# An Electron Microscope Study of the Salamander Thyroid during Hormonal Stimulation*

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PLATES 64 TO 72

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ABSTRACT

Cytological changes in thyroid glands following administration of thyroid-stimulating hormone (TSH), were studied in adult salamanders, *Ambystoma tigrinum*, *Triturus torosus*, and *Triturus viridescens* by electron and light microscopy. Thyroids from untreated salamanders contained large follicles, faintly basophilic colloid, low follicle cells with flattened nuclei, and scant, slightly basophilic cytoplasm. After TSH administration the cell height and nuclear volume increased. Cytoplasmic basophilia was markedly increased and follicle lumina were reduced. In electron micrographs, stacks of ergastoplasmic lamellae appeared near the nucleus occasionally in contact with the nuclear membrane. In more advanced stages of stimulation, lamellar arrays were largely replaced by small disoriented vesicles and larger vacuoles containing colloid-like material. Sections of obliquely oriented ergastoplasmic membranes contained rows of extremely fine particles. Microvilli increased in size and number and Golgi structures became more extensive. Homogeneous osmiophilic droplets increased in size and abundance. Some of the smaller droplets were seen associated with the Golgi zone. Droplets similar in size and density frequently contained closely packed, whorled lamellae. Mitochondria showed no structural changes but occurred in aggregates interposed between the nucleus and highly folded portions of the basal cell membrane.

## INTRODUCTION

There is considerable interest in changes in the nucleoprotein content of cells during secretion. In view of the importance of ribonucleic acid (RNA) in protein synthesis in the cell, it seems of interest to study the fine structure of a cell type in which levels of protein synthesis can be changed from low to high. The epithelium of the thyroid, a protein-producing structure, seems to fulfill the requirements. It is believed (because of their histological appearance) that the thyroids of amphibians, particularly urodeles, have low secretory and hence, low synthetic activity. The round lumen contains a clear, slightly basophilic material enclosed by epithelial cells which are typically very flat with little cytoplasmic basophilia and elongated nuclei. The classical work of Uhlenhuth indicated that these cells could be activated with pituitary extract, (13, 14, 17–22 inclusive).

This report presents preliminary observations on the fine structural changes of salamander thyroids following the administration of thyroid-stimulating hormone (TSH).

### *Materials and Methods*

Adult salamanders, *Ambystoma tigrinum*, *Triturus torosus*, and *Triturus viridescens*, were used. However, most of the material reported here was obtained from

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one species, *A. tigrinum*. The thyroids of these animals consist of separate glands located at the lateral margins of the lower jaw just beneath the skin and immediately anterior to, or in some species (*T. torosus*), covered by the hyoid cartilage. Since these are two distinct glands, one thyroid, usually the right, was removed at the beginning of each experiment and the other removed at the end of a series of injections. In later experiments it seemed desirable to compare thyroids from the same animal with light and electron microscopes; in these instances the right thyroid was fixed for light microscopy.

In an early set of experiments animals received subcutaneously 0.05, 0.1, or 2.0 mg. per day thyroid-stimulating hormone, TSH (Parke, Davis and Co., lot No. 50P4[1]). The 2.0 mg. series proved to be too high a dose for sequential observations and was discontinued in later experiments. Animals were killed daily from day 1 to day 18. In another set of experiments animals were injected with 0.1 mg. TSH on alternate days and then sacrificed; *i.e.*, animals receiving injections on 5 alternate days would be sacrificed on day 10; animals receiving injections on 10 alternate days would be sacrificed on day 20, etc. At the end of each injection period the left thyroid was exposed and fixed *in situ* by dropping buffered osmium tetroxide (8) on the gland. After 10 minutes the left thyroid was gently excised and further fixed for an additional 2 hours in cold buffered osmium tetroxide. The right thyroid was removed and fixed in acetic acid (one part)—absolute alcohol (three parts) for light microscope studies.

In other experiments an attempt to study the detail of the membranes of ergastoplasmic sacs was made. The right thyroid was excised and placed in distilled water for 10 minutes and then fixed for electron microscopy. This procedure produced a dilation of cell structures.

Tissues for light microscopy were sectioned at either 7 or 10 $\mu$ and stained with azure B (5) and the Feulgen reagent. For the study of nucleolar and nuclear RNA, sections were treated with 0.2 mg/ml. crystalline deoxyribonuclease (DNase[2]), at pH 6.5 for 1 hour at room temperature before staining with azure B. The specificity of azure B staining of RNA was controlled by placing some slides in ribonuclease (RNase).

Tissues for electron microscopy were rapidly dehydrated and embedded in butyl methacrylate. Sections, cut on a Porter-Blum microtome, were viewed with an RCA electron microscope, model EMU-2D and RCA EMU-3C. Some grids carrying sections were stained in barium hydroxide or in a mixture of lead acetate and sodium hydroxide (24). Sections approximately 0.5 to 2.0 $\mu$ thick were also cut from the same methacrylate block, mounted on albumin-coated glass slides, and stained with azure B and the Feulgen reagent without removing the methacrylate (6). Such slides were used for comparative purposes and general light microscope orientation.

[1] Dr. D. A. McGinty of Parke, Davis and Co., Detroit, kindly supplied the TSH preparation.

[2] Worthington Biochemical Corp., Freehold, New Jersey.

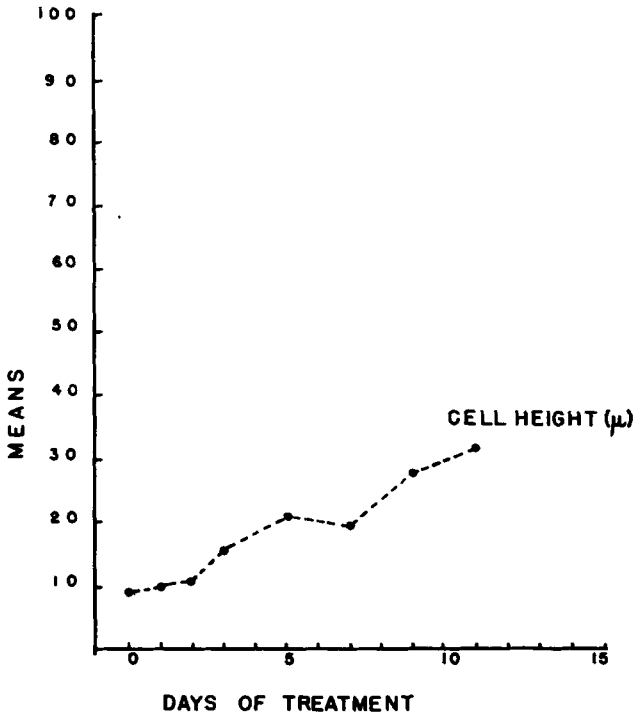
## RESULTS

### *A. Light Microscope Observations:*

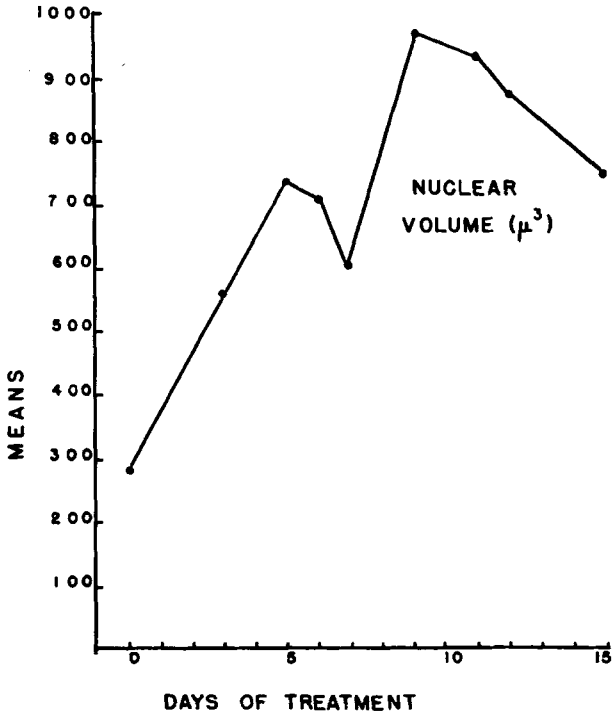
In the normal and in the unilateral thyroidectomized adult salamander the thyroid gland consisted of a group of follicles lined with low cuboidal cells. Lumina were large, round, and contained slightly basophilic colloid. The follicle cell nuclei were flat. A small amount of cytoplasm surrounded the nuclei and exhibited a faint and diffusely granular basophilia (Fig. 1). Most nucleoli were minute, below one micron in diameter.

