

ELECTRON MICROSCOPIC OBSERVATIONS OF THE CYST OF ULTIMOBRANCHIAL ORIGIN FOUND IN THE THYROID GLAND OF A DOG

MASAHARU TASHIRO

*Department of Anatomy and 1st Department of Surgery Nagoya University School
of Medicine (Directors: Prof. Shooichi Sugiyama and Prof. Yoshio Hashimoto)*

The investigations of ultimobranchial tissue still remain to be some contradictory questions on its fate and function. Some investigators insist the contribution of ultimobranchial tissue to formation of thyroid tissue and some others deny it. Autoradiographic studies using radioiodine (Gorbman, '47) elucidated a fact in mice that the follicles of ultimobranchial origin are not able to collect I^{131} and are physiologically different from the thyroid follicles. On the other hand, the concept of transformation of ultimobranchial tissue into thyroid tissue has been supported by studies of alkaline glycerophosphatase activity of branchiogeneous endocrine organs in rats (McAlpine, '55). It has been observed that, when incorporated into the developing thyroid gland, the ultimobranchial tissue is indistinguishably transformed into thyroid-like follicles which appear to function as such during periods of thyroid activity (van Dyke, '58). Furthermore, parafollicular cells (interfollicular cells) found in the mammalian thyroid glands have been recently described to be derived from residual ultimobranchial tissue (Godwin, '36/37, in dogs; van Dyke, '45, in sheep; Sato, '59, in hamsters).

In the electron microscopic study of this article, special attention is paid to (1) the nature of the cytoplasmic components in ultimobranchial tissue, (2) the cytological evidence of glandular activity and (3) the possibility or non-possibility of the same function as that found in thyroid tissue.

MATERIAL AND METHODS

The thyroid gland of a three years old male dog was removed immediately after sacrifice by a sharp blow, cut into small pieces and immersed in 1% osmium tetroxide solution buffered with veronal acetate to pH 7.4 for one hour at 0°C. Following the fixation the blocks of tissue were dehydrated through ethanol and embedded in Epoxy Resin. Sectioning was made with an ultramicrotome, JUM 5 Type. Thin sections on silica-coated grid were stained with a saturated solution of uranyl acetate for three hours. Samples were examined in an electron microscope, Model JEM-5 G Type. Electron micrographs were taken at original magnifications of 3 000 to 7 000 and thereafter photographically enlarged.

OBSERVATIONS

Light microscopic observations showed that the ultimobranchial cyst consisted of simple and stratified epithelia and contained faintly eosinophilic granular substance. Superficial epithelial cells were cubical to columnar and some of them were ciliated. Underlying epithelial cells were polygonal. This held almost the same in electron micrographs and the following interesting findings were further observed.

Superficial epithelial cells had many short and plump microvilli. Some of them had numerous cilia together with microvilli. Microvilli found in these ciliated cells were far longer than usual ones and sometimes branched. Two or more microvilli arose from the same base (Figs. 1 and 3). The cilia arose from the basal corpuscles in the apical zone and showed no characteristic pictures different from those found in the cells of the other organs (Figs. 1 and 3).

The nuclei were as well very irregular-shaped and appeared multilobulated like those of neutrophilic leucocytes, but were sometimes regularly oval (Fig. 3). They were located almost centrically or slightly towards the base of the cell body. The nuclear envelope consisted of double membranes (Figs. 1 to 5). The nucleoplasm was moderately electron dense. The nucleolus was prominent (Fig. 5).

Endoplasmic reticulum was poorly developed and found in small numbers. They appeared usually as small vesicles associated with Palade's ribonucleoprotein granules, and rarely as a few slightly arched or branched filaments consisting of membranes associated with the same granules. They were not dilated to cisterns or lakes and contained no visible materials.

The mitochondria showed round, shortly oval or sometimes rod-shaped profiles and were distributed at random throughout the cell body (Figs. 1 to 5). Cristae mitochondriales were in general orderly arranged and perpendicular to the long axis of the mitochondria. The mitochondria showed no suggestive relation to the endoplasmic reticulum.

