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THE CYTOPHYSIOLOGY OF THE ANTERIOR PITUITARY GLAND*

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IT IS KNOWN that the anterior lobe of the pituitary gland is responsible for secreting six of the nine pituitary hormones: These are somatotrophin (STH or growth hormone), corticotrophin (ACTH), thyrotrophin (TSH), prolactin (lactogenic hormone or luteotrophin), follicle-stimulating hormone (FSH), and luteinizing hormone (LH, or interstitial cell-stimulating hormone). One of the intriguing problems to the cytologist is to relate the secretion of a hormone (or hormones) to a specific cell type in the pars distalis. The evidence from studies of the higher vertebrate pituitary appears to favor the hypothesis that each cell type secretes one particular hormone.[1] However, information has been presented on the amphibian pituitary gland which indicates that more than one hormone may be elaborated by a single cell type.[2-4] It is expected that more studies on the comparative cytophysiology of the pituitary will be rewarding in gaining an understanding of the cytology of this gland.

The extensive literature on the light microscopy of the pituitary gland will not be reviewed in this lecture, but a brief description of the cell types in the rat is necessary. It was recognized early that the cell types of the anterior pituitary could be divided into chromophils and chromophobes by utilizing routine stain, such as hematoxylin and eosin, or with Mallory aniline blue or azan. The chromophils could be further divided into acidophils and basophils on the basis of their tinctorial response to acidic and basic dyes, respectively. It is assumed generally that the secretory granules of the cells are responsible for the specific staining reaction.

Purves and Griesbach[5] utilizing the periodic acid-Schiff technique and Gomori's aldehyde-fuchsin stain, were able to distinguish the thyrotrophs as PAS-positive cells which stained with aldehyde fuchsin and the gonadotrophs as PAS-positive cells which did not stain with AF. These workers also divided the gonadotrophs into cell types which were believed to secrete FSH and LH. The secretion of growth hormone (STH) has been related to the orange G. acidophil and in some species the secretion of prolactin has been related to an acidophil which stains with azocarmine. The staining properties of the corticotroph is unclear.

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The first electron micrographs of pituitary cells were published by Fernandez-Moran and Luft[6] but their studies were hampered by lack of refined ultrathin sectioning techniques. Nevertheless, these workers were able to present some information on the secretory granules of the cells.

Farquhar and her colleagues[7-9] reported their studies on the normal rat anterior pituitary gland and various experimentally altered glands utilizing techniques of ultrathin sectioning for electron microscopy. They demonstrated that it was possible to distinguish the cell types of the rat pituitary on the basis of the size and density of the secretory granules per cell. Thus, the thyrotrophs were classified as having 140 mu granules; somatotrophs, 360 mu; gonadotrophs, 200 mu granules; and luteotrophs, 600 mu.

Pituitary glands from other animals have been investigated and it is possible to separate the cell types in these forms also.[2-4,10-14] Table I is a summary of these results and it is obvious from this table that the size of the granules associated with a particular cell type varies from species to species.

Up to this point considerable emphasis has been placed on the secretory granules and it has been assumed that the secretory granules contain the hormone of the particular cell under observation. Recently, definitive evidence has been presented by Hartley, *et al*[15] that this is a correct assumption. These workers homogenized rat pituitary glands and by differential centrifugation isolated a fraction which contained only 200 mu granules. The hormonal content of this fraction was assayed and it was found to possess the major portion of the gonadotrophic activity. It is reasonable to believe, therefore, that conclusions based on observations of the formation and release of secretory granules may also be pertinent to hormone synthesis and release. This will be discussed in more detail later in the presentation.

Figure 1 is a survey electronmicrograph of secretory cells from the salamander pituitary gland and illustrates the differences in the various cell types. Without going into the details of the various cell types, it is obvious that there are several differences between these cells. The size and shape of the cells vary considerably as do that of the nuclei. These differences are difficult to interpret because of the different planes through which a thin section (less than 500 A thick) may pass, thereby producing different-sized and shaped nuclei and cells. These differences are not used as a primary criterion for differentiating the cell types. Some cells have more secretory granules than others and there is variation in other cellular organelles. These are believed to reflect differences in the secretory cytology of the cells (see later discussion) and are not used to separate the cell types. However, the characteristics of the secretory granules within a single cell type are very constant and, as shown in figure 1, there is considerable difference between the secretory granules of one cell type from those of another. Note the three cells in the center of the micrograph (Figure 1): There is an obvious difference in the size of the granules per cell and the cell in the center has more dense-appearing granules. The difference in the density of the secretory granules is illustrated in figure 2. The dense-appearing granules are characteristic of the acidophilic cell types, whereas the less dense granules are associated with basophils (cell in lower part of figure 2).

ANTERIOR PITUITARY GLAND

Figure 3 is an electron micrograph of a cell type in the salamander pituitary which has two distinct sizes of secretory granules. It has been proposed that this cell type may secrete two hormones.[3] The large granules in this cell are highly variable in size and occasionally two or more appear fused (Figure 3).

The general ultrastructural organization of the secretory cell is similar to that of other cells. The cell contains a nucleus which is bounded by a double nuclear envelope consisting of the inner nuclear membrane and the outer nuclear membrane (Figures 4 and 5). The outer nuclear membrane has ribonucleic protein particles (RNP) attached to it and the membrane is continuous with the elements of the endoplasmic reticulum (ER). The nucleus is generally uniformly granular with one or more nucleoli per nucleus. The ER appears tubular and vesicular and has RNP particles. Mitochondria of the oblong variety are present in the cells and they have a smooth outer membrane and highly infolded inner membrane which forms the cristae of the mitochondrion. Usually the Golgi complex occurs in the juxtanuclear position and is composed of flattened sacs, vacuoles, and vesicles characteristic of this organelle.[16] Frequently secretory granules are found within the Golgi complex in varying sizes up to the maximum diameter of the granules present elsewhere in the cell under observation (Figure 5). This morphological evidence has been interpreted to mean that the Golgi zone is the organelle within the cell responsible for the formation of the secretory granules.[1,4,6] This does not necessarily imply that the hormone is actually synthesized within the Golgi zone but rather that the Golgi complex acts as a "condensation center" for the formation of the granules.

The cell is bounded by the plasma membrane which is double and each cell is separated from its neighbor by a small intercellular space.

