Disruption of the Golgi Zone and Inhibition of the Conversion of Proparathyroid Hormone to Parathyroid Hormone in Human Parathyroid Tissue by Tris(hydroxymethyl)aminomethane

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Tris(hydroxymethyl)aminomethane (Tris, Tromethamine, THAM) and other nonamphoteric amines were previously reported to inhibit the conversion of proparathyroid hormone to parathyroid hormone in bovine parathyroid glands incubated *in vitro*. This inhibition correlated with a striking dilation of the Golgi complex. This work has now been extended to normal, hyperplastic, and adenomatous parathyroid glands from human subjects. The tissues were incubated for up to 3 hours with ^{3}H-leucine in physiologic solutions (control) or in the same solutions containing 50 mM Tris. In one case, diethylamine also was tested. Electron microscopy revealed that the amines produced a dilation of the Golgi complex and swelling of vesicles, predominantly in the region of the Golgi zone. Other organelles were normal in appearance. During the same period, Tris reduced by sixfold the ratio of parathyroid hormone to proparathyroid hormone, from a control value of 2:1 to 1:3. It was apparent that Tris exerted the same biochemical and morphologic actions in human parathyroid tissues as it was previously shown to do in bovine glands. These studies support the concept that the Golgi zone is that region in the parathyroid gland in which proparathyroid hormone to parathyroid hormone conversion is initiated and that Tris inhibits this conversion through disruption of the converting site. (Am J Pathol 87:553–568, 1977)

IT WAS REPORTED PREVIOUSLY that when bovine parathyroid tissue slices were incubated in physiologic buffers containing Tris or other nonamphoteric amines such as glycinamide and diethylamine, there occurred a time-dependent and dose-dependent swelling of the Golgi zone of the chief cells.[1,2] The swelling was paralleled by inhibition of parathyroid hormone formation and an accumulation of its precursor, proparathyroid hormone. This action of Tris required the intact tissue, since the amine did not affect the conversion of proparathyroid hormone to parathyroid hormone in a cell homogenate. Based on these and other

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data, it was postulated that Tris blocked the conversion of proparathyroid hormone to parathyroid hormone by mechanically disrupting the converting site of the Golgi zone.[3-6]

Although the mechanism by which Tris and the other amines act is unknown, it was suggested [1] that, if these agents produced similar structural alterations in other tissues and cells, they might comprise a unique series of specific probes for the study of structure–function relationships in secretory cells. To this end, it seemed worthwhile to first establish that the disruptive action of Tris on the parathyroid Golgi zones was not confined solely to the bovine gland. We report herein that, in normal, hyperplastic, and adenomatous parathyroid glands from humans, Tris causes extensive vesicle formation that, as in bovine tissue, appears to be of Golgi origin and that this morphologic change correlates with the inhibition of parathyroid hormone formation from proparathyroid hormone.

Materials and Methods

Preparation and Incubation of Tissue Slices

Fresh normal parathyroid glands were obtained at autopsy from 10 subjects less than 3 hours postmortem. Fresh hyperplastic or adenomatous parathyroid tissue from 6 subjects was obtained less than 15 minutes after removal at surgery. Each tissue was cut into 2 mm × 2 mm × 1 mm pieces and incubated in either Earle's balanced salt solution (EB solution), Minimum Essential Medium–10% fetal calf serum (MEM solution), or Hanks' solution–5% fetal calf serum for 60 minutes as indicated. The tissues were then divided into two portions. One was further incubated for 30 minutes in fresh buffer solution; the other was incubated in the same solution with the addition of either 50 mM Tris, pH 7.4, or 50 mM diethylamine, pH 7.4. The tissues were then incubated in the same fresh media but containing ^{3}H-leucine or ^{3}H-lysine for 2 to 3 additional hours. Portions of the tissue were removed at appropriate times for electron microscopic examination. The osmotic strength of the control and experimental solutions was not normally equalized since we found earlier that these small differences had no effect on either the morphology or measured biochemical activity of the tissues.[1]

Isolation of Proparathormone and Parathormone

After incubation the tissue and medium were homogenized together in 5 ml of 8 M urea–0.2 M HCl–0.1 M cysteine after which 25 ml of a similar 10% homogenate of frozen bovine glands were added to serve as carrier protein. The mixture was processed as described previously [7] through the carboxymethylcellulose (CM-cellulose) chromatography step in order to separate radioactive proparathyroid hormone and parathyroid hormone. Aliquots of each fraction were assayed for radioactivity using Phosphor Solution B. The recovery of the radioactive hormonal peptides was followed by inclusion of bovine ^{14}C-proparathyroid hormone and ^{14}C-parathyroid hormone as markers in the initial homogenate. Recovery was about 50% during the course of the extraction procedure and was unaffected by the presence of Tris in the incubation media. The recoveries of proparathyroid hormone and parathyroid hormone from CM-cellulose columns averaged 55% and 85%, respectively, and the values of the prohormone and hormone, unless stated otherwise, were not adjusted for these losses. The incorporation of ^{3}H-amino acids into

total proteins of the tissue was measured in the insoluble 10% trichloroacetic acid precipitate derived from a sample of the original tissue homogenate. The precipitate was collected by centrifugation, washed several times with 10% trichloroacetic acid, extracted with 5% hot trichloroacetic acid, dissolved in a tissue solubilizer (NCS, Amersham/Searle) and assayed for radioactivity using Phosphor Solution A.

Radioactive assay was conducted by liquid scintillation spectroscopy using either 5 g/liter of 2,5-diphenyloxazole (PPO) and 0.12 g/liter of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) in toluene (Phosphor Solution A) or 3.33 g/liter of PPO and 0.08 g/liter of POPOP in a solution of toluene/Triton X-100 (2:1) (Phosphor Solution B). The efficiency of counting and degree of quenching for samples of interest were determined by means of an internal standard, either ^{14}C-toluene, ^{3}H-toluene, or $^{3}H_2O$.