In animals receiving injections of TSH the cell height increased fourfold during the first 12 days, the epithelium changing from low cuboidal to columnar (Fig. 2). The luminal edge of the follicle cell frequently appeared dome-shaped. This dome-shaped surface thus partially occluded the lumen (Fig. 2). With progressive stimulation the nuclei changed in shape from typically oblate spheroids to spheres and prolate spheroids with their long axes now perpendicular to the basement membrane (Fig. 2). Nuclear dimensions reached a maximum—about three to four times the control level—at 9 days. Nucleoli also reached a maximum diameter at 9 days. Micrometer measurements of changes in cell height and nuclear volume are plotted in Text-Figs. 1 and 2. With continued TSH administration, chromosomal RNA, as measured by a visual estimation of azure B, increased. After prolonged stimulation (12 to 16 days) necrotic epithelial cells appeared in the lumina and among the follicular epithelium (Fig. 2).

"Clumped" or condensed areas of cytoplasmic basophilia (probably equivalent to the ergastoplasm visualized in the electron microscope) were first seen after 3 days of treatment, usually in the basal corners of the cell. These were frequently, but not always, found against the nuclear margin. At day 4 to 5 the nuclear margin became basophilic in many cells and a well defined cap of ergastoplasm appeared on the apical margin of the nucleus. By day 12 to 14 most cells exhibited diffuse basophilia although in some cells ergastoplasmic clumps persisted throughout the series. The basophilic staining was removed with RNase treatment at all stages.




TEXT-FIG. 1. Changes in cell height with successive TSH injections.




TEXT-FIG. 2. Changes in nuclear volume with successive TSH injections.

Characteristic of the follicle cell cytoplasm were vacuoles or vesicles filled with material resembling that seen in the lumen. After 16 days of treatment, cells of *Triturus* typically showed one or two huge vacuoles which occupied most of the cytoplasm and frequently bulged out into the lumen. In *Ambystoma*, at 12 days, the vacuoles were usually smaller and much less frequent (Fig. 2).

*B. Electron Microscope Observations:*

*1. Nuclear Changes:*

The nucleoli enlarged and after 3 days, they were prominent, appearing as irregularly anastamosing strands, occasionally vacuolated. Areas of clustered electron-dense particles were observed in the nucleus (Figs. 10 and 17). Feulgen-stained adjacent methacrylate sections indicated that some of these regions contained DNA. In electron micrographs it was frequently difficult to distinguish between such DNA-containing areas and nucleolar regions. There seemed to be no relationship between amounts of electron-dense granulation and progressive TSH administration.

The nuclear membrane appeared double during stimulation (Figs. 15 and 16). In most follicle cells the nuclear boundary was frequently hazy in outline or obscured by electron dense granules (Figs. 6, 10, 11, and 17).

Apart from the increased nuclear volume seen with TSH administration, little change was noted. Stained sections resulted in the enhancement of heterochromatic regions within the nucleus. This was particularly noticeable in obliquely sectioned nuclei. Fragments of stained, fine, filamentous "strands" could be seen in the obliquely sectioned nuclear membranes. The filaments extended, on occasion, between the heterochromatic areas and the cytoplasmic RNA granules (Fig. 17).

*2. Cytoplasmic Changes:*

*Microvilli.*—Microvilli appeared infrequently as short straight projections along the luminal edge of follicle cells in control thyroids (Figs. 5 and 7). After TSH treatment they appeared larger in width and height and in greater numbers (Figs. 8 to 10), sometimes as straight, pointed projections (Fig. 10), and sometimes as long, bulbous structures containing droplets of materials (Figs. 8 and 9).

*Basement Membrane.*—The basement membrane of controls and of the early stages of stimulation appeared as a straight or slightly folded band of dense material lying between follicle cells (Fig. 3). With prolonged TSH administration (11 to 12 days), the basement membrane and the adjacent basal cell membrane contained many folds (Fig. 15).

*Cytoplasmic Droplets.*—Round or oval droplets varying in size from 0.2 to 2 $\mu$ were visible in the cytoplasm of untreated and treated tissues. TSH administration resulted in an increase in the number of these structures (Figs. 4 and 10). The smaller droplets were frequently seen near or within the Golgi zone (Fig. 18). In stimulated thyroids the droplets were usually homogeneously dense and were frequently surrounded by a clearly defined outer membrane (Fig. 10). A few of the less homogeneous bodies often appeared to contain small internal structures frequently seen as densely wrapped whorled lamellae with light oval centers (Figs. 11 to 13). The diameter of these lamellae closely approximated those of the Golgi membranes. After treatment in distilled water some of the whorled lamellae within droplets were unraveled (Fig. 14).

*Mitochondria.*—No differences were observed between the mitochondria of control and treated thyroids. The basal cell region, in which the cell membrane was deeply folded, seemed to have more mitochondria (Fig. 15).

*Ergastoplasm.*—Control and treated thyroid epithelium both contained ergastoplasmic regions. These were of two types: (1) the lamellated ergastoplasm: large vesicles often flattened and joined to each other, corresponding to basophilic "clumps" seen with the light microscope (Fig. 4); and (2) the vesicular ergastoplasm: individual smaller vesicles equivalent to the diffuse basophilia in the light microscope (Fig. 15). The "clumped" areas were small in unstimulated or slightly stimulated thyroids and restricted to the lower basal corners of the cell adjacent to either end of the flattened nucleus (Fig. 3). In the TSH-injected series the amounts and types of ergastoplasm varied with the state of stimulation. At later stages the vesicular ergastoplasm predominated with some lamellated ergastoplasm persisting (Fig. 4). The vesicles were generally small, round, and dispersed in the cytoplasm. Some granule-studded membrane enclosed vesicles contained a homogeneous material (Fig. 16).

Oblique sections of vesicle walls revealed a fine particulate structure. This appeared as discrete particles assuming a variety of patterns and they were on, or embedded within, the ergastoplasmic

membrane. They sometimes were arranged in circles or sets of parallel granules (Figs. 18 and 19). A section cut normal to these cytoplasmic membranes revealed the more common type of ergastoplasm (Fig. 16).

*Vacuoles.*—Vacuoles containing moderate amounts of a homogeneous material with low electron scatter became visible as early as the second day of treatment (Fig. 3). This material resembled that found in the lumen. These vacuoles varied in size and appeared to be distinctively different, because of the lighter electron-scattering material, from smaller ergastoplasmic vesicles. Frequently they were located in an ergastoplasmic region. Coalescence of variably dense ergastoplasmic vesicles, especially in later stages of stimulation, suggested an origin for these vacuoles possibly producing a dilution of the heavier electron-scattering material of the smaller vesicles.

*Golgi Apparatus.*—This cytoplasmic structure was visible in control and treated thyroids. It appeared as thin, long, closely stacked membranes frequently woven through clusters of small vesicles (Fig. 10). These Golgi membranes were straight (Fig. 10), or horseshoe-shaped (Fig. 6). The Golgi body usually occupied a perinuclear position (Fig. 10), although some electron micrographs showed clusters farther out in the cytoplasm (Fig. 6). The untreated follicle cells contained small restricted Golgi zones (Fig. 5). TSH treatment resulted in a more extensive Golgi area containing more prominent Golgi structures. Small droplets approximately 0.1 to 0.2 $\mu$ in size and described earlier, were seen associated with Golgi vesicles (Fig. 18).

## DISCUSSION

Continued TSH stimulation results in increased amounts of ergastoplasm. The origin of ergastoplasmic membranes or its frequently associated granules remains speculative. Early changes in ergastoplasm seem to be associated with perinuclear regions. It has been previously demonstrated that the outer nuclear membrane is continuous with ergastoplasmic membranes (23).

Lead acetate–sodium hydroxide staining aided in visualizing fine strand-like structures which extended between the nucleus and cytoplasm. They were best seen in obliquely sectioned nuclear membranes. The association with dense, chromatin granules at its nuclear end and RNA granules at its cytoplasmic end suggests a role in nuclear cytoplasmic interaction. These strands may be related to recently observed nuclear "pores" (23) and their suggested role in the transfer of material between nucleus and cytoplasm. They may also be similar or equivalent to the filaments observed by Swift (15).

It has been observed that ergastoplasm may exist in two states: as an aggregate of lamellae or as independent vesicles, each with its characteristic membrane-associated granules. This granule-membrane complex has been assumed to be equivalent to the basophilia or RNA-containing structures seen with the light microscope (10). In thyroid tissue it seems likely that the lamellar aggregates are the equivalent of condensed perinuclear basophilic regions seen with the light microscope and the independent ergastoplasmic vesicles constitute the diffuse basophilia. Because diffuse basophilia is seen in later stages of TSH administration, it would seem that the separated vesicles are derived from the connected ones of the ergastoplasmic aggregates. These individual vesicles which are limited by granule-studded membranes may have synthesized thyroid secretion as evidenced by higher electron scatter of their content. The variably dense vesicles which become numerous at 7 days of stimulation may later discharge their product or fuse to give rise to the huge vacuoles seen in thyroids after 12 to 16 days of TSH stimulation. The relationship of ergastoplasm to protein synthesis has been suggested previously for thyroid tissue (1, 3). The similarity in electron density between follicular colloid and intracellular vacuolar content suggests such a function.