The Golgi zone consisted of closely arranged, smooth membranes and smooth round vesicles (Figs. 2 and 5). Some of the membranes and vesicles were electron dense and suggested transitions to secretory granules.

The ground substance of the cell body was moderately dense in underlying epithelial cells but less dense in superficial ones. The density was due to two components, (1) dense granules comparable in appearance to ribonucleoprotein granules and (2) a fine stippling. The former were found accumulated in groups and distributed in the form of islets. Secretory granules were found in small numbers in superficial epithelial cells. The secretory granules appeared at first in the Golgi zone, grew moving towards the apical zone and faded slightly in density. They reached the plasma membrane of the apical margin of the cell, which was found among the microvilli, and finally they opened to the cavity to release their content. Besides these components of the ground substance, amorphous granules were sometimes found accumulated in superficial epithelial cells and were not stained with uranyl acetate (Figs. 4 and 5). No characteristic feature to be suggestive of microapocrinal secretion was found in superficial epithelial cells.

The intercellular spaces were narrow and bounded by two plasma mem-

branes of adjacent cells. In the superficial zone of the cyst wall terminal bars were well developed and in the deeper zone desmosomes were well-formed. Plasma membranes were seen often forming peg-like interdigitations with membranes of adjacent epithelial cells and the interdigitations were more complicated at angles where more than two epithelial cells were in contact. The basement membranes found in underlying epithelial cells were composed of linear amorphous material of moderate electron density and collagen fibrilles (Fig. 1). Desmosomes were frequent here (Fig. 1).

DISCUSSION

Electron microscopic observations of follicle cells of the thyroid gland in dogs have been made by Tashiro ('62). Many of the follicle cells of dogs have thin and delicate microvilli in the apical margin, regularly round or oval nuclei, many rod-shaped mitochondria, numerous secretory granules and well-developed rough-surfaced endoplasmic reticulums. This finding was also confirmed in the thyroid glands of lower vertebrates by Muramoto ('62), and is in sharp contrast with the present finding of ultimobranchial cells. Most of the ultimobranchial cells have very irregular-shaped nuclei, round or short oval mitochondria, small numbers of poorly developed rough-surfaced endoplasmic reticulums and a few secretory granules (Figs. 1 to 5). The rough-surfaced endoplasmic reticulums appear as small numbers of vesicles and rarely as a few filamentous structures (Fig. 3). This may suggest that ultimobranchial cells are different in function from thyroid follicle cells.

Electron microscopic observations of parafollicular cells of the thyroid gland in dogs have been made by Tashiro ('62). According to him, the parafollicular cells have somewhat irregular-shaped nuclei, short mitochondria, small numbers of secretory granules and poorly developed rough-surfaced endoplasmic reticulums. The endoplasmic reticulums are similar in appearance to those of ultimobranchial cells, and are usually small vesicles but rarely a few filaments. This may support the concept of derivation of parafollicular cells from ultimobranchial tissue (Godwin, '36/37, van Dyke '45, Sato, '59). On the other hand, the parafollicular cells have been reported to be residuals of embryonic thyroid cells (Bozzi, 1895) or to be of embryonic nature (Wölfler, 1880). Fujita *et al.* ('62) described in embryonic chicks that endoplasmic reticulums of thyroid cells are not distinct and appear as small vesicles.

Amorphous granules found in the superficial epithelial cells of the ultimobranchial cyst are of unknown origin and nature (Fig. 5). Machida *et al.* ('62) found glycogen in ultimobranchial cysts of embryonic guinea pigs. The experiments to determine whether the amorphous granules correspond with the glycogen or not were not carried out in this study.

SUMMARY

The cyst of ultimobranchial origin in the thyroid gland of a dog was studied by the use of the electron microscope and the following result was obtained.