Electron Microscopic Examination

Fresh tissue and tissues after incubation were fixed in 4% glutaraldehyde, 0.1 M sodium phosphate-buffered at pH 7.2, postfixed in 1.33% *sym*-collidine-buffered osmium tetroxide (pH 7.4), dehydrated in ethanol, and embedded in Araldite-Epon.[8] Thin sections were cut with a diamond knife on an LKB ultramicrotome and were stained with lead citrate [9] and uranyl acetate.[10]

Materials

^{3}H-leucine was purchased from New England Nuclear Corporation, Boston, Mass.; Tris from Sigma Chemical Company, St. Louis, Mo.; and carboxymethylcellulose (CM-52) from Reeve Angel, Clifton, N.J. Radioactive bovine ^{14}C-proparathyroid hormone and ^{14}C-parathyroid hormone markers were biosynthesized and isolated as earlier described.[11] All other chemicals were reagent grade and were purchased from various chemical suppliers.

Results

Ultrastructural studies were performed on the human parathyroid tissue before and after *in vitro* incubation. The results with four representative samples—a normal parathyroid gland, one with chief cell hyperplasia, an oxyphil adenoma, and a chief cell adenoma—will now be described.

Figure 1 shows the ultrastructural appearance of normal parathyroid tissue removed 3 hours after death and before incubation. Except for mild degenerative changes such as swollen mitochondria, the cellular structures—including plasma membrane, nucleus, endoplasmic reticulum, secretory granules and Golgi complex—appeared normal. Figure 2 shows normal parathyroid tissue following incubation for 2 hours in MEM solution. All cellular structures including the mitochondria appeared normal. Thus, incubation in the control buffer appeared to improve the morphologic integrity of the tissue. The tissue maintained this normal appearance up to at least 3 hours of incubation. Identical results were obtained with the other incubation solutions. Figure 3 shows parathyroid tissue after incubation for 1 hour in the control MEM solution followed by 1 hour in the same buffer containing 50 mM Tris. The cells contained

multiple membrane-limited vacuoles which were approximately spherical and ranged from 0.15 to 1.7 μ in diameter. The membranes of the vacuoles were sometimes incomplete and coalesced with adjacent vacuoles. Most of the vacuoles appeared empty, but they occasionally contained distorted membranous material, secretory granule-like material, or lipid-like material. The vacuoles appeared to be mostly concentrated in the apparent region of the Golgi apparatus. Normal Golgi apparatuses were rarely if ever identified in the cells of any of the tissue that were incubated in Tris solutions for periods of 1 hour or more. Furthermore, secretory granules were rarely identified in these cells. In contrast, the remainder of the cell organelles presented a normal appearance. Figure 4 shows the tissue after incubation for 1 hour in MEM solution followed by 1 hour in the same buffer containing diethylamine. Multiple vesicles were seen that resembled those resulting from incubation with Tris and had a configuration suggestive of Golgi origin.

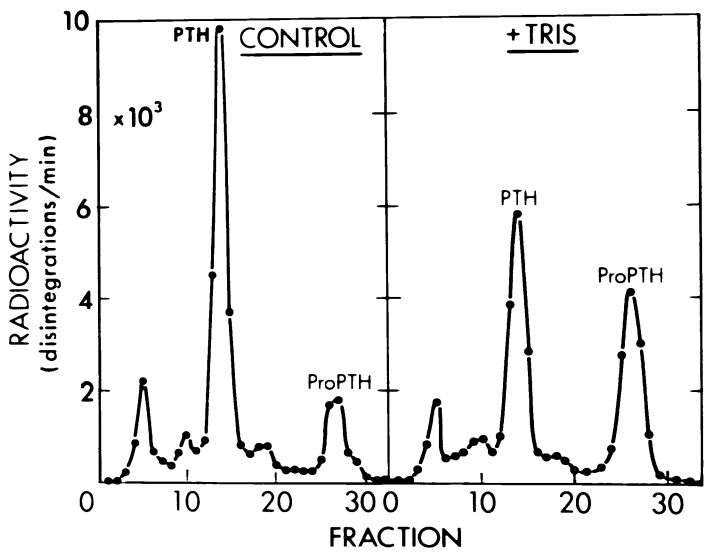
The ultrastructural appearance of parathyroid tissue with primary chief cell hyperplasia after removal at surgery was normal except for occasional mild nuclear disruption and mitochondrial swelling. After incubation of a sample of this tissue for 2 hours in EB solution (Figure 5), it was somewhat improved in appearance over the unincubated sample. Following incubation for 1 hour in EB solution containing Tris, however, the tissue contained multiple vesicles; and normal Golgi complexes were not observed (Figure 6). All other cellular structures appeared relatively normal.

The ultrastructural appearance of a functioning oxyphil adenoma immediately after removal at surgery is presented in Figure 7. In general, all cells—including the oxyphil cells, the transitional oxyphil cells, and the rare chief cells—were morphologically intact. Likewise, all of the cellular organelles including the Golgi complexes appeared to be normal. After 2 hours in EB solution, the tissue continued to exhibit a normal appearance. When a sample of the adenoma was incubated in EB solution containing Tris, multiple vesicles formed (Figure 8), and there was an absence of most recognizable Golgi complexes. Secretory granules were rarely identified. All other structures appeared normal. At some sites, structures intermediate between normal Golgi vacuoles and the fully distended vacuoles were seen.

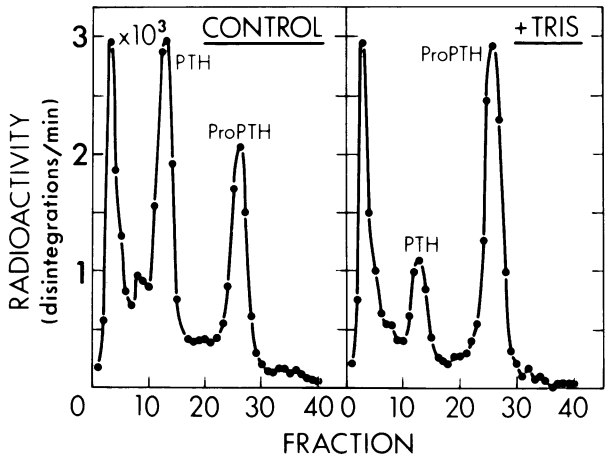
The ultrastructural appearances of a sample of a chief cell adenoma examined immediately after removal at surgery and after 60 minutes of incubation in EB solution are shown in Figures 9 and 10, respectively. The cellular structures were generally intact. Figures 11 and 12 show the tissue incubated for 30 and 60 minutes, respectively, in EB solution containing Tris. At 30 minutes there appeared to be mild to moderate distension of

Golgi apparatuses and focal vesicle formation which was more prominent at 60 minutes.

