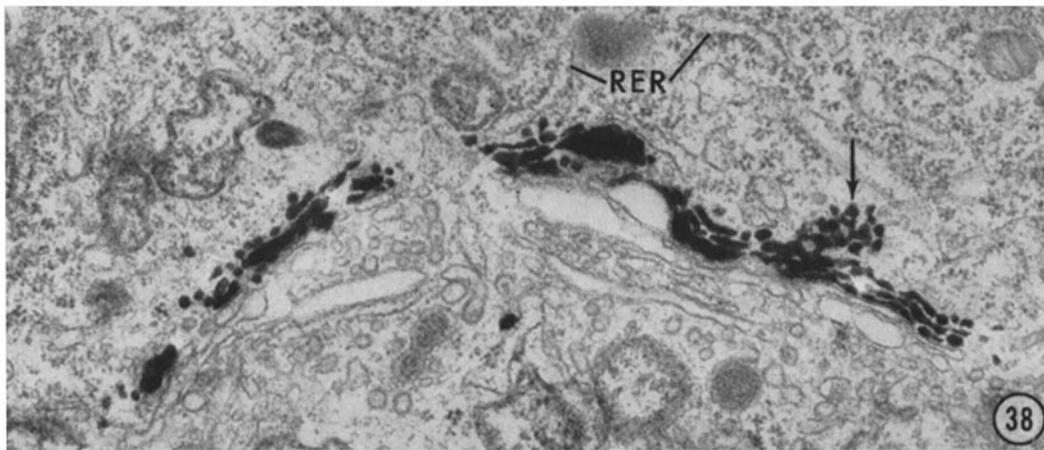
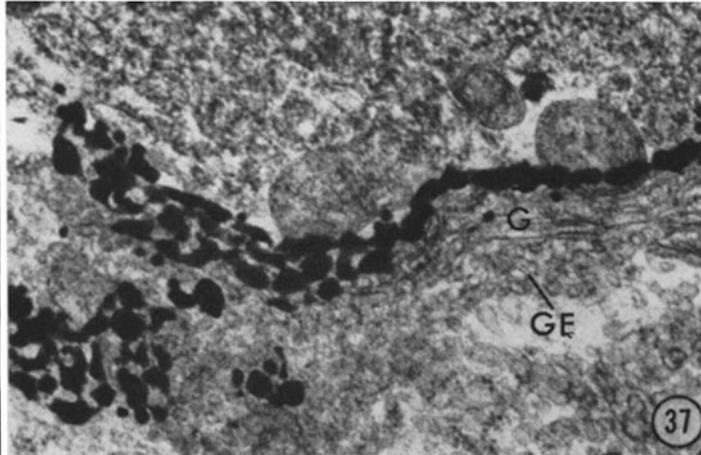
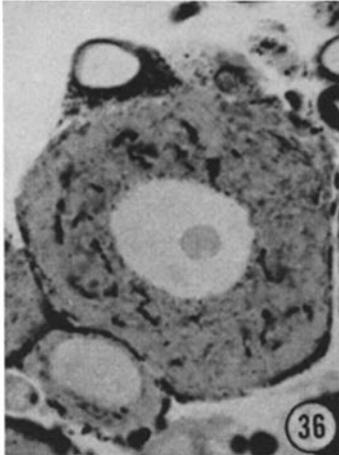
FIGURE 32 In this micrograph both cisternal (*C*) and tubular (*T*) portions of GERL are evident. An autophagic vacuole is indicated at *AV* (see legend to Fig. 30) and a dense body at *L*. The Golgi stack (*G*) shows no reaction product. A portion of the nucleus is seen at *N*. $\times 41,000$.

FIGURE 33 A small portion of GERL is shown, with some of its tubular and cisternal structures evident. *CV* shows a coated vesicle with reaction product (cf. references 57 and 58). $\times 42,000$.

FIGURE 34 The AcPase-positive structures at the right of this micrograph are interpreted as dense bodies in formation, from swellings of GERL. To the left is an AcPase-positive autophagic vacuole (see Fig. 30) with a connection (arrow) to a positive tubular structure, probably part of GERL. $\times 44,000$.

FIGURE 35 This micrograph shows a coated vesicle (*CV*) in continuity with a tubule of GERL. The arrows indicate regions where the delimiting membrane of a GERL tubule is evident. $\times 80,000$.





many adrenalin-containing granules arise from GERL. It has been suggested by Ma and Biempica (61) that in human hepatocytes, GERL is a site of VLDL concentration. In epithelial cells of rat thyroid, "B" granules, thought to contain uniodinated thyroglobulin, appear to be packaged by GERL (62). A probable origin from GERL of other granules is indicated by published micrographs from other laboratories. Thus, the impressive publication of Herzog and Miller (74) suggests to us that the peroxidase-rich secretory granules in cells of the rat parotid gland arise from GERL. In all cases but that of the "B" granules of thyroid, the granules arising from GERL show AcPase activity.

From the earlier observations in our laboratory on thin sections of neurons, we proposed that GERL concentrates and packages AcPase into dense bodies. This proposal is greatly strengthened by the numerous suggestive images seen in this

study in thick sections, and in thin sections as well. In addition, the new observations suggest that dense bodies arise by swelling and separation of portions of GERL cisternae as well as from tubules of GERL.

The observation of Holtzman et al. (57) that coated vesicles in the region of GERL possess AcPase activity are confirmed in this study. This type of lysosome also appears to derive from GERL by outgrowth from a tubule, although an origin from a cisternal portion is not excluded.