It has been previously noted that the granules frequently associated with the ergastoplasmic membrane (endoplasmic reticulum) are approximately 10 to 15 m$\mu$ in diameter (9). These granules probably contain RNA and have been seen in this study. When membranes are cut obliquely, patterned granules are seen which are arranged predominantly in parallel double rows and occasionally in circles. Such patterned arrays have only been seen in stimulated thyroids and are persistent with increased synthesis at this time. Their patterns resemble those described for the normal mouse thyroid (4), and for an alga, *Chlamydomonas* (12).

The cytoplasmic droplets or granules may possibly be derived from separated ergastoplasmic vesicles which have gathered dense material. However, the fact that similar yet smaller structures are seen near or even within Golgi vesicles lends more support to the Golgi zone as being a

possible source of origin. If they represent secretory granules, their role in the secretory cycle has not been revealed in this study. Some of the cytoplasmic droplets contain whorled lamellae and are seen only with TSH administration. The origin of these internal concentrically arranged lamellae and their function remain unknown. These thyroid droplets or granules superficially resemble the "big droplets" of Rhodin (11), the fetal mouse kidney droplets described by Clark (2), and those of mouse thyroid noted by Ekholm and Sjöstrand (4). Clark proposes that the fetal mouse kidney droplets represent intracellular structures which segregate particulate or macromolecular substances. The TSH, being mammalian in origin, may represent a "foreign" substance which becomes localized or segregated within these formed thyroid droplets and their whorled lamellae.

Golgi bodies were found in control and stimulated thyroids. Classical cytology has associated these bodies with some function in the secretory process. With TSH stimulation Golgi structures become larger and more prominent. The intimate association of small secretory granules or homogeneous droplets with Golgi vesicles suggests that the granules might originate within. Such small granules could conceivably enlarge through accumulation of material within and move out into other cytoplasmic areas where they could then participate in the secretory activities of the cell.

In the 16-day-stimulated *T. viridescens* thyroids, vacuoles occurred in the center of lamellate ergastoplasmic regions. What appears to be remnants of vesicle walls projected into the cavity. The location of these relatively large areas with variable densities suggest they may arise as a result of fusion of ergastoplasmic vesicles.

No observable changes in numbers of mitochondria were seen. The aggregation of mitochondria about folded portions of the membrane at the base of the cell and in close proximity to the capillary, may indicate an important role for mitochondrial enzymes in transport mechanisms.

Braunsteiner *et al.* (1) and Monroe (7) believed that microvilli functioned as absorptive structures moving colloid from the lumen to the cell. The possibility that some secretory function might be attached to these structures still exists. TSH administration has appreciably increased the follicular cell volume. The formation and prevalence of microvilli on the free luminal cell surface in later stages of stimulation might represent a physical phenomenon of cellular expansion and might, together with the dome-shaped protusions, represent that region of the cell which most easily compensates this demand for expanded volume. The functional significance for the morphologically differing microvilli observed in this study remains unknown.

The foldings of the basal cell membrane may represent a simple physical phenomenon due to increased cell volume within a restricted area. This would be similar to luminal cell surface changes described earlier. Thus, there may be no attached physiological significance. However, the basal cell region is undoubtedly important for the transportation of material from the lumen or follicle cell to the capillary. The complex folding of this cell membrane in association with a localized concentration of mitochondria in the basal cell region may thus suggest an adaptation designed to transfer material between the lumen or follicle cell and the blood.

## Bibliography


1. Braunsteiner, H., Fellinger, H. K., and Pakesch, F., *Endocrinology*, 1953, **53,** 123.
2. Clark, S. J., Jr., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 349.
3. Dempsey, E. W., and Peterson, R. R., *Endocrinology*, 1955, **56,** 46.
4. Ekholm, R., and Sjöstrand, F. S., *J. Ultrastruct. Research*, 1957, **1,** 178.
5. Flax, M. H., and Himes, M. H., *Physiol. Zool.*, 1952, **25,** 297.
6. Houck, C. E., and Dempsey, E. W., *Stain Technol.*, 1954, **29,** 207.
7. Monroe, B. G., *Anat. Rec.*, 1953, **116,** 345.
8. Palade, G. E., *J. Exp. Med.*, 1952, **95,** 286.
9. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 59.
10. Palade, G. E., and Siekevitz, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 171.
11. Rhodin, J., Karolinska Institutet, Stockholm, Aktiebologet Godvil, 1954, 1.
12. Sager, R., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 463.
13. Schwartzbach, S. S., and Uhlenhuth, E., *Physiol. Zool.*, 1933, **6,** 236.
14. Schwartzbach, S. S., and Uhlenhuth, E., *Endocrinologie*, 1936, Band 16, Heft 6, 412.
15. Swift, H., A Symposium on the Chemical Basis of Development, (William D. McElroy and Bentley Glass, editors), Baltimore, Johns Hopkins Press, 1958.

16. Uhlenhuth, E., personal communication.
17. Uhlenhuth, E., Transactions of the American Association for the Study of Goiter, *Western J. Surg., Obstet. and Gynecol.*, Portland, Oregon, 1936.
18. Uhlenhuth, E., and Schwartzbach, S. S., *Proc. Soc. Exp. Biol. and Med.*, 1928, **36,** 152.
19. Uhlenhuth, E., and Schwartzbach, S. S., *Endocrinologie*, 1935, Band 16, Heft ½, 9.
20. Uhlenhuth, E., Schenthal, J. E., Thompson, J. U., Mech, K. F., and Algin, G. H., *J. Morphol.*, 1945, **76,** 1.
21. Uhlenhuth, E., Schenthal, J. E., Thompson, J. U., and Zwilling, R. L., *J. Morphol.*, 1945, **76,** 45.
22. Uhlenhuth, E., Thompson, J. U., and Schenthal, J. E., *Anat. Rec.*, 1935–36, **64,** supplement, 52.
23. Watson, M. L., *Biochim. et Biophysica Acta,* 1954, **15,** 475.
24. Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1958, **4.** 727.

## Explanation of Plates

| | | | |
|---|---|---|---|
| *B.M.* | basement membrane | *Id.* | nuclear indentation |
| *Cap.* | capillary | *Lu.* | lumen |
| *Cl.* | collagen | *Mit.* | mitochondria |
| *C.M.* | cell membrane | *Miv.* | microvilli |
| *Con.* | contaminant | *N.* | nucleus |
| *Dr.* | secretion droplet or granule | *N.M.* | nuclear membrane |
| *Er.* | ergastoplasm or endoplasmic reticulum | *Nu.* | nucleolus |
| *Fil.* | filamentous strand | *T.B.* | terminal bar |
| *Gr.* | ergastoplasmic granules | *Vac.* | vacuole |
| *G.Z.* | Golgi zone | *Ves.* | vesicle |
| *HC.* | heterochromatin | *W.L.* | whorled lamellae |

The solid line in each figure represents one micron unless otherwise indicated.