The capacity of the various parathyroid samples to synthesize proparathyroid hormone and parathyroid hormone upon incubation in the control and Tris-containing buffers was assessed. Among the individual experiments, there was substantial variability in incorporation of radioactivity into total protein. Overall parathyroid hormone and proparathyroid hormone represented from 1 to 7% of the total acid-insoluble protein. The appearance of CM-cellulose chromatograms from two typical studies—normal parathyroid tissue (Text-figure 1) and an oxyphil adenoma (Text-figure 2)—are shown. After 2 to 3 hours of incubation in control buffer, the majority of the radioactivity in the sample was in parathyroid hormone and proparathyroid hormone, with about twice as much radioactivity in the former than in the latter. When samples of the tissues were incubated in the Tris-containing EB solution, the amount of radioactivity in parathyroid hormone was substantially less and the amount of proparathyroid hormone was greater by about the same amount. The radioactivity in the other (unidentified) peaks in the elution profile was about the same. In the total of 16 normal, hyperplastic, and



TEXT-FIGURE 1—Carboxymethyl cellulose ion exchange elution profiles of parathyroid hormone, proparathyroid hormone, and related peptides derived from a normal human gland after incubation in control or Tris-containing buffer. Approximately 65 mg of tissue were incubated in Earle's balanced salt solution with 25 μCi of ^{3}H-lysine for 3 hours after a 1-hour preincubation as described in Materials and Methods. Tris was added to the experimental buffer at a concentration of 50 mM. The elution positions of parathyroid hormone (PTH) and proparathyroid hormone (ProPTH) are indicated.



TEXT-FIGURE 2—Carboxymethyl cellulose ion exchange elution profiles of parathyroid hormone, proparathyroid hormone, and related peptides derived from a human parathyroid oxyphil adenoma after incubation in control or Tris-containing buffer. The conditions were similar to those described in the legend to Text-figure 1 except that 50 μCi of ^{3}H-leucine was used and the buffer was Hanks' solution–5% fetal calf serum.

adenomatous parathyroid glands, the average amount of radioactive parathyroid hormone as a percentage of the total hormonal peptides was reduced from 76 ± 3% (SD) to 27 ± 3% (SD) when the tissues were incubated in the amine-containing buffer.

Discussion

Our data show that Tris (and when tested, diethylamine) produced large single membrane–limited vacuoles when normal, hyperplastic, and adenomatous human parathyroid tissues were incubated in solutions containing this amine. In contrast, incubation of samples of the same tissue in the physiologic buffers that did not contain Tris yielded normal appearing tissues. Indeed, the tissues incubated in the control solutions appeared structurally as good or better than did the unincubated autopsy specimens. The amine-induced vesicles were detected in smaller dimensions at 30 minutes and were generally fully distended by 1 hour of incubation. The impression from viewing many sections similar to those in Figures 1–12 is that these vesicles represent at least in part swollen cisternae and vesicles of Golgi origin. This impression is strengthened by the almost total disappearance of recognizable Golgi organelles in the cells. The uniqueness of the morphologic alteration brought on by Tris is emphasized by the normal appearance of the other major cellular organelles, except for the apparent decrease in number of identifiable secretory granules. Regarding this latter finding, it is felt that the number of granules and extent of tissue sampling was not sufficient to quantitate or determine statistical significance of this alteration.

The morphologic changes were accompanied by a marked accumulation of newly-synthesized proparathyroid hormone and an equivalent

lessening in the amount of newly-synthesized parathyroid hormone in the tissue, in accord with the results reported earlier with bovine tissue.[1] Thus the present data support the previous hypothesis [1] that Tris disrupts the Golgi structure—the site of conversion of proparathyroid hormone to parathyroid hormone [3-6]—and in so doing inhibits the enzymatic conversion of the prohormone to hormone. It will be of interest to determine if other compounds such as the antibiotic ionophore X537A, reported to cause swelling of the Golgi apparatus, also are associated with inhibition of this enzymatic conversion.[12]

One additional point of interest is the superficial similarity in ultrastructure between human primary water-clear cell hyperplasia [13] and the parathyroid tissue of the present study following incubation with Tris. Both types of tissue have many intracytoplasmic spherical membrane-limited vacuoles. The mechanism of the multiple vesicle formation in those two processes appears to be different, however, because a) in contrast to our Tris-incubated tissue, water-clear cells demonstrate readily identifiable and nondistended Golgi zones and b) evidence reported elsewhere [14] suggests that inhibition of the conversion of proparathyroid hormone to parathyroid hormone is not a feature associated with the vesicle formation of water-clear cell hyperplasia since the percentage of proparathyroid hormone [proparathyroid hormone/(proparathyroid hormone + parathyroid hormone)] in tissue from 1 case of clear cell hyperplasia was 49%, whereas the mean percentage of proparathyroid hormone in 6 cases of adenoma was 61% and in 4 bovine glands was 50%.

Our findings may have substantial clinical significance. Tris has been recommended for the correction of metabolic acidosis associated with cardiac bypass surgery and cardiac arrest and the correction of acidity of acid-citrate-dextrose (ACD) blood used in cardiac bypass surgery [15] and exchange transfusion.[16] It would appear that the agent has direct toxic actions possibly induced by the basicity of the unneutralized amine [15,17,18] or due to its metal-chelating property.[19,20] These actions, however, are expressed almost immediately and may not be related to the Golgi changes we observed. On the other hand, in longer term studies (that is, those extending more than a few minutes), Tris may exert different pharmacologic effects. For example, it has been reported to cause hepatic injury (possibly unrelated to alkalinity of solutions) when infused into infants via the umbilical vein to combat idiopathic respiratory distress syndrome.[21] *In vitro*, Tris is known to be toxic to cells in culture,[22] to cause release of specific membrane-associated proteins from *Escherichia coli*, suggesting that it may react uniquely with cell membranes,[23] and to specifically increase secretion of insulin by pancreatic islet cells in cul-

ture.[24] In such situations, it is possible that the action of Tris might be related to a disruption of the Golgi or other subcellular organelles in cells at large. Such data reinforce the need to investigate more closely the interaction of Tris with cells and organs *in vitro* and *in vivo*.