A question which has intrigued endocrinologists for many years is, "How is the hormone released from the cells and placed in the blood stream?" To the cytologist this means following the release of the secretory granules from the cell and discovering their fate in the capillaries. This requires information on the ultrastructure of the capillary and the relationship of the secretory cell to the capillary. The first electron micrographs of the capillary organization were published by Rinehart and Farquhar[17] on the rat pituitary and since then the capillary organization in the pituitary has been studied by several other workers. These results shall be illustrated with electron micrographs of capillaries from the salamander pituitary gland and from a human pituitory tumor.*

The capillary consists of endothelial cells which unite to form a completely enclosed vessel (Figures 6 and 7). The endothelial cell varies in thickness from several micra in the region of the nucleus (Figure 7) to a very thin layer less than 500 A (Figure 10). Fenestrations occur frequently in the endothelial cell (Figure 6) and the endothelial membranes continue across the fenestrations (Figures 9 and 10). Surrounding the capillary and extending between the secretory cells is a space

*This work represents a portion of a study on the ultrastructure of human pituitary tumors with Dr. R. J. Knighton and will be published in detail later. A preliminary report has been given.[18]

(Figures 6-10) which has a basement membrane adjacent and closely opposed to the endothelial cells of the capillary and a similar basement membrane adjacent to the secretory cells. This space, which has been termed the pericapillary space, contains many fibrils and frequently pericapillary cells or pericytes are seen (Figure 10). The secretory cells have an intimate relationship to the pericapillary space (Figure 10) and from the abundance of capillaries in the pars distalis it is very probable that each secretory cell borders a pericapillary space in one or more places.

Figure 11 is a diagramatic summary of the structure of the capillary and pericapillary organization and shows the relationship of the secretory cells to the capillary.

The release of the granules from the secretory cells has been studied by several workers and many proposals have been made for the mechanism of placing the hormone in the blood stream. Farquhar[1] has published the most convincing electronmicrographs of the release of granules from the cell and her results will be described. As the mature secretory granule approaches the cell membrane, the granule membrane becomes less tightly bound to the granule. The granule membrane breaks and fuses with a similar break in the plasma membrane of the secretory cell thereby placing the contents of the granule outside of the secretory cell and in the pericapillary space (Figure 12). The granule dissolves while within the pericapillary space and the hormone is released. The hormone diffuses through the endothelial cell into the lumen of the capillary.

Figure 12 is a drawing which illustrates the ultrastructure of a pituitary secretory cell and shows the stages in the formation and release of the secretory granules.

Finally, some consideration to the secretory cycle of the secretory cell will be given. If one studies a large number of electronmicrographs from secretory cells in the pars distalis it is apparent that different cells of the same cell type exhibit variation in their ultrastructure. For example, some cells are almost filled with secretory granules whereas others are practically devoid of these granules. It is believed that this variation is the cytological expression of a cell in a different phase of the secretory cycle. An understanding of the secretory cycle of an individual cell is difficult to obtain because the cells are not phased in such a way as to facilitate this study. In other words, at a given time each cell may be in a different phase of the secretory cycle; and in view of the fact that the anterior pituitary is secreting six different hormones one can readily appreciate the complexity of trying to analyze the secretory phase of a particular cell.

It is possible to stimulate the pituitary to secrete a specific hormone by experimentally altering the balance of hormones. Thus, if an animal is thyroidectomized the level of thyroxin in the blood falls and the thyrotrophs of the pituitary are stimulated to secrete more thyroid stimulating hormone (TSH). It was reasoned that a study of the ultrastructure of salamander pituitary glands from a thyroidectomized animal might throw some light on the secretory cycle of the thyrotrophs.

Early thyroidectomy changes in the salamander pituitary are difficult to interpret because few cells are affected and with the small area one can study in an electron microscope these changes are hard to find. However, forty-five to sixty days after the thyroids are removed from an animal the pituitary cells show striking changes which are believed to reflect increased hormone production and release. Figures 13 and 14 illustrate the changes which occur in the thyroidectomy cells. The most obvious change seen with the light microscope is an enlargement of the cell. The cell loses almost all of the secretory granules and granules are not seen forming in the Golgi complex (Figure 13). The rough and smooth ER are much more abundant and prominent in the cell. The rough ER appears to first align in parallel arrays, then the cisternae enlarge, and finally vesiculation of the ER occurs (Figure 14). The ER has an abundance of RNP particles attached to the outer surface of the ER. There is a striking increase in the number and size of the mitochondria. Golgi material is scattered throughout the cytosome and is much more abundant. This occurs despite the fact that the cell is not forming secretory granules.

Figure 15 is a diagramatic summary of the pituitary secretory cell cycle in the salamander pituitary gland and a similar secretory cycle is probably present in other pituitary glands. The first reaction of the cell to the stimulus to secrete hormone is the release of secretory granules. This may trigger the synthesis of additional hormone which is indicated in the electronmicrographs by an increase in the rough ER. The variations in the number and size of the mitochondria per cell is related to the increased demands on the cell for energy production in protein synthesis. The increase in the prominence of the Golgi complex is easily understood during the time when the cell is forming granules. However, when the cell is apparently not forming secretory granules the increased amount of Golgi material is difficult to explain. Perhaps, the Golgi zone is involved in the actual synthesis of the hormone in addition to its role in the formation of secretory granules.

In summary, an attempt has been made to present a general review of the cytophysiology of the anterior pituitary gland with primary emphasis on the ultrastructure of the cell types. No attempt has been made to review exhaustively the literature and in certain unsettled areas all points of view have not been presented. In the same manner many details have been ommitted for the sake of brevity and clarity of presentation.

Abbreviations

Aldehyde-fuchsin	— AF	Nuclear envelope	— NE
Capillary lumen	— CL	Nucleus	— N
Corticotrophin	— ACTH	Pericapillary Cell	— PC
Endothelial cell	— EC	Pericapillary Space	— PS
Fenestration	— F	Rough endoplasmic reticulum	— RER
Follicle-stimulating hormone	— FSH	Secretory cell	— SC
Golgi Apparatus	— GA	Secretory granule	— SG
Luteinizing hormone	— LH	Somatotrophin	— STH
Luteotrophin	— LTH	Smooth endoplasmic reticulum	— SER
Mitochondria	— M	Thyrotrophin	— TSH