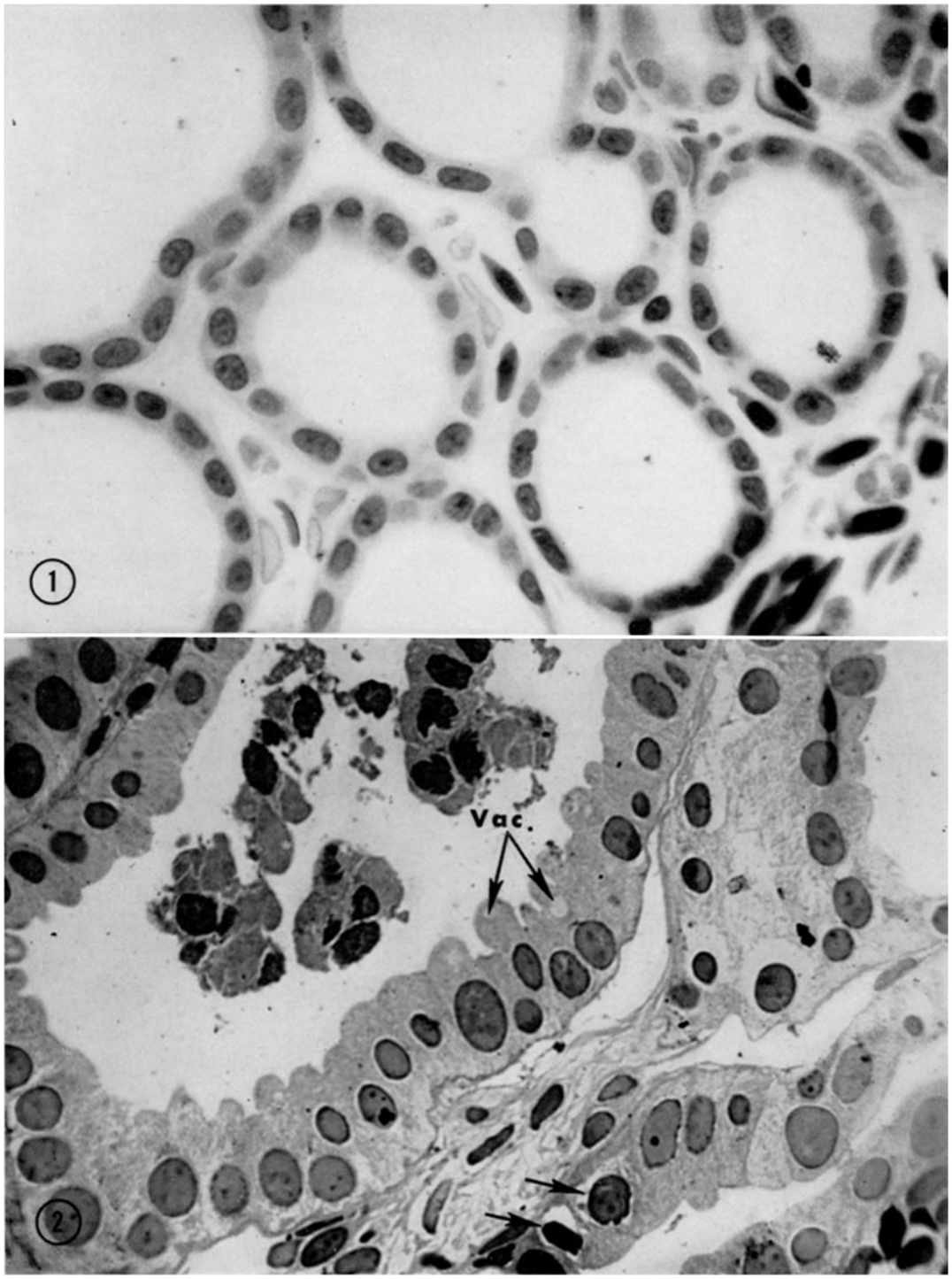
## Plate 64

### Photomicrographs

*A. tigrinum*, Osmium-fixed, methacrylate-embedded. Azure B–stained, × 320.

Fig. 1. Untreated control. Note the oval or round follicles with low cuboidal cells; elongated nuclei; inconspicuous nucleoli; and scant lightly stained cytoplasm.

Fig. 2. Treated with TSH for 12 days. Note the occluded slit-like lumen in the lower right; the conversion of the low cuboidal follicle cell to that of the columnar type; the increased nuclear size with larger, more prominent nucleoli; the protruding "dome-shaped" luminal edge of the follicular cell; and the presence of large vacuoles (*Vac.*) in the more heavily stained cytoplasm of some cells. In the larger lumen are aggregates of necrotic cells which were sloughed off from the secretory epithelium. These seem to be derived from similar cells still lining the follicle lumen (arrows).



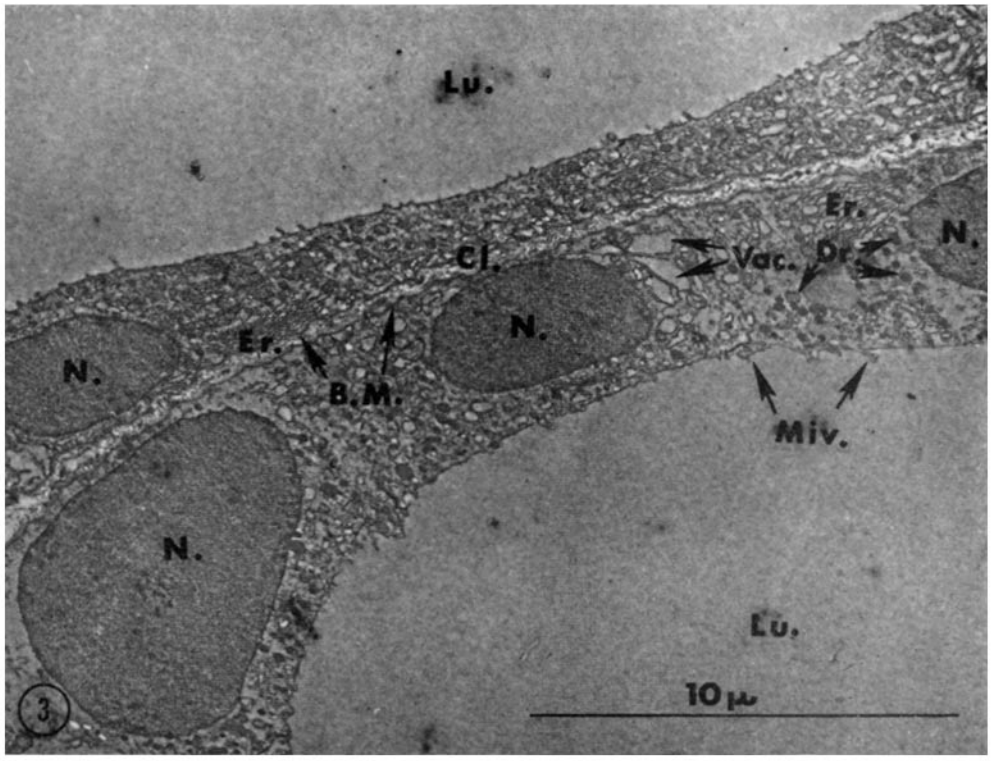

(Herman: Salamander thyroid during hormonal stimulation)

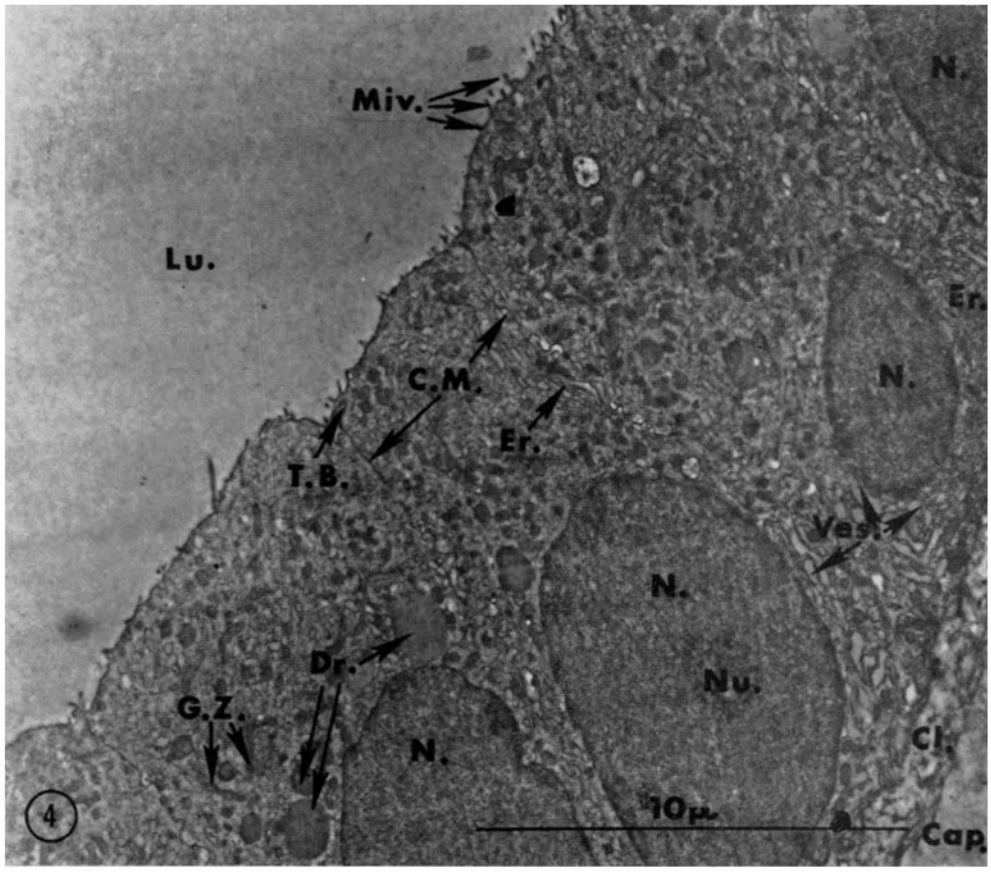
## PLATE 65

A comparison of unstimulated and stimulated thyroid cells, × 5,700.

FIG. 3. *A. tigrinum* thyroid. Received 0.1 mg. TSH daily for 2 days. Two adajcent follicles. Note the flat cells; elongated nuclei, (*N*); scant cytoplasm; relatively little ergastoplasm, (*Er.*), with occasionally expanded, slightly dense vacuoles (*Vac.*). Small homogeneously dense droplets (*Dr.*) are scattered about; a thin, relatively straight basement membrane (*B.M.*) lies on either side of an area separating the two follicles and containing strands of collagen (*Cl.*). Microvilli (*Miv.*) are seen as short, infrequently occurring projeetions on the free, luminal surface of the follicle cell. Parts of two lumina (*Lu.*) are seen.