As referred to earlier, one benefit of such a study might be to learn if Tris can serve as a specific intracellular probe of Golgi function. This would be the case if it could be shown that it produces morphologic alterations in organs other than the parathyroid and in the processing of other peptide molecules (such as collagen, insulin, pancreatic and parotid digestive enzymes, immunoglobulins, and albumin) believed to follow the Golgi route.

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Acknowledgments

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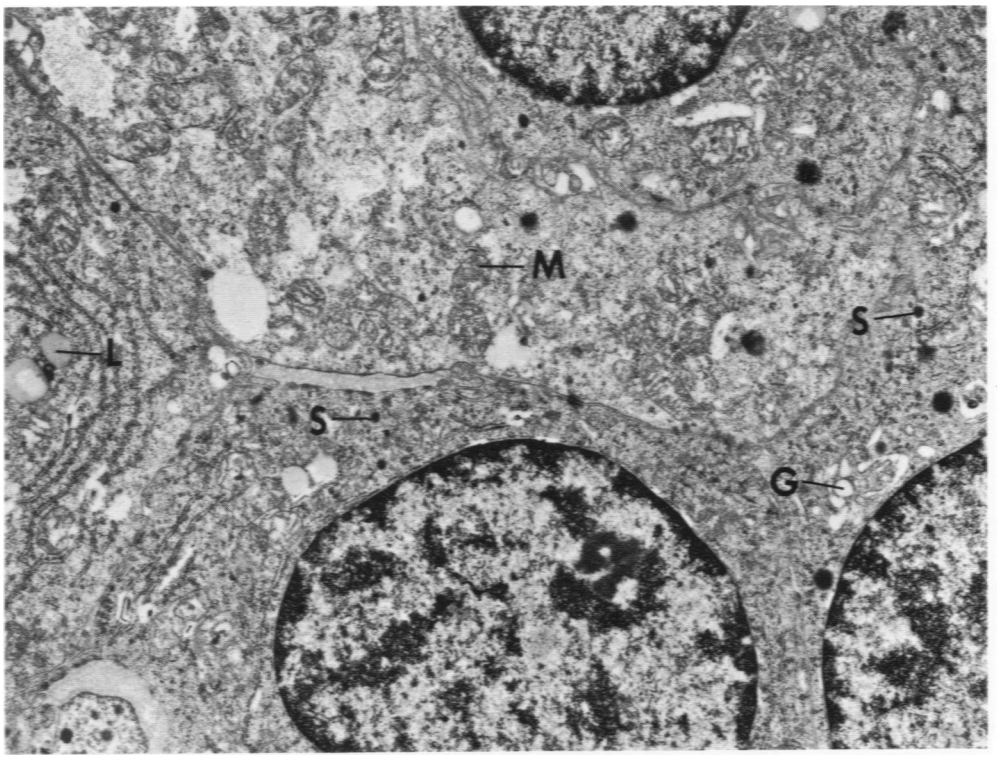
All illustrations are electron micrographs of sections stained with lead citrate and uranyl acetate.



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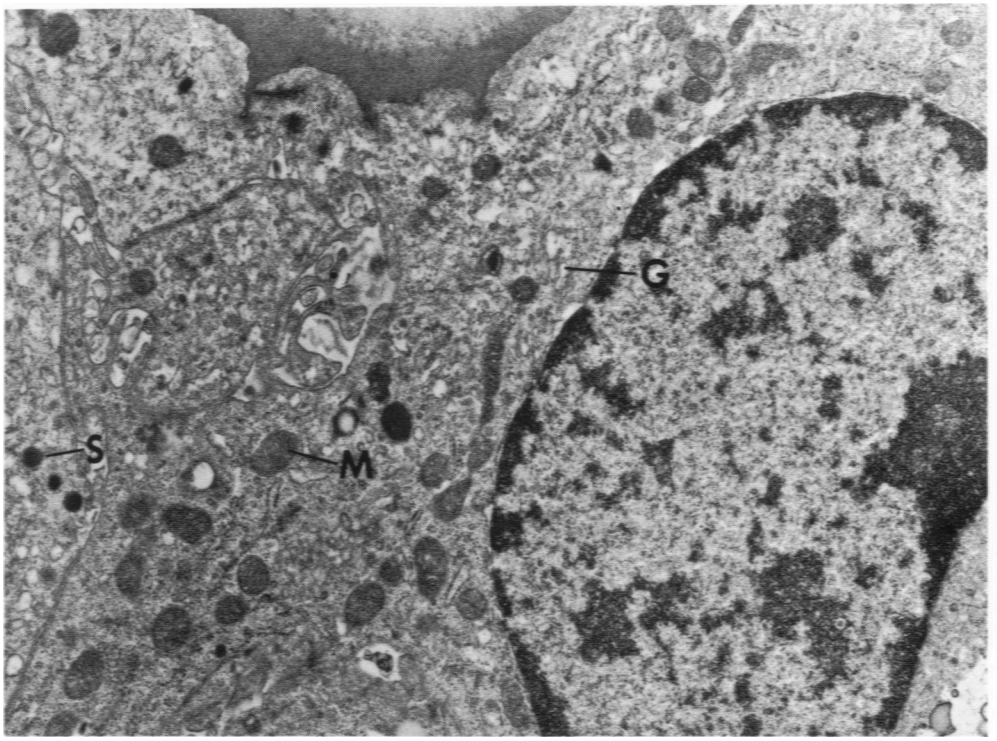

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Figure 1—Normal parathyroid gland, 3 hours postmortem, before incubation. Mitochondria (*M*), Golgi apparatus (*G*), secretory granules (*S*), and other cellular structures appear normal except for focal mild degenerative changes. *L* = lipid. (× 9520) **Figure 2**—Normal parathyroid gland, same as Figure 1, following incubation for 2 hours in control MEM solution. All cellular structures, including mitochondria (*M*), Golgi apparatus (*G*), and secretory granules (*S*), appear normal. (× 14,000)