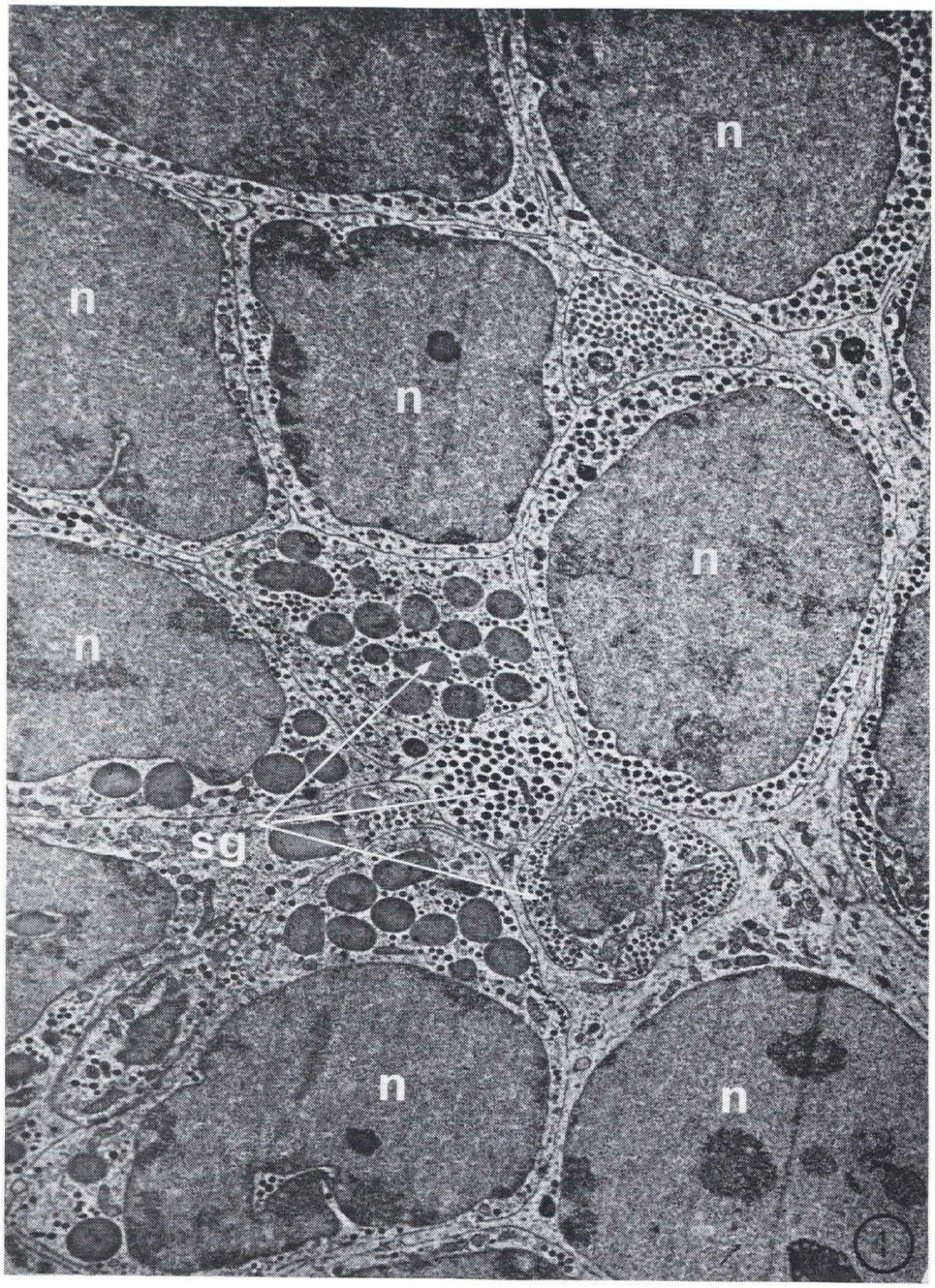

Figure 1

A survey electronmicrograph of pituitary secretory cells from the pars distalis of the salamander pituitary gland. Magnification 5,000X.

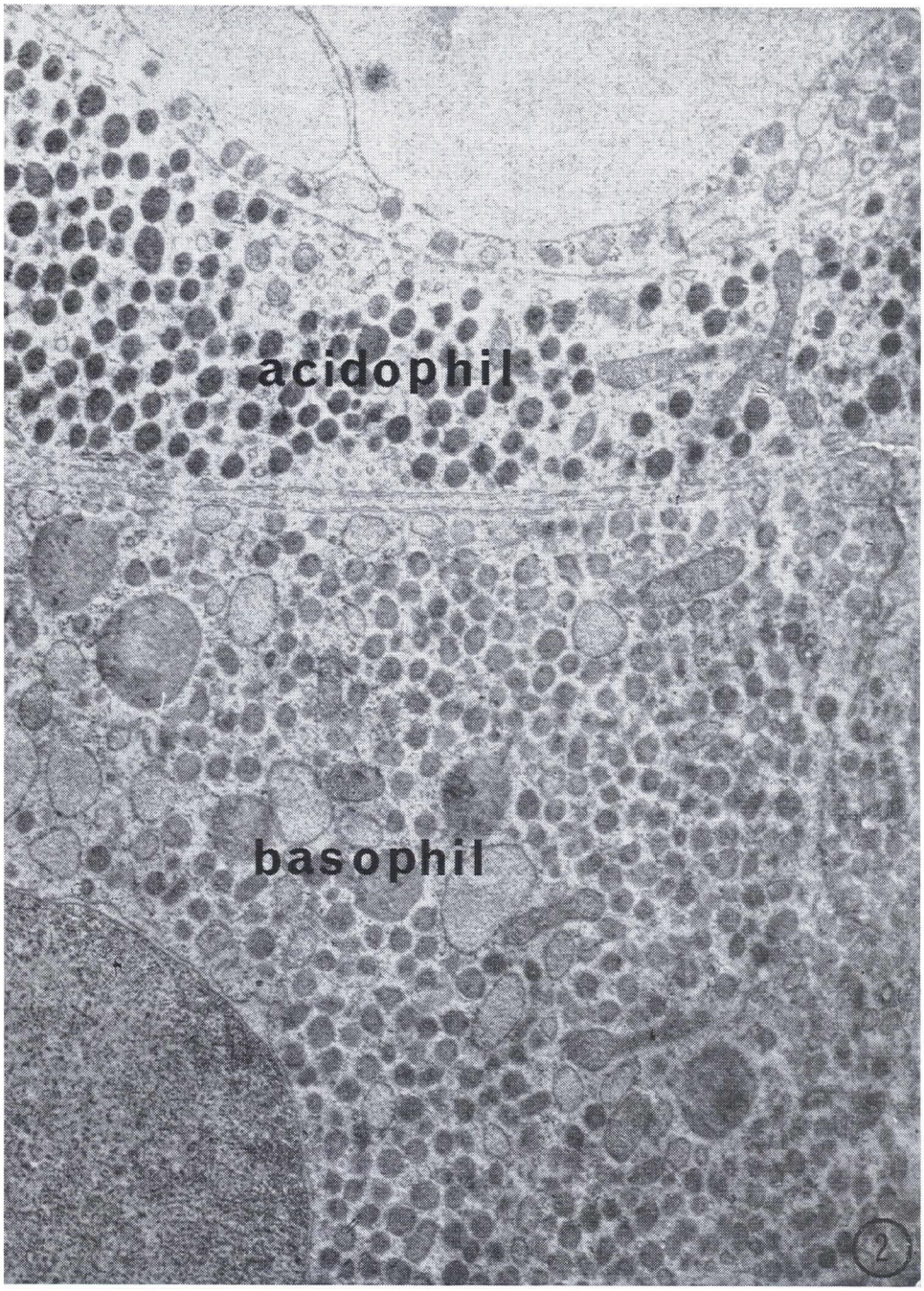

Figure 2

An electronmicrograph which illustrates the difference between the acidophilic and basophilic cell types of the anterior pituitary gland. Magnification 21,000X.