FIG. 4. *A. tigrinum* thyroid: Received 0.1 mg. TSH daily for 13 days. The number and size of the dense homogeneous droplets (*Dr.*) have increased. The Golgi zone (*G. Z.*) is prominent. A large nucleolus (*Nu*) is present. The ergastoplasm (*Er.*) is extensive and some of its vesicles (*Ves.*) have expanded. Part of a capillary (*Cap.*) appears in the extreme lower right corner. It is bordered by a narrow space containing connective tissue fibrils (*Cl.*). At the luminal juncture of adjacent cells a terminal bar (*T. B.*) may be seen.



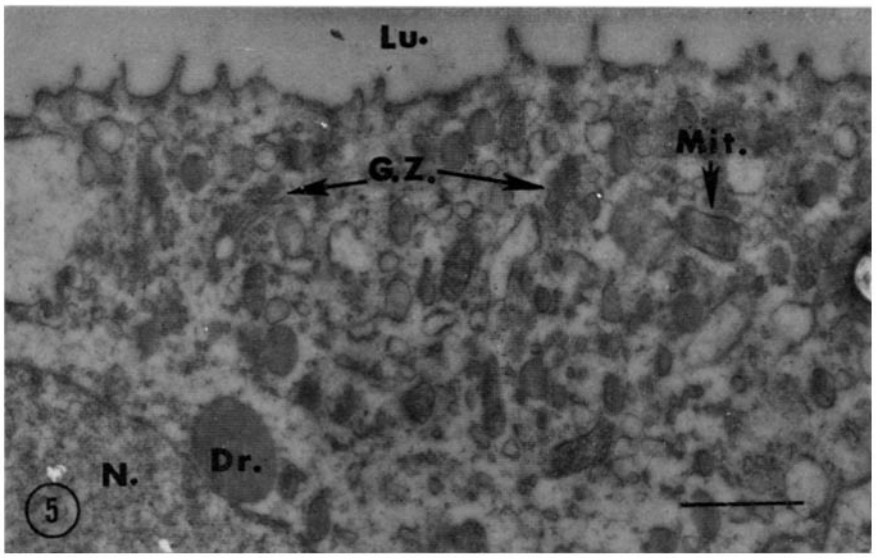



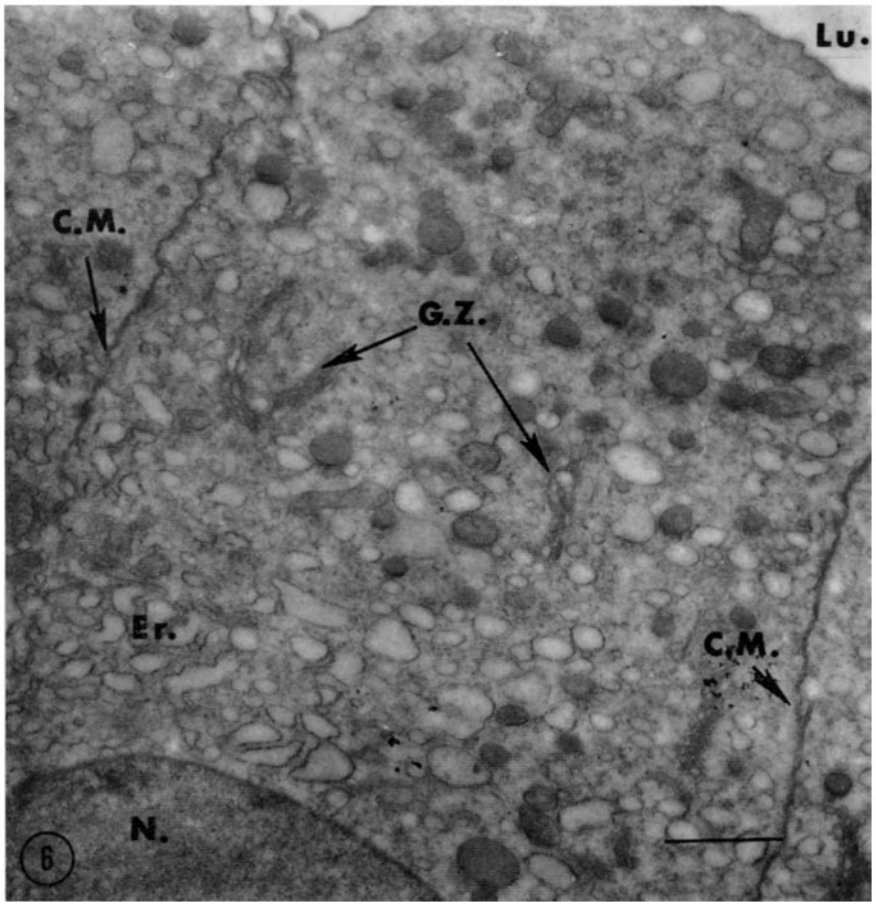

(Herman: Salamander thyroid during hormonal stimulation)

PLATE 66

FIG. 5. *A. tigrinum* thyroid. Uninjected control. Higher magnification of part of an uninjected follicle cell. A portion of the lumen (*Lu*) runs across the upper part of the micrograph. Part of the nucleus (*N.*) appears in the lower left. The Golgi zone (*G.Z.*) is relatively small and inconspicuous. A mitochondrion (*Mit.*) with parallel arranged cristae is present. × 16,000.

FIG. 6. *A. tigrinum* thyroid. Received 0.1 mg. TSH for 7 days. Micrograph is at the same magnification as Fig. 5. The closely apposed cell membranes (*C.M.*) of adjacent follicular cells may be seen. Part of the lumen (*Lu*) is seen in the extreme upper right. The Golgi zone (*G. Z.*) has become more extensive. The ergastoplasm (*Er.*) is seen as a collection of vesicles near a portion of the nucleus (*N.*) × 16,000.




(Herman: Salamander thyroid during hormonal stimulation)

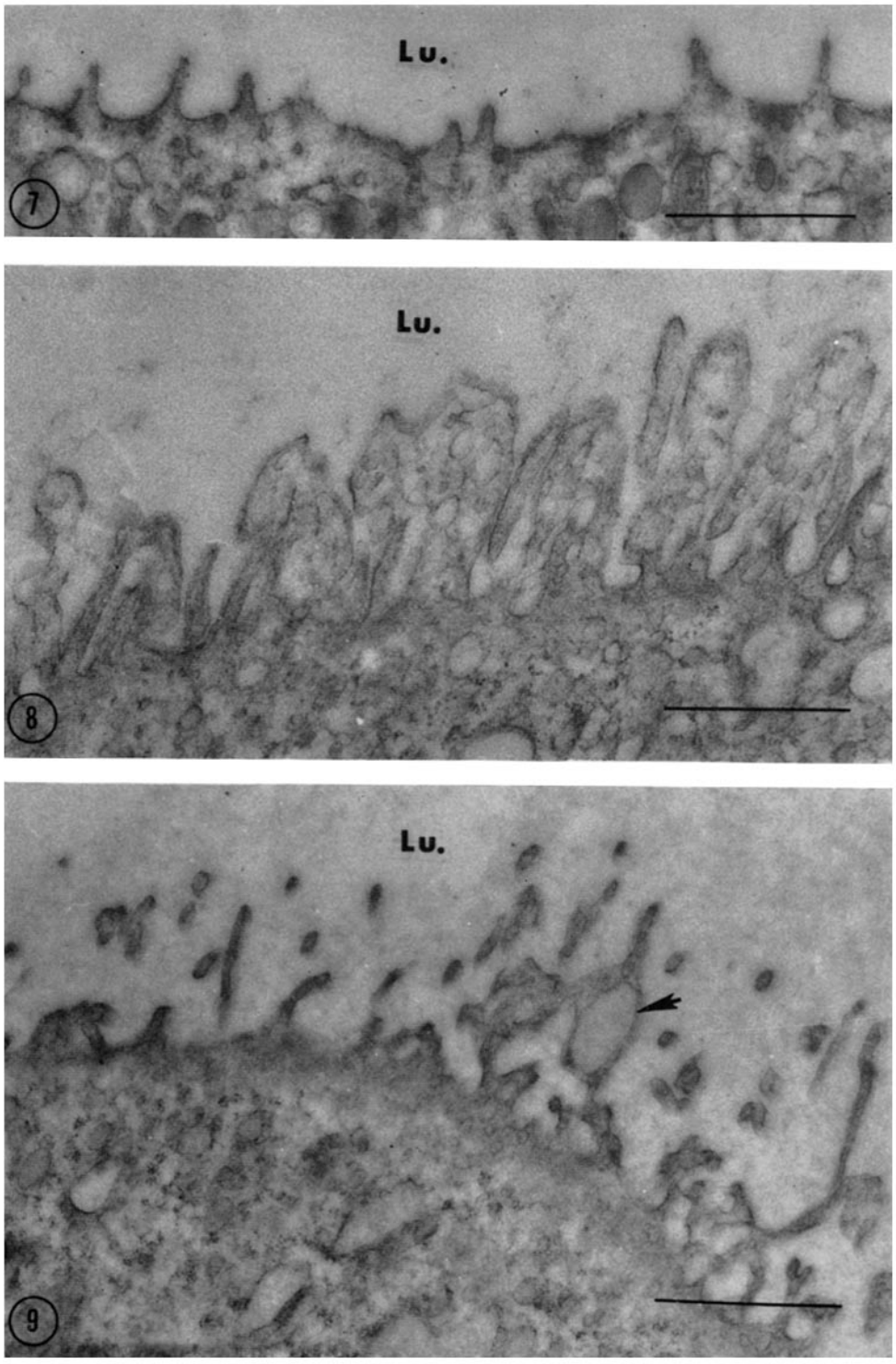
PLATE 67

Changes in microvilli of epithelial cells following TSH administration. All at × 27,000.