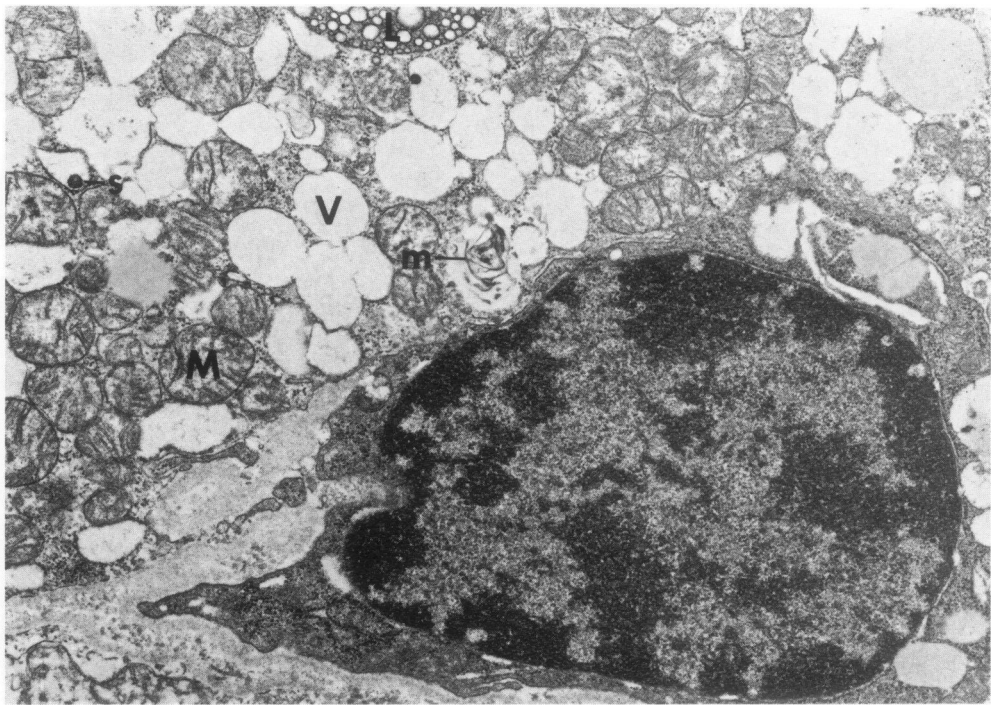

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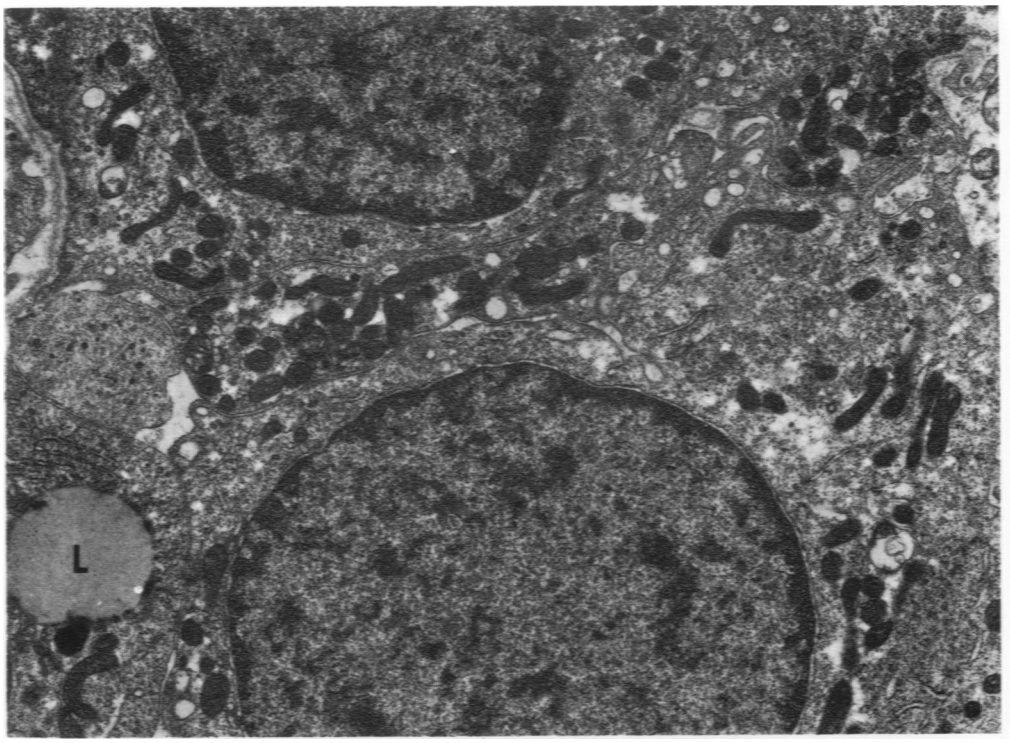
Figure 3—Normal parathyroid gland, same as Figure 1, following incubation for 1 hour in control MEM solution then 1 hour in the same buffer containing 50 mM Tris. Multiple membrane-limited vacuoles (*V*) 0.15 to 1.7 μ in diameter appear to be concentrated in the region of the Golgi apparatus, occasionally contain membranous (*m*) or secretory granule-like (*s*) material, and sometimes coalesce with an adjacent vacuole. Normal secretory granules are not identified. Other structures, including mitochondria (*M*), appear normal. *L* = lipid. (× 15,000) **Figure 4**—Normal parathyroid gland, same as Figure 1, following incubation for 1 hour in control MEM solution, then 1 hour in the same buffer containing diethylamine. Multiple vesicles (*V*) similar to those resulting from incubation with Tris are suggestive of Golgi origin and occasionally contain membranous material (*m*). (× 28,000)