The epithelial cells of the ultimobranchial cyst were different in appearance from thyroid follicle cells in the following points—multilobulated nuclei, short mitochondria, a few secretory granules and poorly developed rough-surfaced endoplasmic reticulums. The rough-surfaced endoplasmic reticulums appeared usually as a number of small vesicles and rarely as a few filaments, and were characteristic of the ultimobranchial cells.

In superficial epithelial cells short and plump microvilli were seen, and in some of them cilia were found together with long microvilli.

The possibility and non-possibility of the same function as that found in thyroid follicle cells and those of development of parafollicular cells from ultimobranchial cells were discussed.

REFERENCES

1. BOZZI, E. *Beitr. path. Anat.* **18**: 125, 1895.
2. FUJITA, H., M. MACHINO AND S. NAGATA. *Fol. Endocrin. Jap.* **38**: 725, 1962.
3. GODWIN, M. C. *Am. J. Anat.* **60**: 299, 1936/37.
4. GORBMAN, A. *Anat. Rec.* **98**: 93, 1947.
5. MACHIDA, Y. AND S. SUGIYAMA. *Fol. anat. Jap.* **38**: 73, 1962.
6. MCALPINE, R. J. *Am. J. Anat.* **96**: 191, 1955.
7. MURAMOTO, K. *Acta. Anat. Nipp.* **37**: 63, 1962 (Abstract).
8. SATO, T. *Fol. Anat. Jap.* **33**: 225, 1959.
9. TASHIRO, M. *Acta. Anat. Nipp.* **37**: 52, 1962 (Abstract).
10. TASHIRO, M. *Acta. Anat. Nipp.* **37**: 63, 1962 (Abstract).
11. VAN DYKE, J. H. *Am. J. Anat.* **76**: 201, 1945.
12. VAN DYKE, J. H. *The ultimobranchial body. Gorbman's Comparative Endocrinology*, 320. New York: John Wiley and Sons, Inc., 1958.
13. WÖLFLE, A. Über die Entwicklung und den Bau der Schilddrüse mit Rücksicht auf die Entwicklung der Kröpfe. Berlin, 1880 (cited from C. Wegelin: Schilddrüse, Henke-Lubarsch: *Handb. spez. path. Anat. u. Hist.*, 8: 1, Berlin: Julius Springer, 1926).

EXPLANATION OF FIGURES

- Am: Amorphous granules
- Bc: Basal corpuscle
- Bm: Basement membrane
- C: Cilia
- Ca: Cavity of an ultimobranchial cyst
- D: Desmosome
- Er: Rough-surfaced endoplasmic reticulum
- G: Secretory granule
- Gol: Golgi zone
- M: Mitochondria
- mv: Microvilli
- N: Nucleus
- n: Nucleolus
- T: Terminal bar

FIG. 1. A ciliated cell. The ciliated cell has two kinds of process, cilia and microvilli. The microvilli are longer and often branched. Rough-surfaced endoplasmic reticulums are poorly developed. $\times 21\,000$.

- FIG. 2. An ordinary superficial epithelial cell. Microvilli are short and plump. In the Golgi zone some of vesicles and membranes are seen containing electron dense material. Mitochondria show round and oval or sometimes rod-shaped profiles. Cristae mitochondriales are in general orderly arranged. Rough-surfaced endoplasmic reticulums are poorly developed. $\times 15\ 000$.
- FIG. 3. A ciliated cell and three ordinary superficial epithelial cells. Rough-surfaced endoplasmic reticulums are poorly developed and appear as small vesicles and filaments. Nuclei are irregular-shaped. $\times 12\ 000$.
- FIG. 4 and FIG. 5. Secretory granules with a distinct limiting membrane leave the Golgi zone and are moving towards the apical margin fading in electron density, and some of them reach completely the apical plasma membrane found among microvilli. Amorphous granules are seen. $\times 15\ 000$.