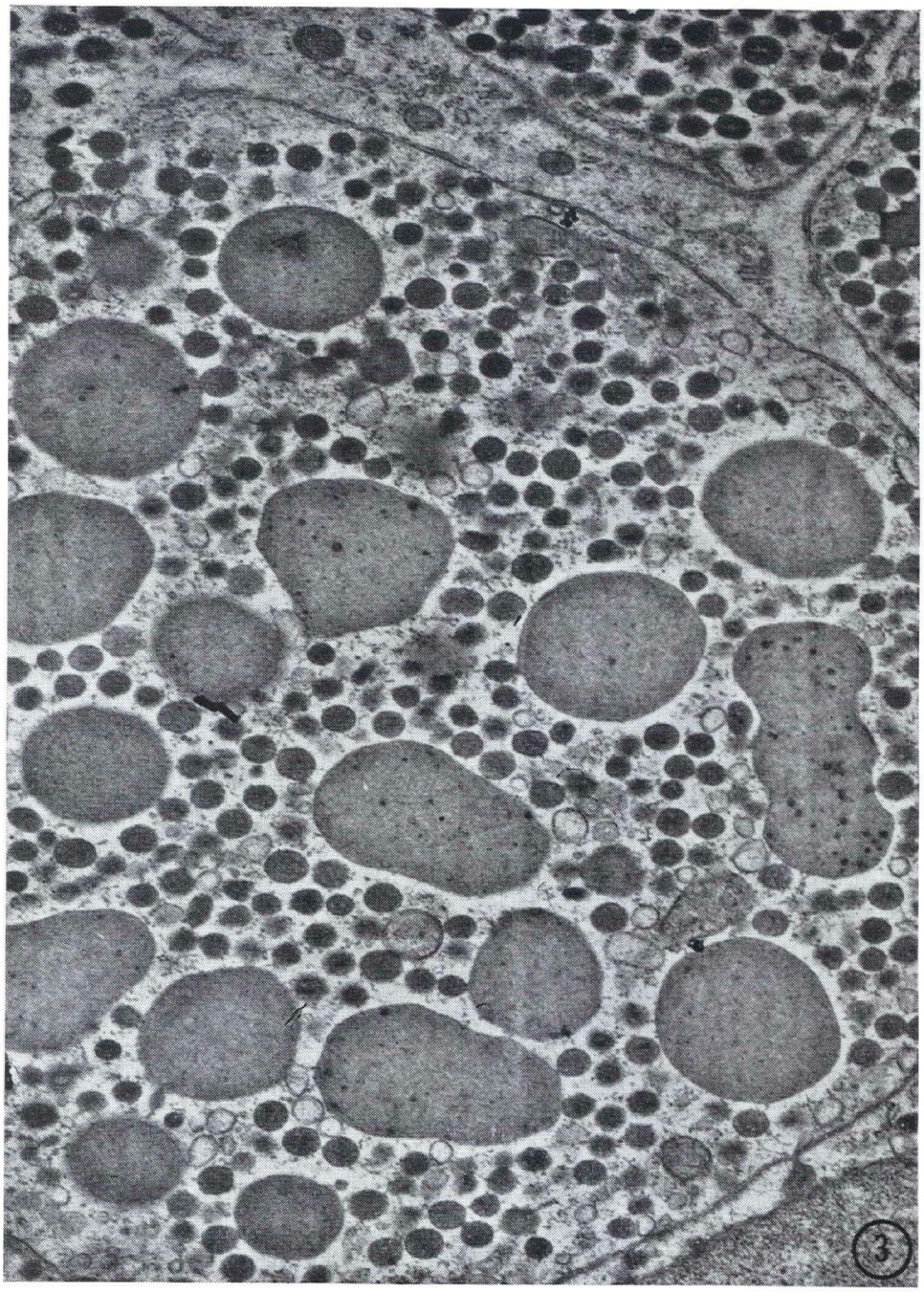

Figure 3

A secretory cell from the salamander pituitary which contains two classes of secretory granules. Magnification 21,000X.