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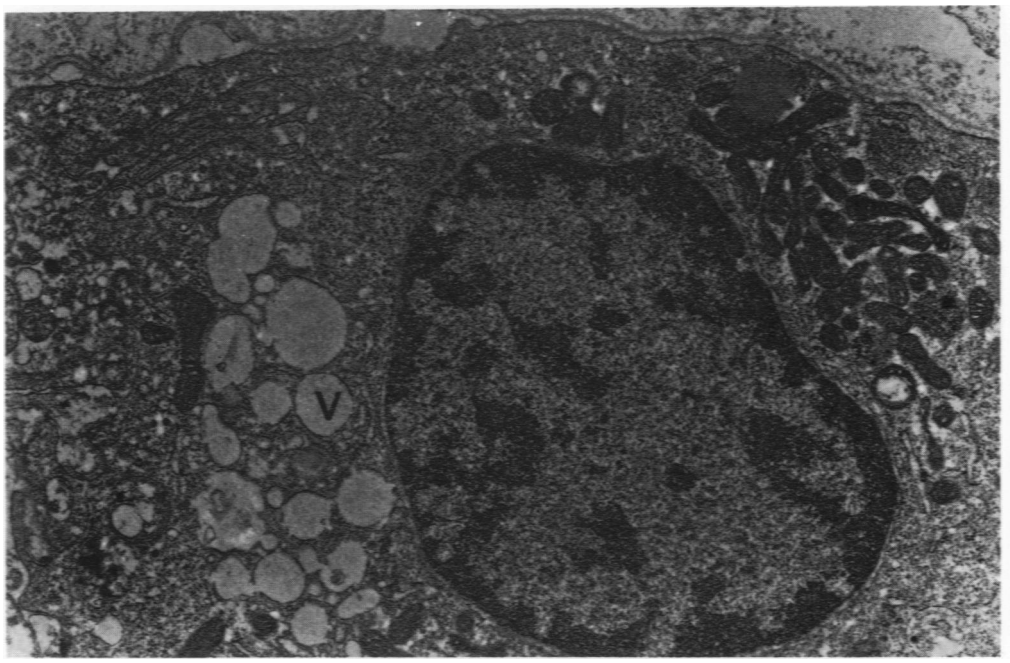

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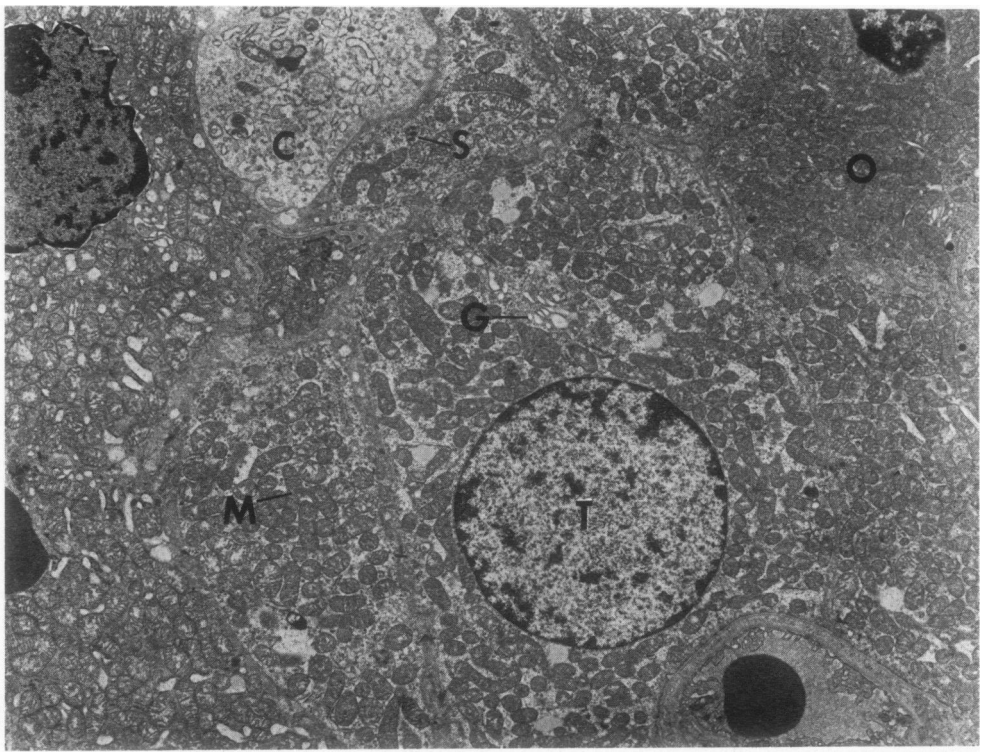
Figure 5—Chief cell hyperplasia of parathyroid gland, surgically excised; following incubation for 2 hours in control EB solution. Cellular structures appear intact. *L* = lipid. (× 11,480) **Figure 6**—Chief cell hyperplasia of parathyroid gland, same as Figure 5, except following incubation for 1 hour in control EB solution then 1 hour in the same buffer containing 50 mM Tris. Multiple vesicles (*V*) present in the cytoplasm are suggestive of Golgi origin and are similar to those seen in Figures 3 and 4. Other cellular structures appear intact. (× 14,000)



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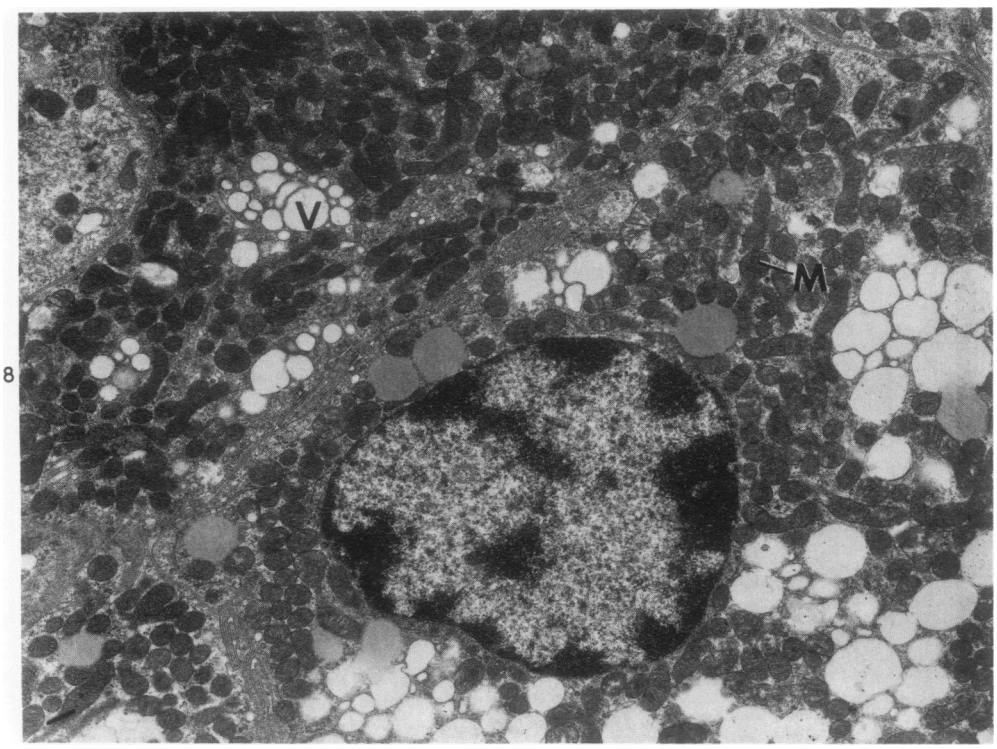