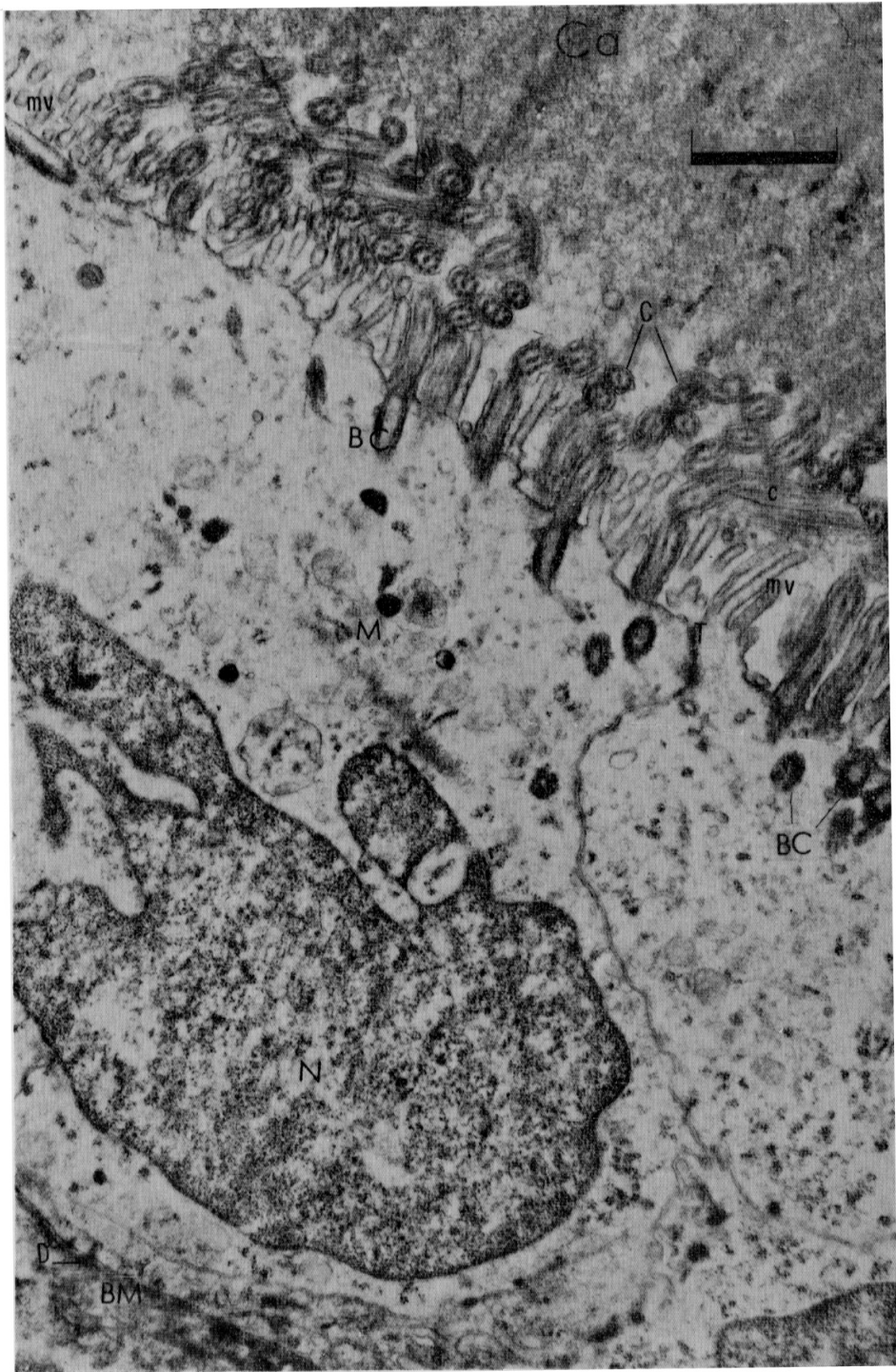


FIG. 1