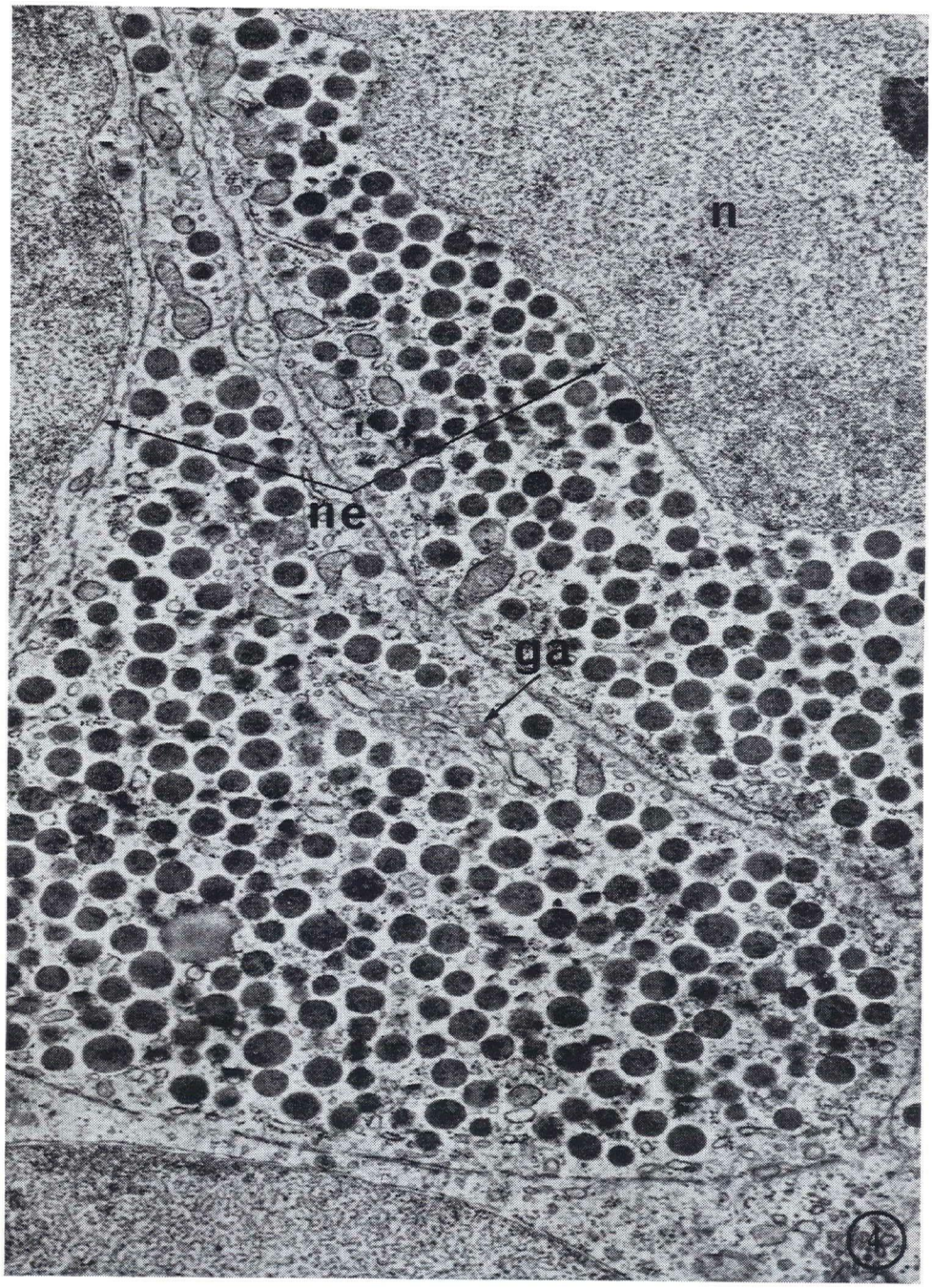

Figure 4

Two acidophilic cell types with a Golgi complex (ga) located in the cytoplasm of one of the cells. Magnification 19,000X.

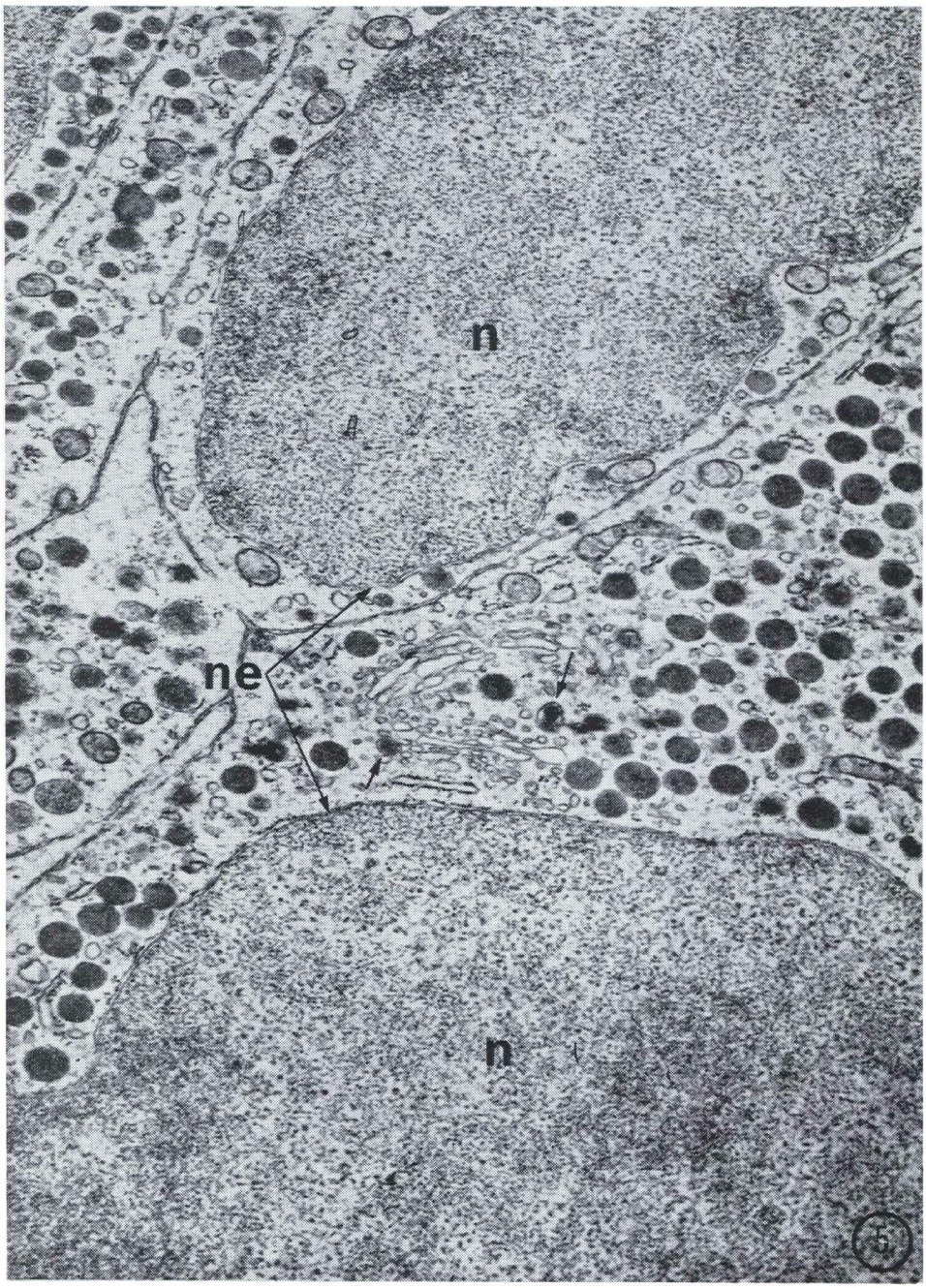

Figure 5

An electronmicrograph showing the Golgi complex with secretory granules (arrows) within the Golgi vacuoles. Magnification 20,000X.