FIG. 7. *A. tigrinum:* Untreated control. The apex of a follicle cell is shown bordering on the lumen (*Lu*). Note the short, stubby, infrequently occurring microvilli.

FIG. 8. *T. viridescens:* Received 0.1 mg. TSH daily for 12 days. Note the change in number, length, and morphology of the microvilli. Such bulbous microvilli are frequently seen with TSH administration.

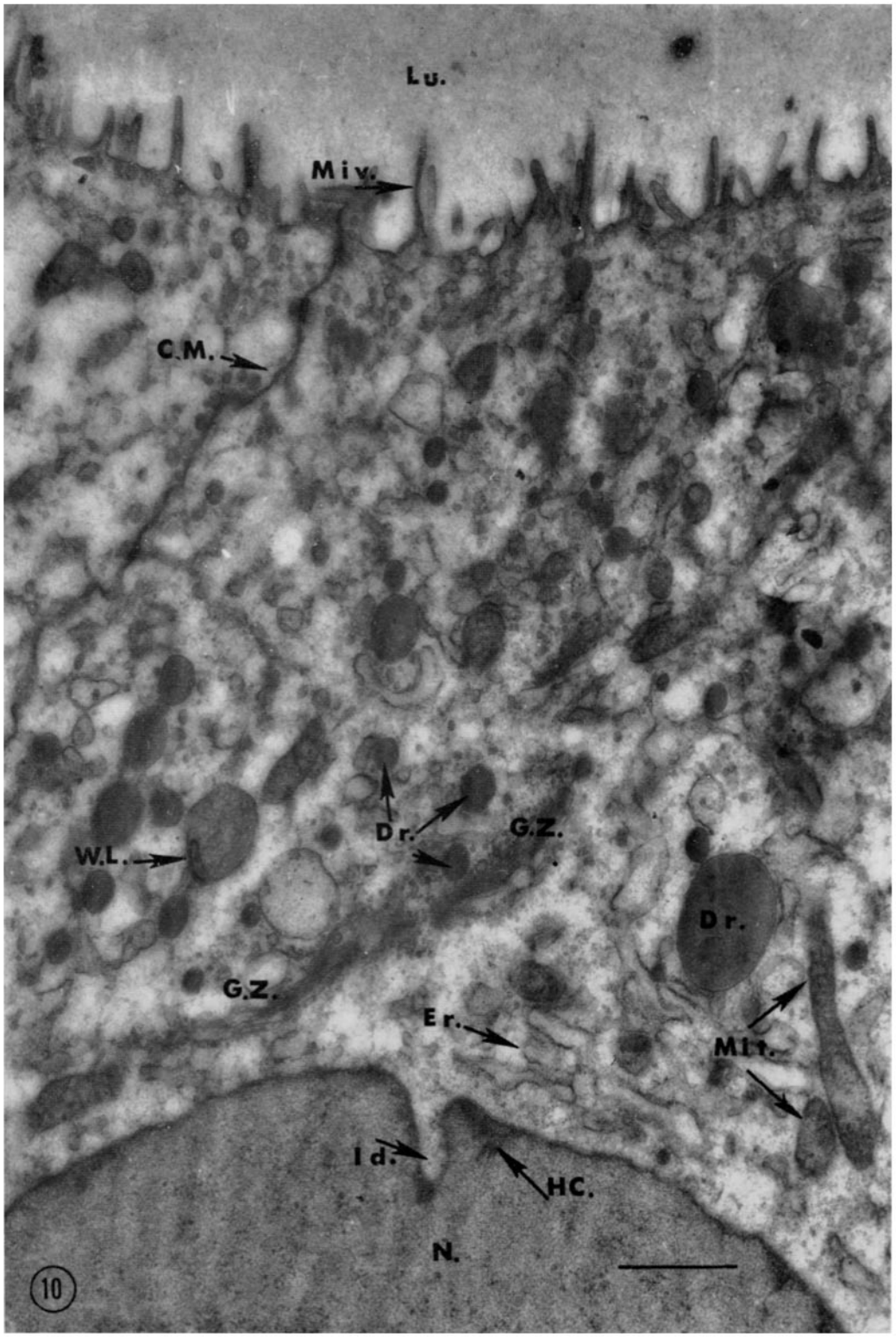
FIG. 9. *T. viridescens:* Received 0.1 mg. TSH daily for 12 days. Another form of microvilli which becomes visible with TSH is shown by these narrow, frequently straight, finger-like projections. Some microvilli may contain droplet-like structures within (arrow).




(Herman: Salamander thyroid during hormonal stimulation)

PLATE 68

FIG. 10. *A. tigrinum* thyroid: Received 0.1 mg. TSH daily for 5 days. The microvilli (*Mv.*) appear as straight, long numerous, finger-like projections into the lumen (*Lu.*). The apposed cell membranes (*C.M.*) of two cells may be seen. A portion of the nucleus (*N*) contains a slight nuclear indentation (*Id.*), and a region of electron dense granules presumed to be heterochromatin (*HC.*) appears just below the nuclear membrane. The ergastoplasmic vesicles (*Er.*) appear flattened. Membrane-bounded, homogeneous droplets (*Dr.*) are discernible. Sometimes these contain a whorled membranous structure within (*W.L.*). × 18,000.

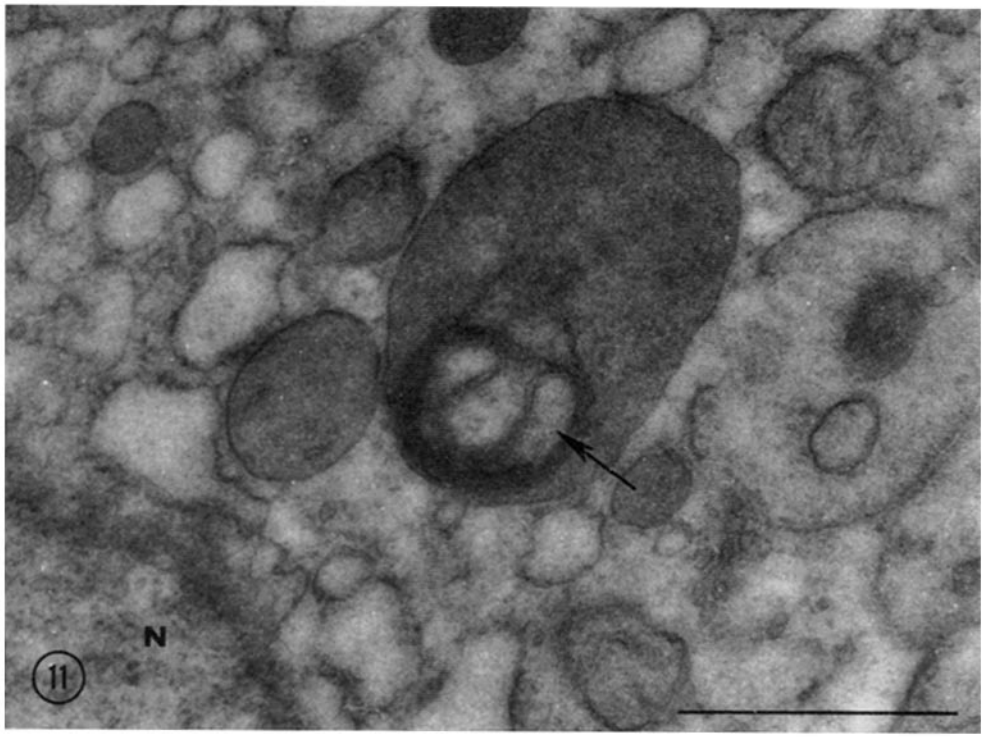


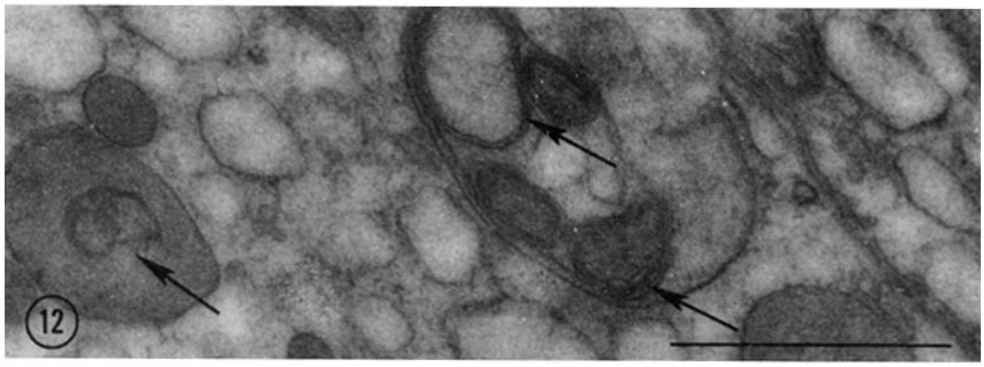

(Herman: Salamander thyroid during hormonal stimulation)