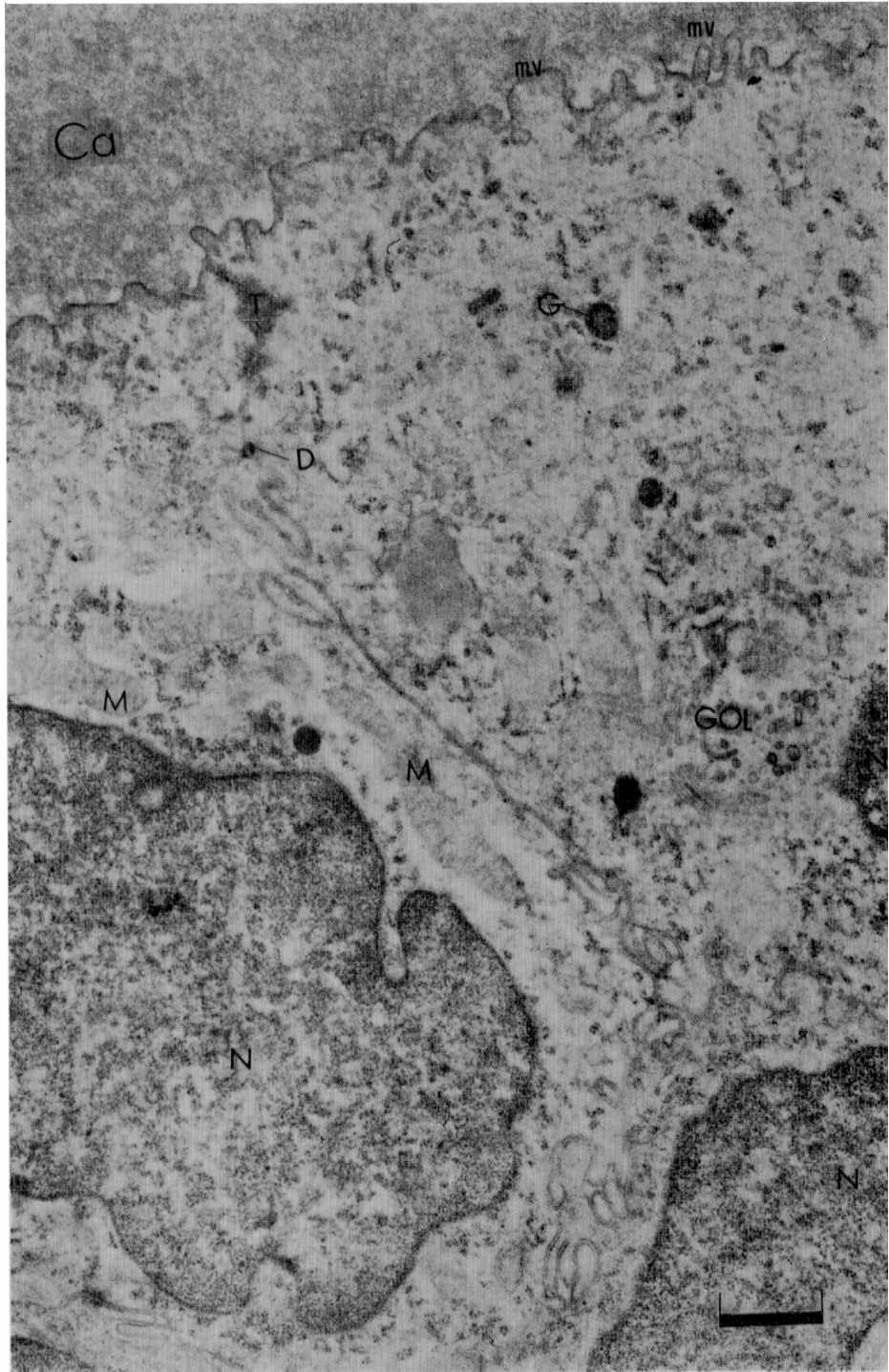


FIG. 2