Value of Thick Section Enzyme Cytochemistry

Study of 0.5- μ sections by high-resolution, high-voltage electron microscopy would aid considerably in elucidating details of the Golgi apparatus and related structures. However, the studies of Rambourg (15), and Rambourg and Chrétien

FIGURES 36-39 Portions of small neurons of adult rat dorsal root ganglia following initial fixation and prolonged osmication at 40°C in 2% aqueous OsO₄. Neurons were selected that showed little or no osmium staining in ER and mitochondria.

FIGURE 36 Light micrograph of 0.5 μ Epon section showing osmium staining of the Golgi apparatus. See diagrams of C. Golgi (32, 33) showing its appearance in paraffin sections and with its appearance in frozen sections incubated for TPPase activity (Figs. 41 and 42 in reference 14). In the areas of 0.5 μ Epon sections where endoplasmic reticulum stains as well as the Golgi apparatus (not illustrated), clear areas are seen. These appear as "negative images" of the Golgi apparatus. Such images result from the absence of reduced osmium in all Golgi elements except the outer one and from the absence of osmium in GERL. $\times 1250$.

FIGURE 37 Electron micrograph, 0.5 μ Epon section. In the left half of the figure the outer Golgi element is seen in face view, revealing its irregular anastomosing nature. At the right it is seen on end. The other Golgi elements (*G*) and GERL (*GE*) are negative. Small vesicle-like structures may represent transverse sections of tubules. $\times 46,000$.

FIGURE 38 Thin section stained with lead. The outer element of the Golgi stack is cut face view at the arrow, revealing its anastomosing nature. At the extreme right it appears as three distinct elements. However, the numerous interruptions and irregularities, coupled with thick section studies, make it highly probable that it is a single irregular element. Small positive structures may be vesicles, but they are more likely to be transverse sections of the tubular portions of the outer element. Rough ER is seen at *RER*. The portion branching towards the left lacks ribosomes on the surface facing the Golgi stack. $\times 32,000$.

FIGURE 39 This thin section demonstrates that only the outer element of the Golgi stack (*G*) reduces osmium. The arrow indicates a region where the outer element has branched (compare the difference in the appearance of the adjacent Golgi elements to those at *G*, where the Golgi stack is cut almost vertically). Osmium staining does not occur in GERL (*GE*) or coated vesicles (*CV*), or in ER generally (*RER*). Note the continuity of smooth ER, part of GERL, with ribosome-studded ER (bottom *RER* label). As in Fig. 38, the positive vesicle-like structures are probably transverse sections of the tubules which compose the outer Golgi element. The small osmium-negative vesicles at *V* probably include "transitional vesicles" (see Fig. 40). $\times 23,000$.



FIGURE 40 Diagram depicting, tentatively, the relations of endoplasmic reticulum, GERL and Golgi apparatus in the small neurons of adult rat dorsal root ganglion and neurons of the 16-day fetal rat ganglion. From the Nissl bodies (*NB* at the outer aspect of the Golgi stack) endoplasmic reticulum (*ER*) presumably carries materials, via transitional vesicles (*TV*) and transitional sheets (*TS*), to the *outer element* (*OE*) of the Golgi stack. At the right the *ER* is shown approaching the Golgi stack as is often seen in sections, in a roughly perpendicular manner, as recently described by Claude (47) in rat hepatocytes. The *outer Golgi element* is composed of irregularly anastomosing tubules or small saccules. Two *fenestrated elements* are shown between the outer and innermost element (*IE*) of the stack. There are often two to three such elements in the fetus and three to four in the adult. The *innermost element* (*IE*) consists of a hexagonal array of tubules. A smooth tubule, at left, of smooth *ER* (coming directly from rough *ER* or from GERL) enters each polygonal compartment (*PC*). From a Nissl body (*NB*, at left) endoplasmic reticulum (*ER*) presumably transports material, including acid phosphatase, to GERL (*GE*) which consists of cisternal portions (*C*) and tubules (*T*). The tubules of GERL form anastomoses which are more regular in the adult (upper portion of the diagram) than in the 16-day fetus (middle part of diagram, where the Golgi stack is shown twisted so that GERL lies above the innermost element and where the other elements are not drawn). In the middle portion, the origin of dense bodies (*DB*) from GERL tubules and cisternae is shown. Coated vesicles (*CV*) are shown arising from GERL tubules. The cisternae of GERL are drawn as connected to each other by tubules. The Golgi apparatus forms a continuous network coursing through the cytoplasm. The unlabeled vacuole, with an external coating, represents an early stage in formation of the second type of autophagic vacuole discussed in the legend to Fig. 30.

(35), and the observations presented in this publication indicate the great fund of knowledge that can be gained by study of such thick sections with stained structures in conventional microscopes, at 80 or 100 kv. Structures with sufficient *intrinsic* electron opacity might also be studied to advantage in thick sections.²

Only after the hexagonal arrays of the diphosphatase-rich structures were dramatically visualized in thick sections did we search for, and find, images suggesting or showing them in thin sections. Indeed, review of micrographs from thin sections, taken by A. Novikoff in 1964, shows small portions of the arrays, but their significance was not appreciated.

Even more dramatic is our experience with AcPase. A. Novikoff had taken a great many micrographs from thin sections of a particular block in 1964, of which one micrograph was published (Fig. 55 in reference 14); in none was a cisternal portion of GERL found. In the course of preparing this publication we took thick (0.5- μ) sections of this same block, and the existence of cisternal portions in small neurons of adult ganglia was established in the first square examined. The thick sections were cut and the negatives for Figs. 28 and 29 were developed within 20 min!

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² The usefulness of such sections in diaminobenzidine cytochemistry is suggested in another publication (78).

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