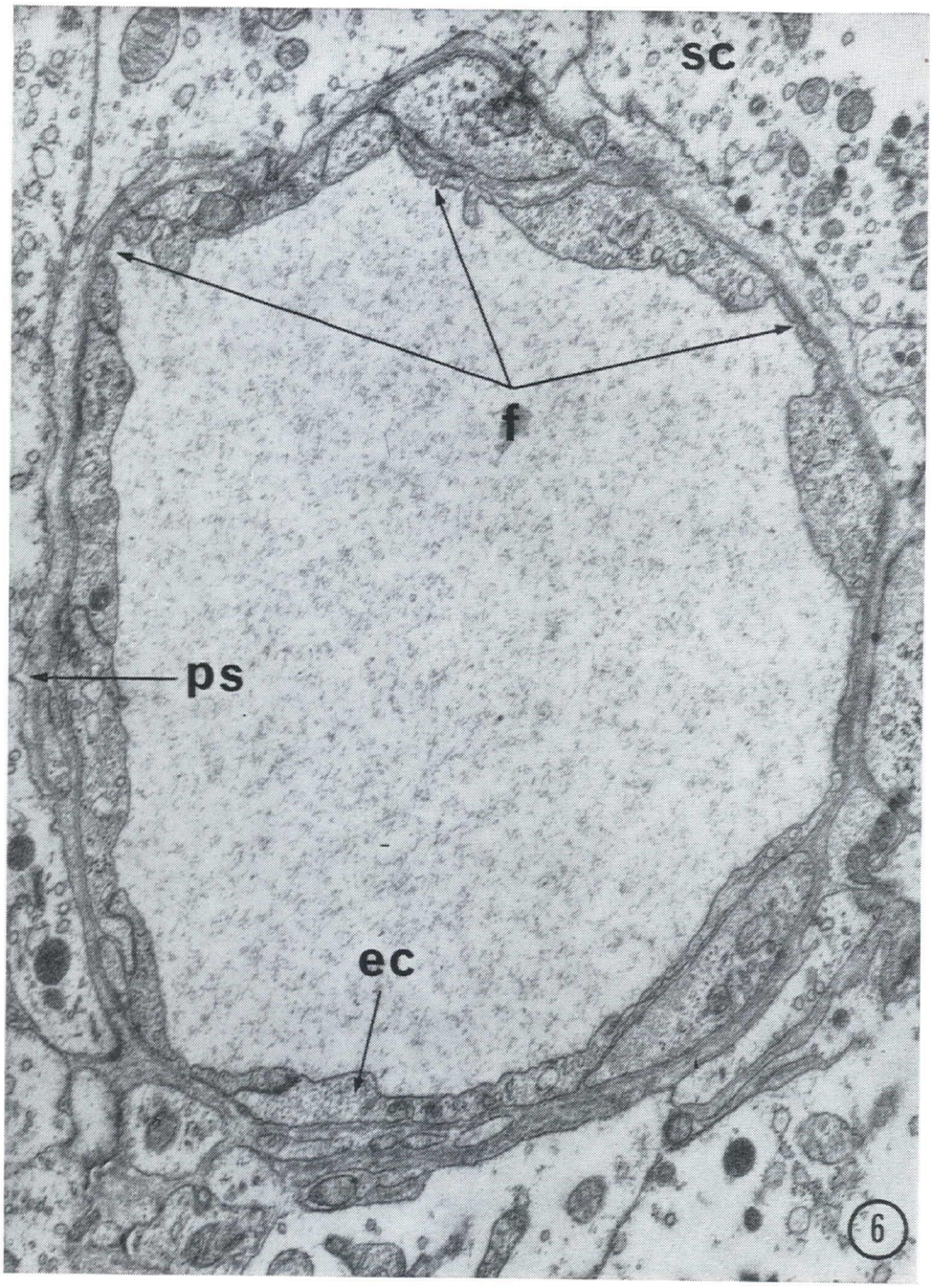

Figure 6

A cross-section through a capillary of a human pituitary. Magnification 18,000X.