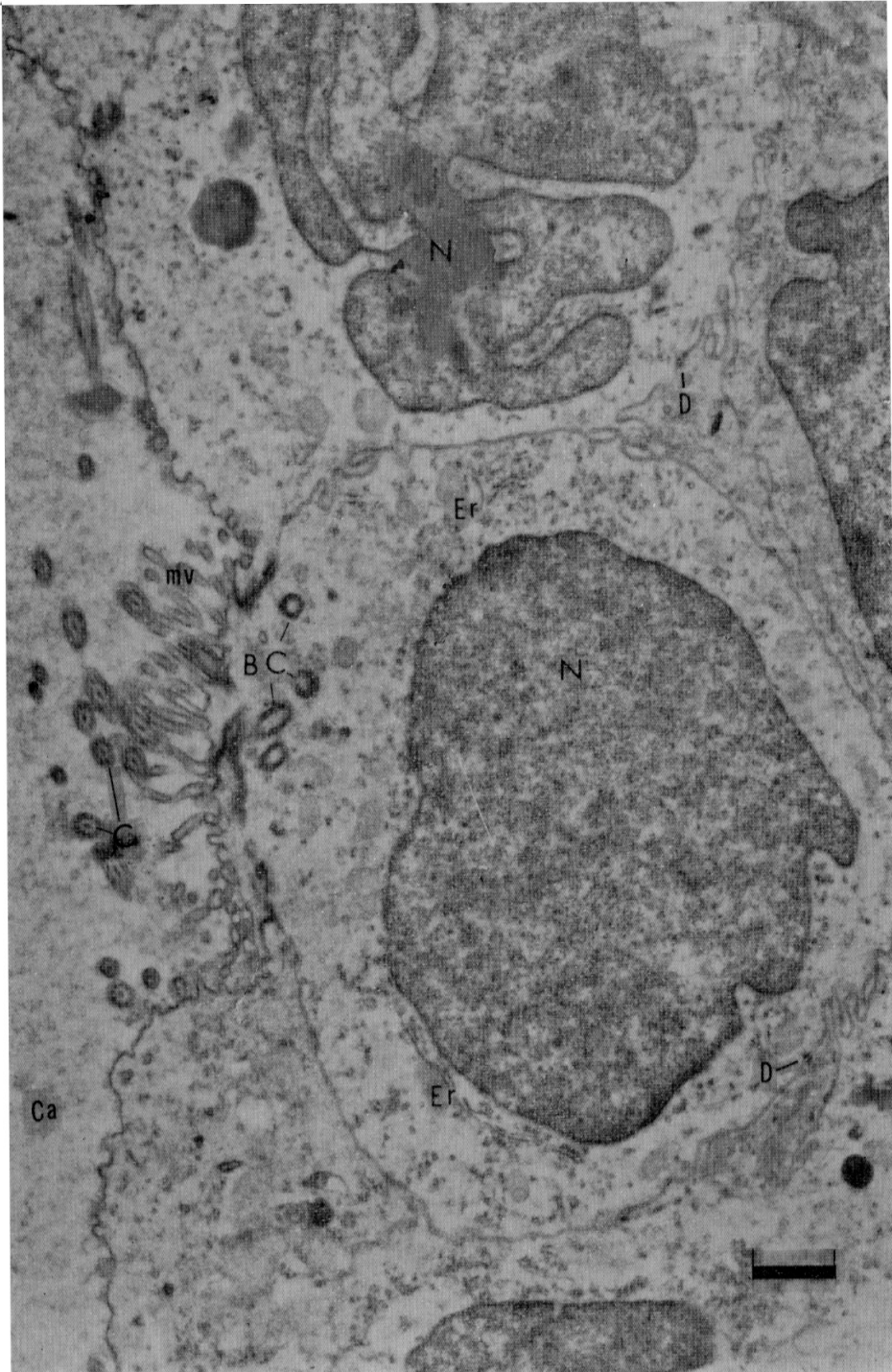


FIG. 3