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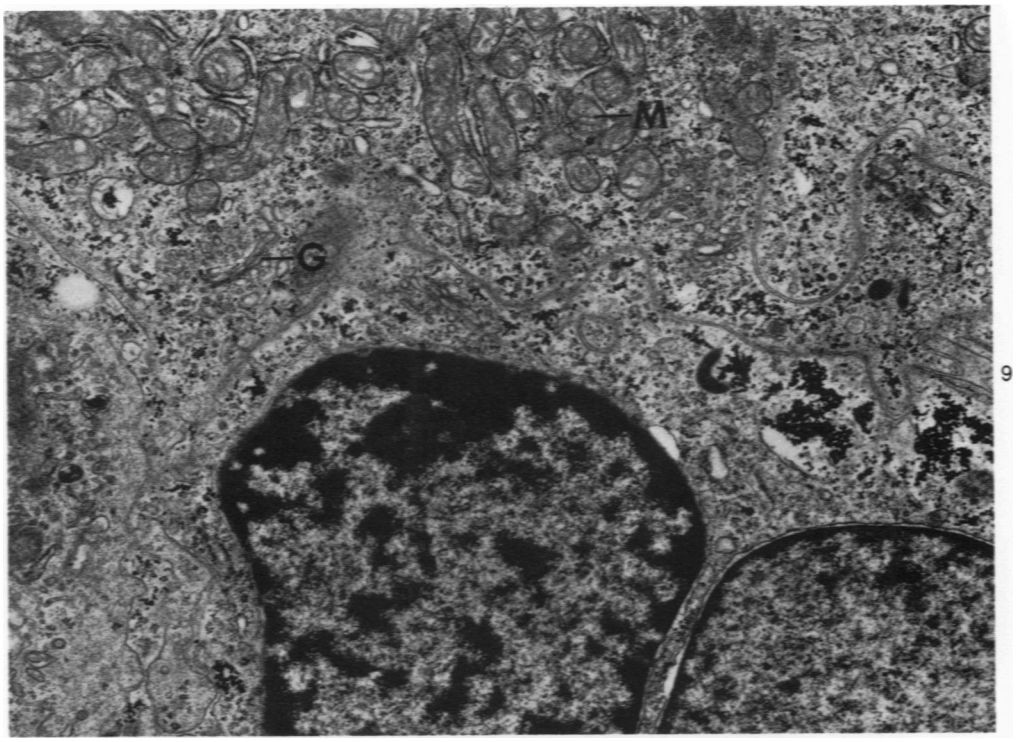
Figure 7—Oxyphil adenoma (functioning) of parathyroid gland, surgically excised; before incubation. Oxyphil cells (*O*), transitional oxyphil cells (*T*), and rare chief cells (*C*) are present. Mitochondria (*M*), Golgi apparatus (*G*), secretory granules (*S*), and other cellular structures appear intact. (× 4620) **Figure 8**—Oxyphil adenoma (functioning) of parathyroid gland, same as Figure 7, following incubation for 1 hour in control EB solution, then 1 hour in the same buffer containing 50 mM Tris. Multiple vesicles (*V*) present in the cytoplasm are suggestive of Golgi origin and are similar to those seen in Figures 3, 4, and 7. Secretory granules are not identified. Mitochondria (*M*) and other cellular structures appear intact. (× 9520)



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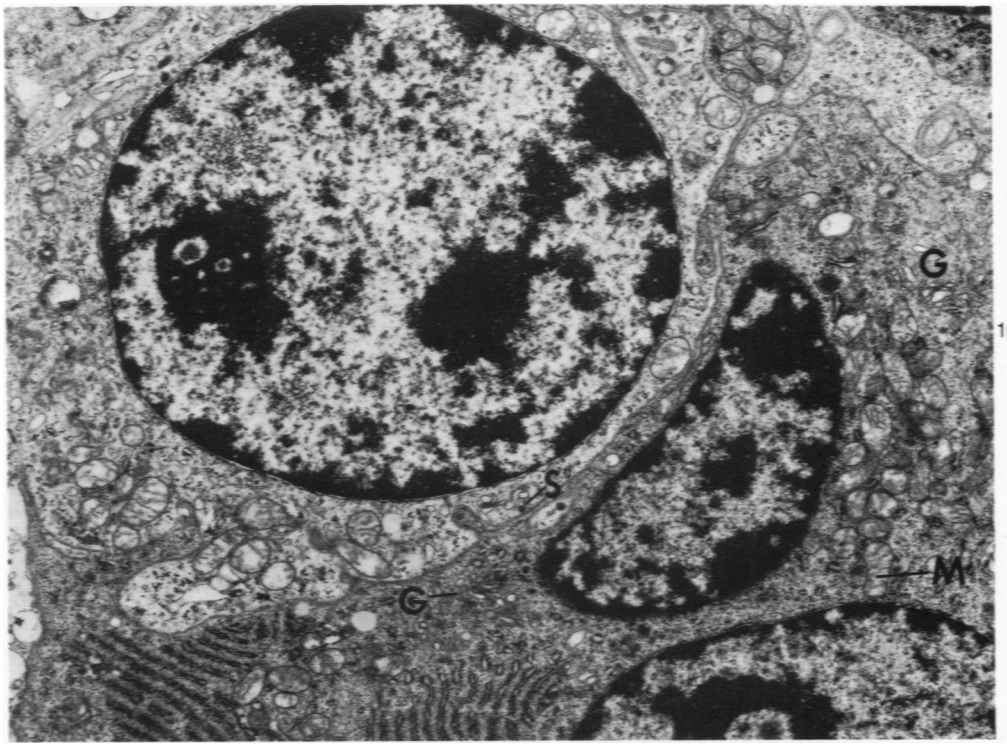