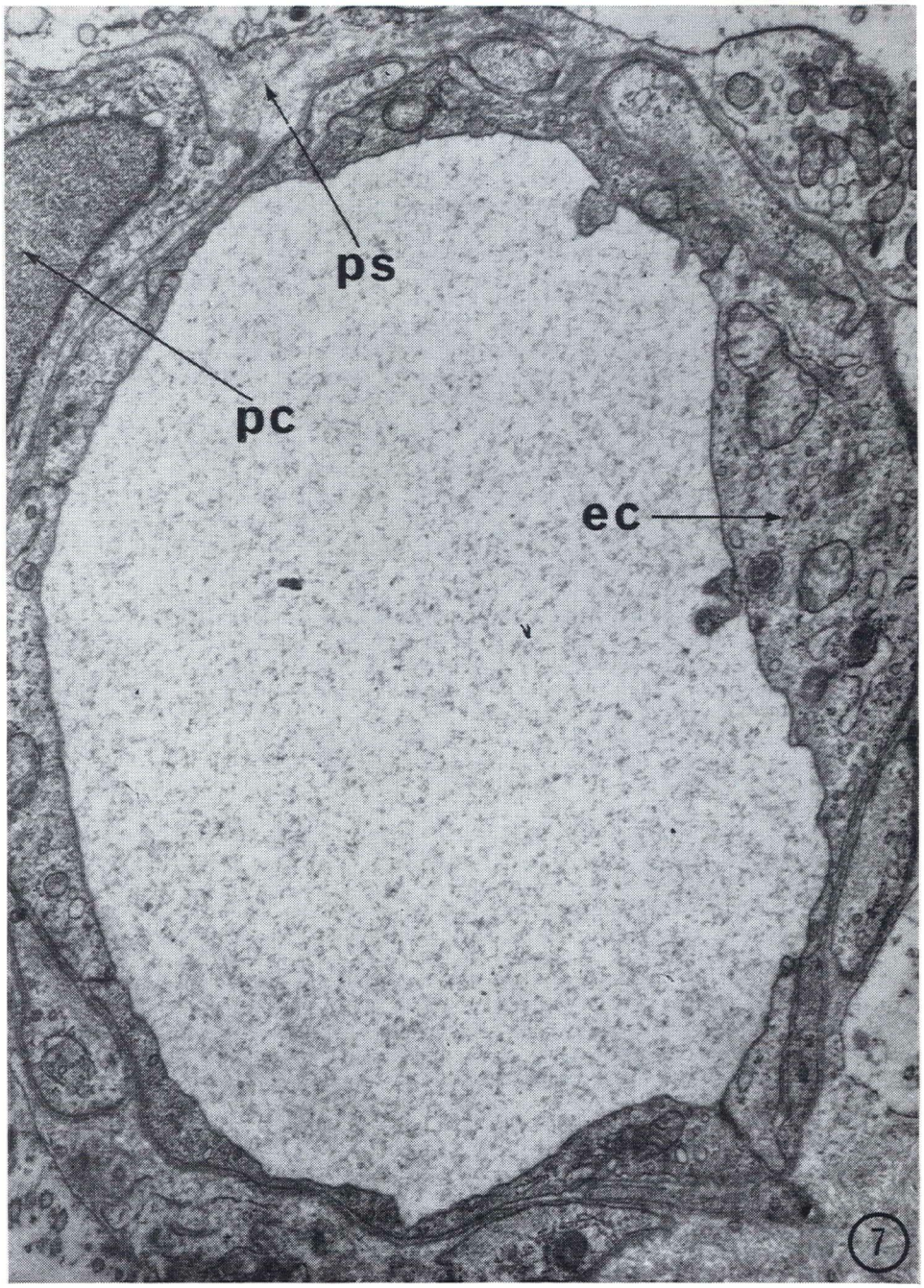

Figure 7

A cross-section of a human pituitary capillary showing the pericapillary cell (pc), pericapillary space (ps) and the endothelial cell (ec) of the capillary. Magnification 15,000X.

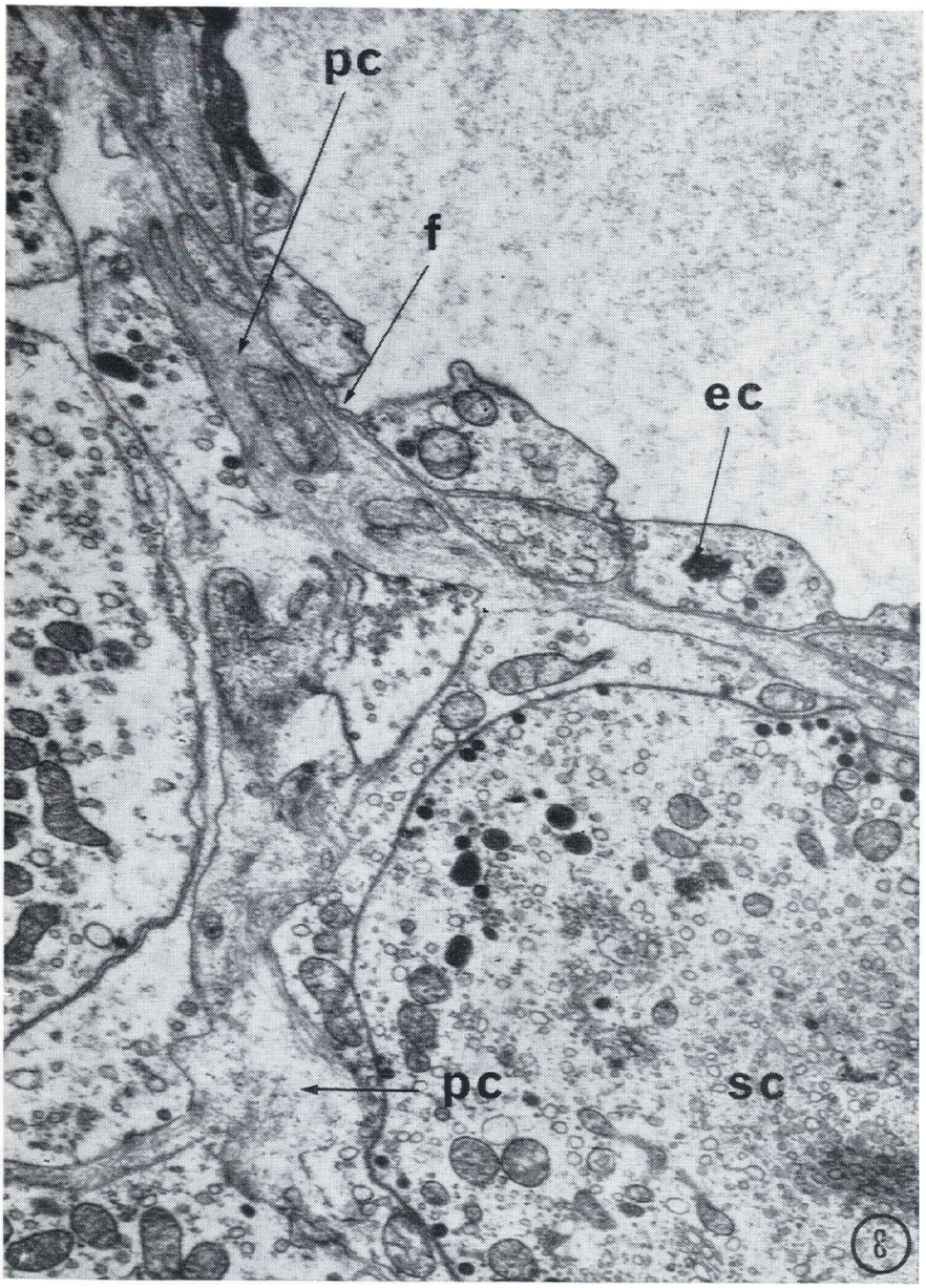

Figure 8

A portion of a capillary and the pericapillary space. Note how the pericapillary space extends between the secretory cells. Magnification 15,000X.