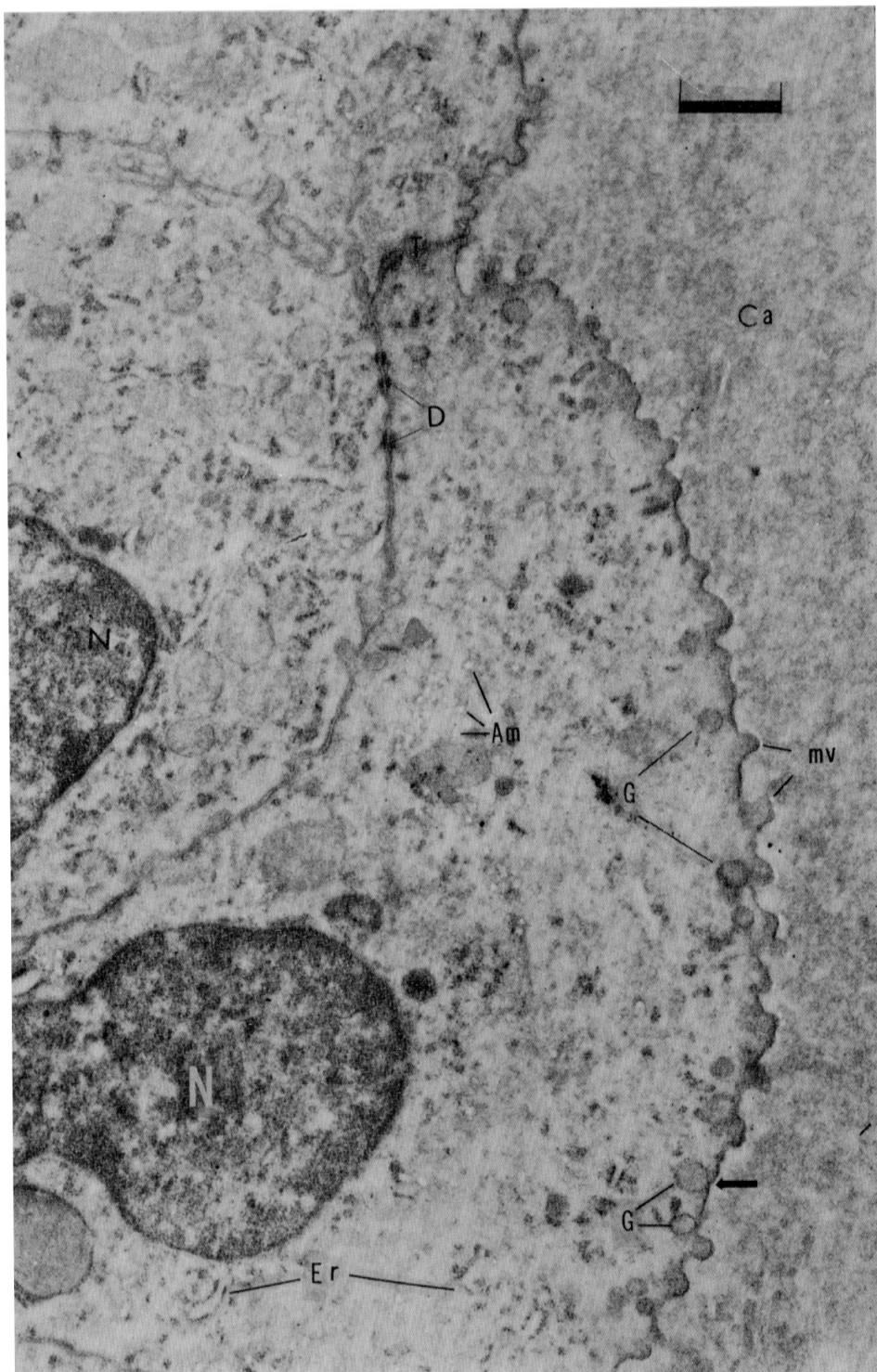


FIG. 4