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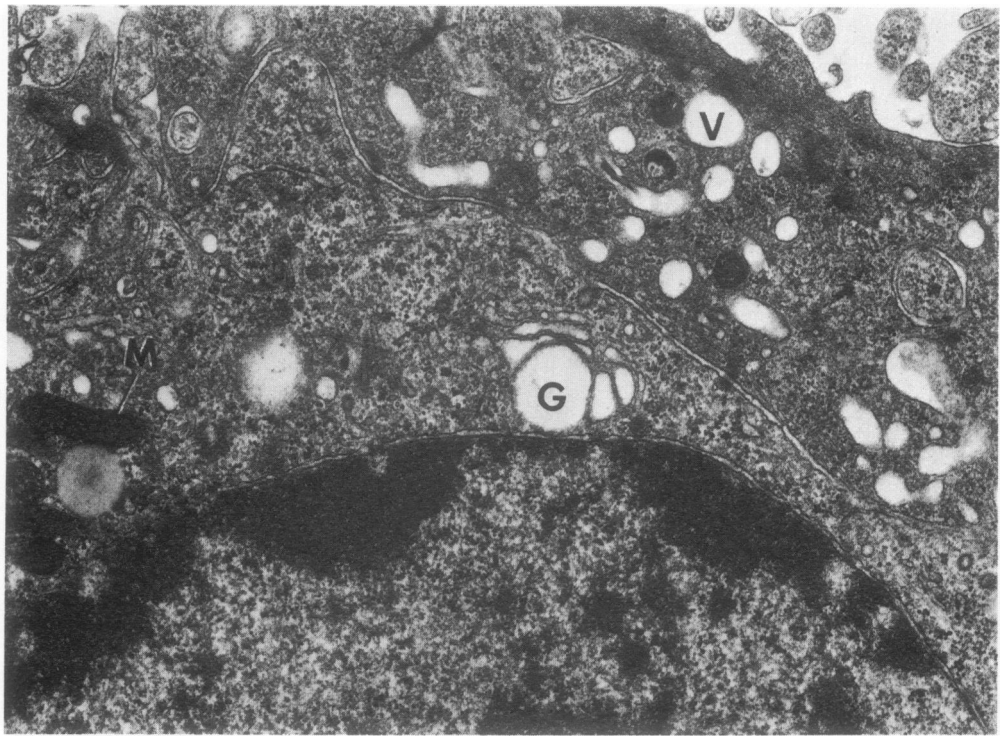
Figure 9—Chief cell adenoma of parathyroid gland, surgically excised; before incubation. Mitochondria (*M*), Golgi apparatus (*G*), and other cellular structures appear intact. (× 14,000) **Figure 10**—Chief cell adenoma of parathyroid gland, same as Figure 9, following incubation for 2 hours in control EB solution. Mitochondria (*M*), Golgi apparatus (*G*), secretory granules (*S*), and other cellular structures appear intact. (× 9520)



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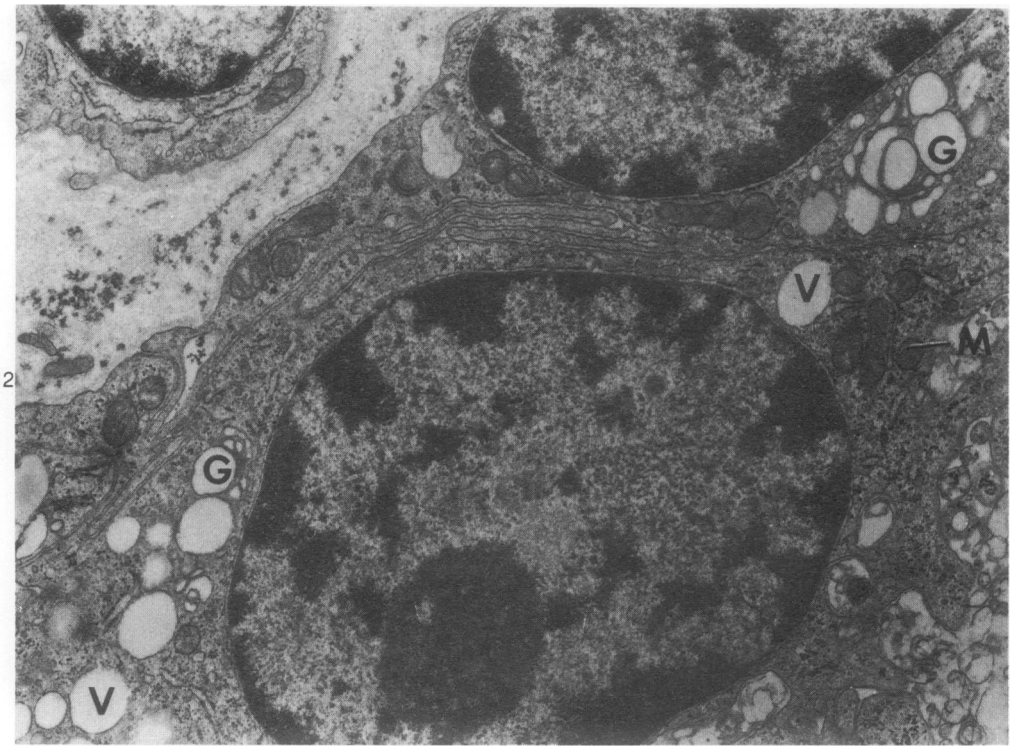

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Figure 11—Chief cell adenoma of parathyroid gland, same as Figure 9, following incubation for 1.5 hours in control EB solution then 0.5 hour in the same buffer containing 50 mM Tris. There is focal vacuolar distension of Golgi apparatus (*G*) in the lower cell and more scattered small vesicles (*V*) in the upper cell. Secretory granules are not identified. Mitochondria (*M*) and other cellular structures appear intact. (× 22,400) **Figure 12**—Chief cell adenoma of parathyroid gland, same as Figure 9, following incubation for 1 hour in control EB solution, then 1 hour in the same buffer containing 50 mM Tris. There is vacuolar distension of Golgi apparatus (*G*) and scattered vesicles (*V*), but these changes are greater in degree and extent than that in Figure 12. Secretory granules are not identified. Mitochondria (*M*) and other cellular structures appear intact. (× 15,000)