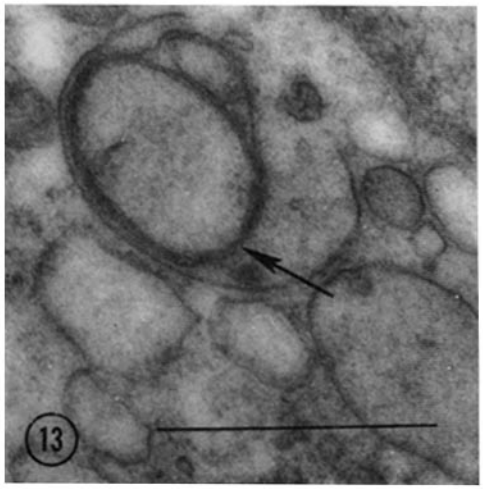
PLATE 69

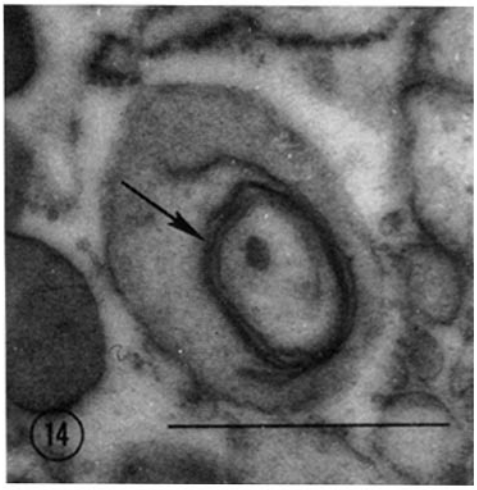
Various forms of cytoplasmic droplets or granules containing varieties of whorled, closely packed membranes. All at × 37,000.

FIGS. 11 through 14. *A. tigrinum* thyroid: Received 0.1 mg. TSH on 13 alternate days over a period of 26 days. Various types of whorled lamellae (arrows) are found within these cytoplasmic structures. Note the comparatively clear area within the whorled membranes of the large granule of Fig. 11, and two darker areas within whorls of the granule in the center of Fig. 12. The granule in Fig. 14 has an unraveled membrane due to treatment of the thyroid with distilled water for 10 minutes prior to fixing in osmium.



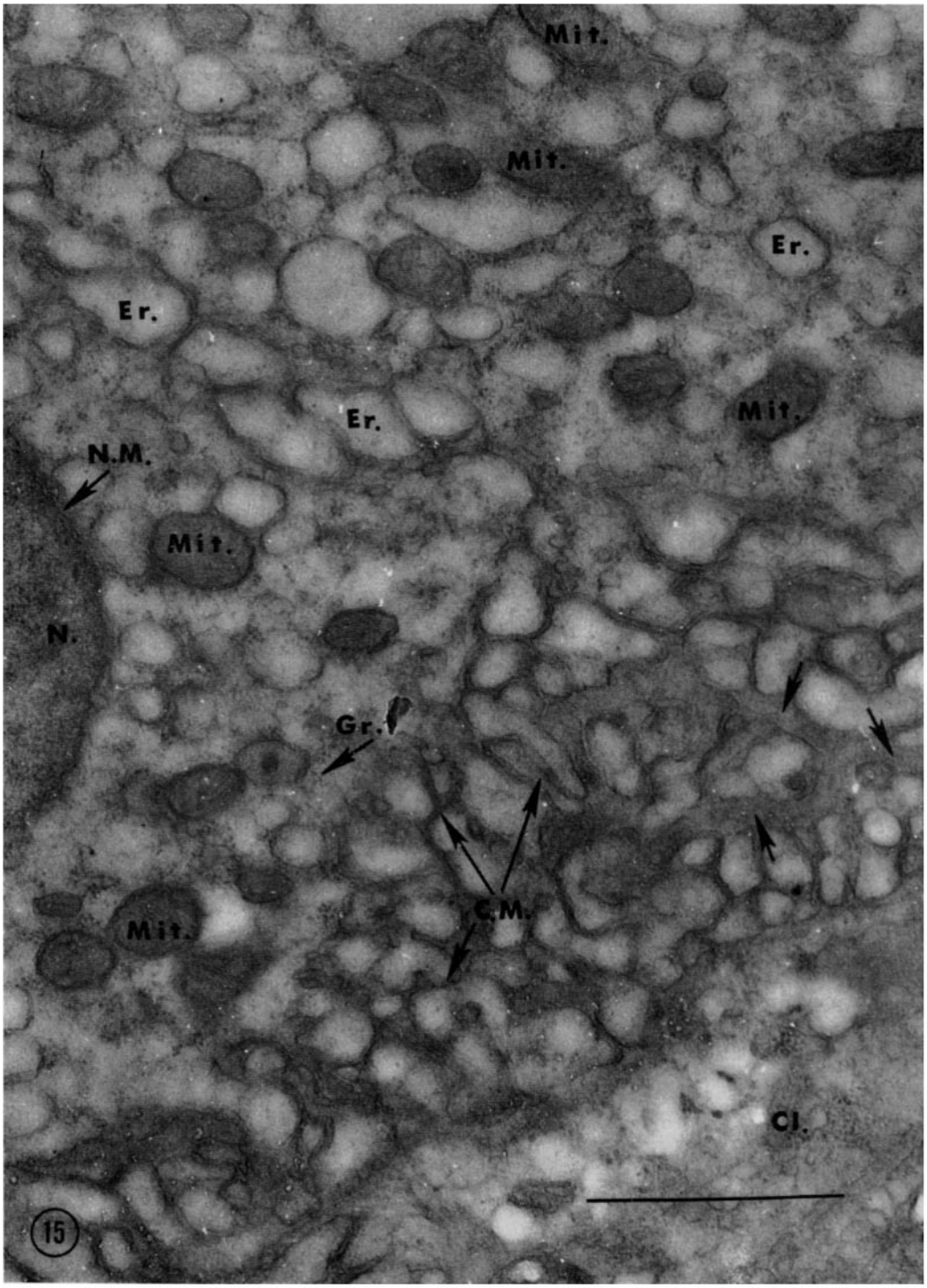




(Herman: Salamander thyroid during hormonal stimulation)