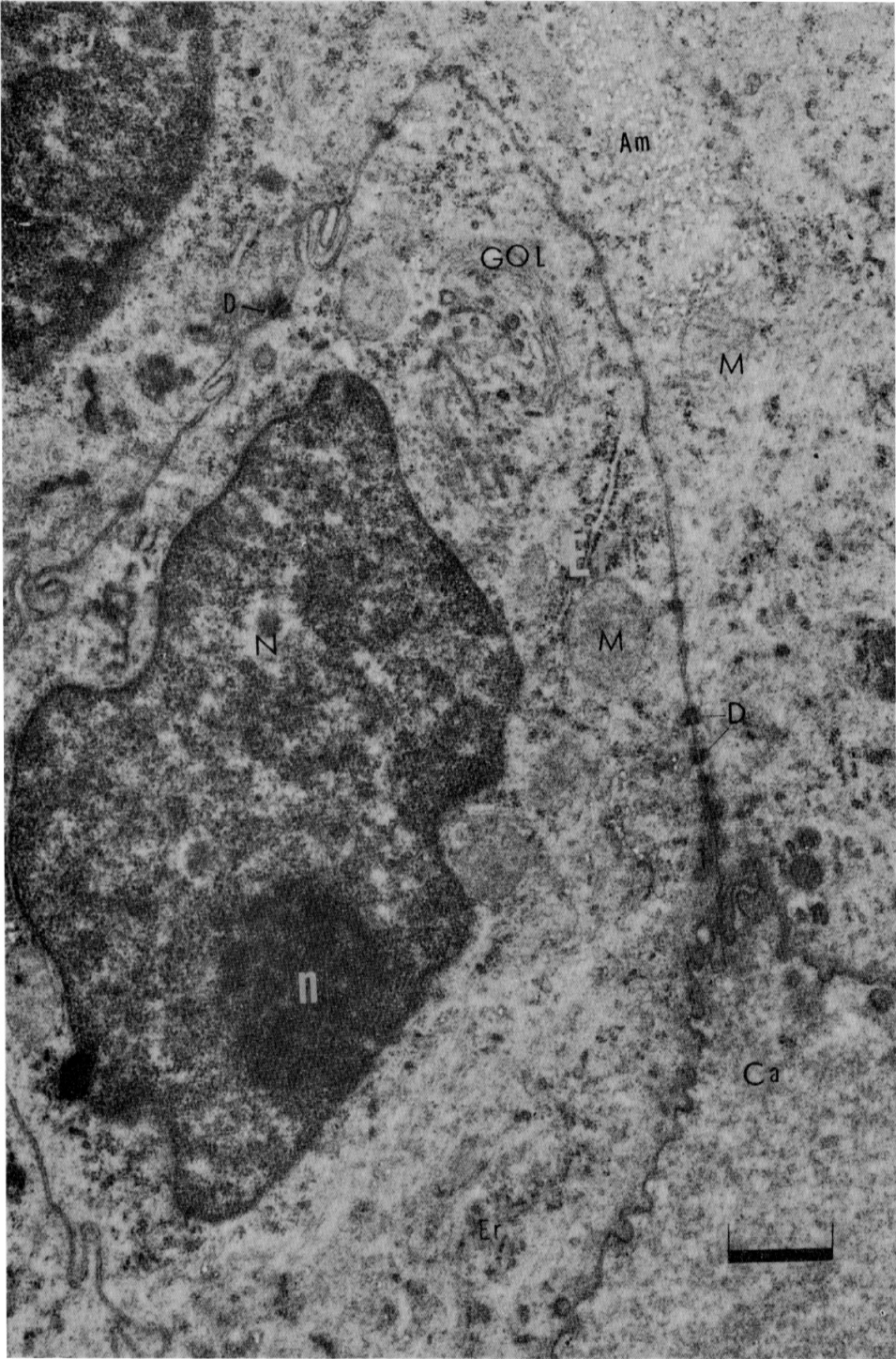


FIG. 5