PLATE 70

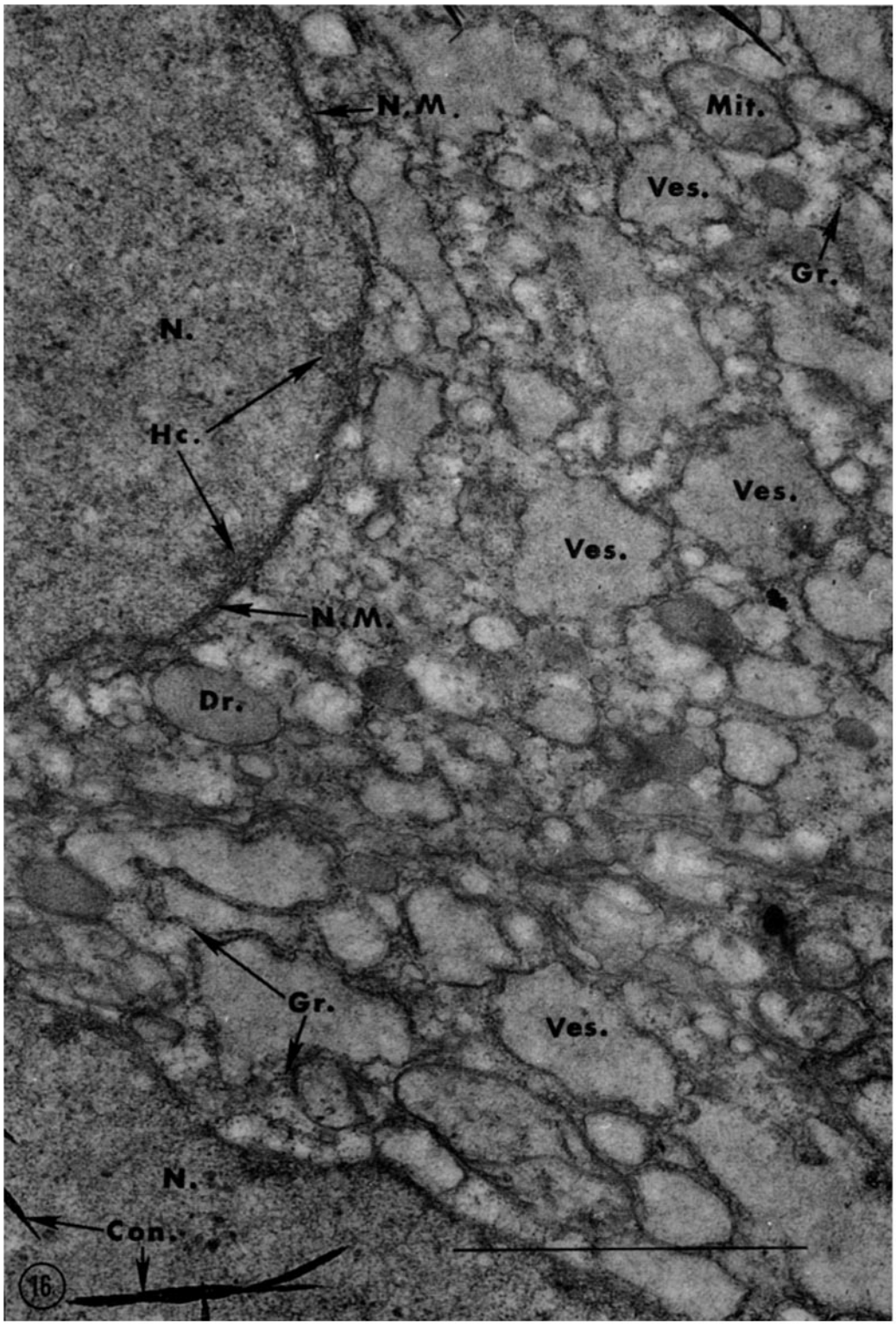
FIG. 15. *A. tigrinum* thyroid: Received 0.05 mg. TSH daily for 16 days. This electron micrograph is in the basal region of the follicle cell. Collagen (*Cl.*) appears in the lower right corner and the cell membrane (*C.M.*) has been thrown into numerous folds occasionally "trapping" some basement membrane within (unmarked arrows). Mitochondria (*Mit.*) frequently appear in the basal cell regions. × 36,000.




(Herman: Salamander thyroid during hormonal stimulation)

PLATE 71

FIG. 16. *A. tigrinum* thyroid: Received 0.1 mg. TSH daily for 6 days. The section was stained for five minutes in barium hydroxide before being viewed with the electron microscope. Some contaminant (*Con.*) probably barium carbonate crystals, appears at the lower left. Clusters of electron dense particles, probably heterochromatin (*HC.*) material, are just beneath the nuclear membrane tending to obscure it. Adjacent to these areas are regions lacking such clustered particles. Here the nuclear membrane (*N.M.*) appears doubled. Granules (*Gr.*) with characteristic linear patterns may be seen appearing freely in the cytoplasm or attached to the outer surfaces of the membrane enclosed vesicles (*Ves.*) of the ergastoplasm. The vesicles at day 6 to 7 have characteristically begun to separate, enlarge, and gather a material of moderate density within. × 54,000.



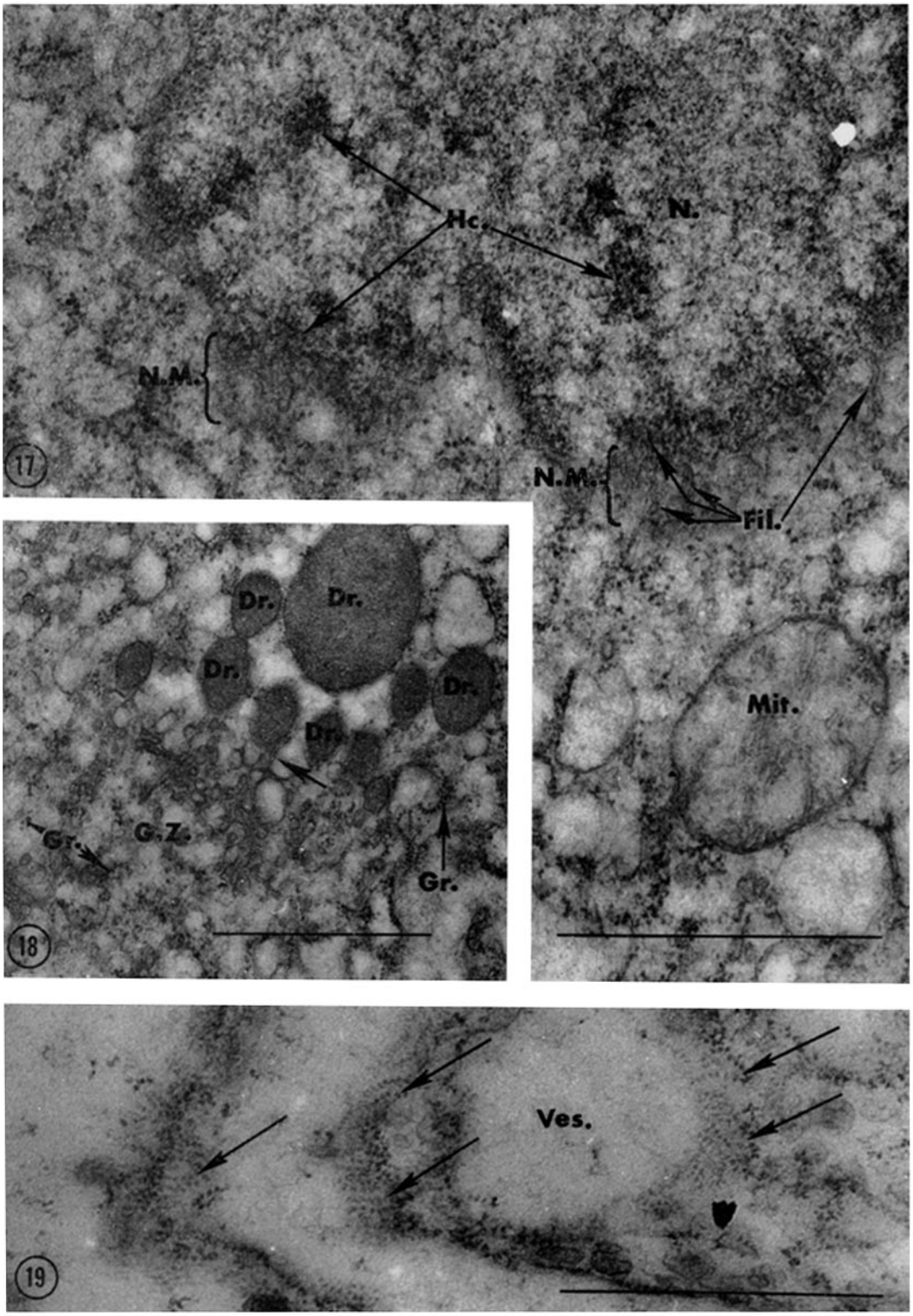

(Herman: Salamander thyroid during hormonal stimulation)

PLATE 72

FIG. 17. *A. tigrinum* thyroid: Received 0.1 mg. TSH daily for 6 days. The section was stained for 5 minutes in a solution of lead acetate and sodium hydroxide. The nucleoprotein granules of the cytoplasm and the heterochromatin (*HC.*) are more intensely stained. The nucleus has been cut obliquely so that the nuclear membrane (*N.M.*), as viewed here, probably exists between the area indicated by the brackets. Within such obliquely sectioned areas of the membrane may be seen parts of filamentous strands (*Fil.*) that appear between nucleus and cytoplasm. Occasionally these strands seem to be associated with nucleoprotein granules on the cytoplasmic side and heterochromatic regions at the nuclear end. × 51,000.

FIG. 18. *A. tigrinum* thyroid: Received 0.1 mg. TSH daily for 6 days. Variously sized homogeneous droplets or granules (*Dr.*) are seen in close association with the Golgi zone (*G. Z.*). A Golgi membrane (unmarked arrow) seems to be intimately associated with a small droplet. Such close association suggests the possible formation of such structures within Golgi vesicles. × 34,000.

FIG. 19. *A. tigrinum* thyroid: Received 0.1 mg. TSH daily for 10 days. Several of the ergastoplasmic membranes have been cut obliquely or "grazed" by the knife. This reveals the patterned array of 10 to 15 mμ granules (arrows) that are associated with the outer surfaces of these cytoplasmic structures. × 51,000.




(Herman: Salamander thyroid during hormonal stimulation)