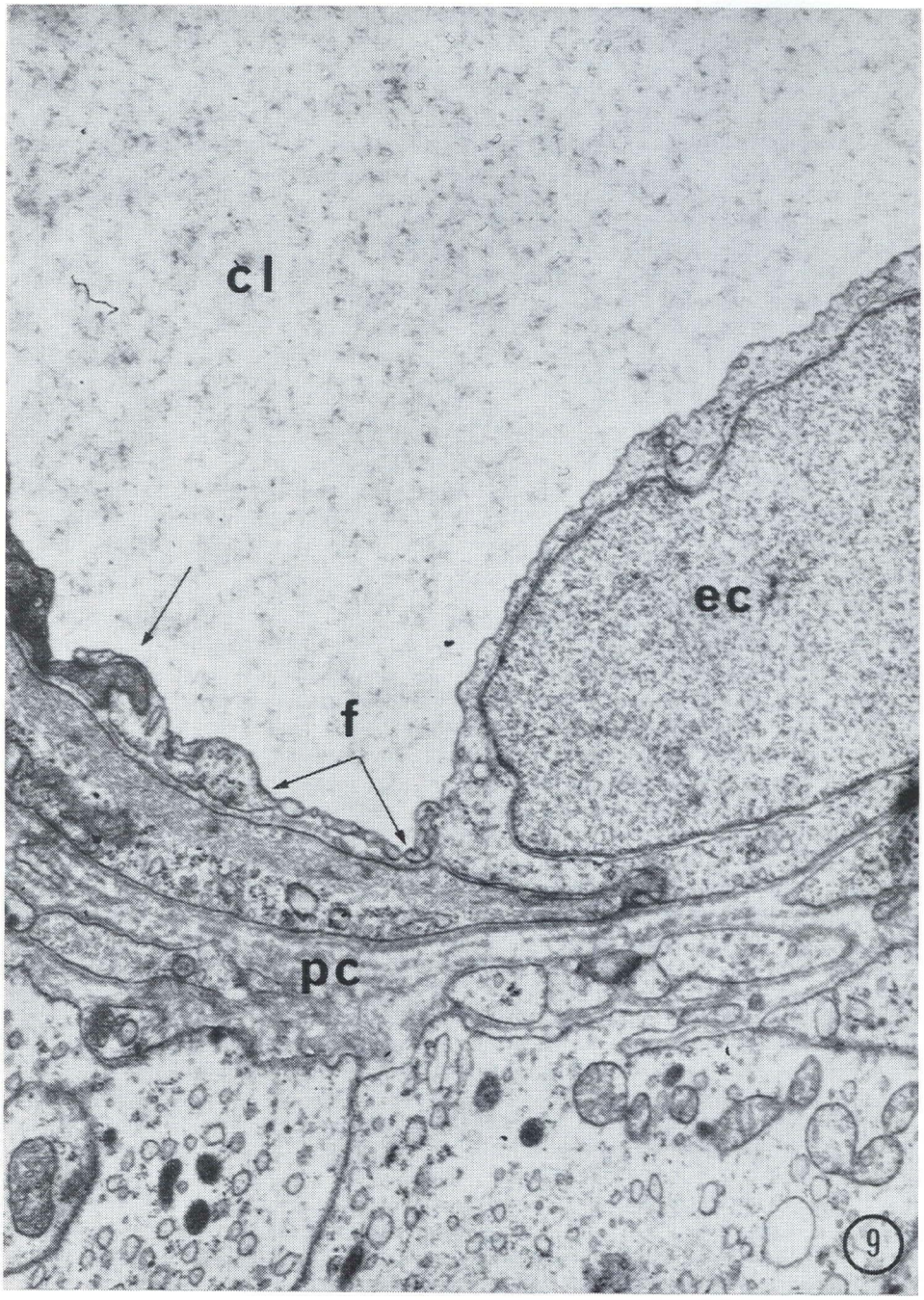

Figure 9

Junction of two endothelial cells (arrow) in the pituitary capillary. Several fenestrations (f) are shown through the endothelial cell. Magnification 24,000X.

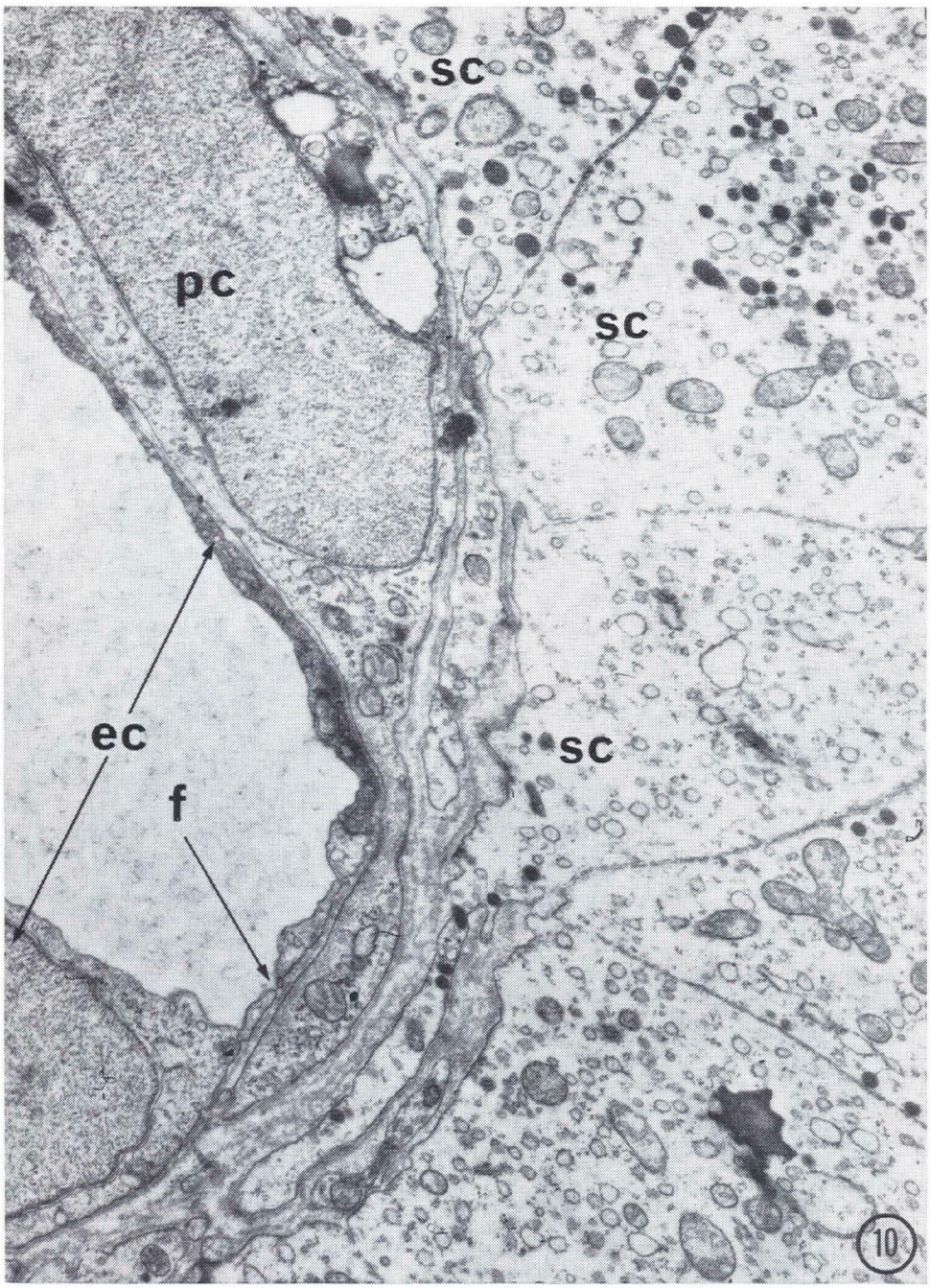

Figure 10

An electronmicrograph of a pituitary capillary with a fenestration (f) which shows the membrane extending across the fenestration. Note the close relationship of the secretory cells (sc) to the pericapillary space. Magnification 18,000X.

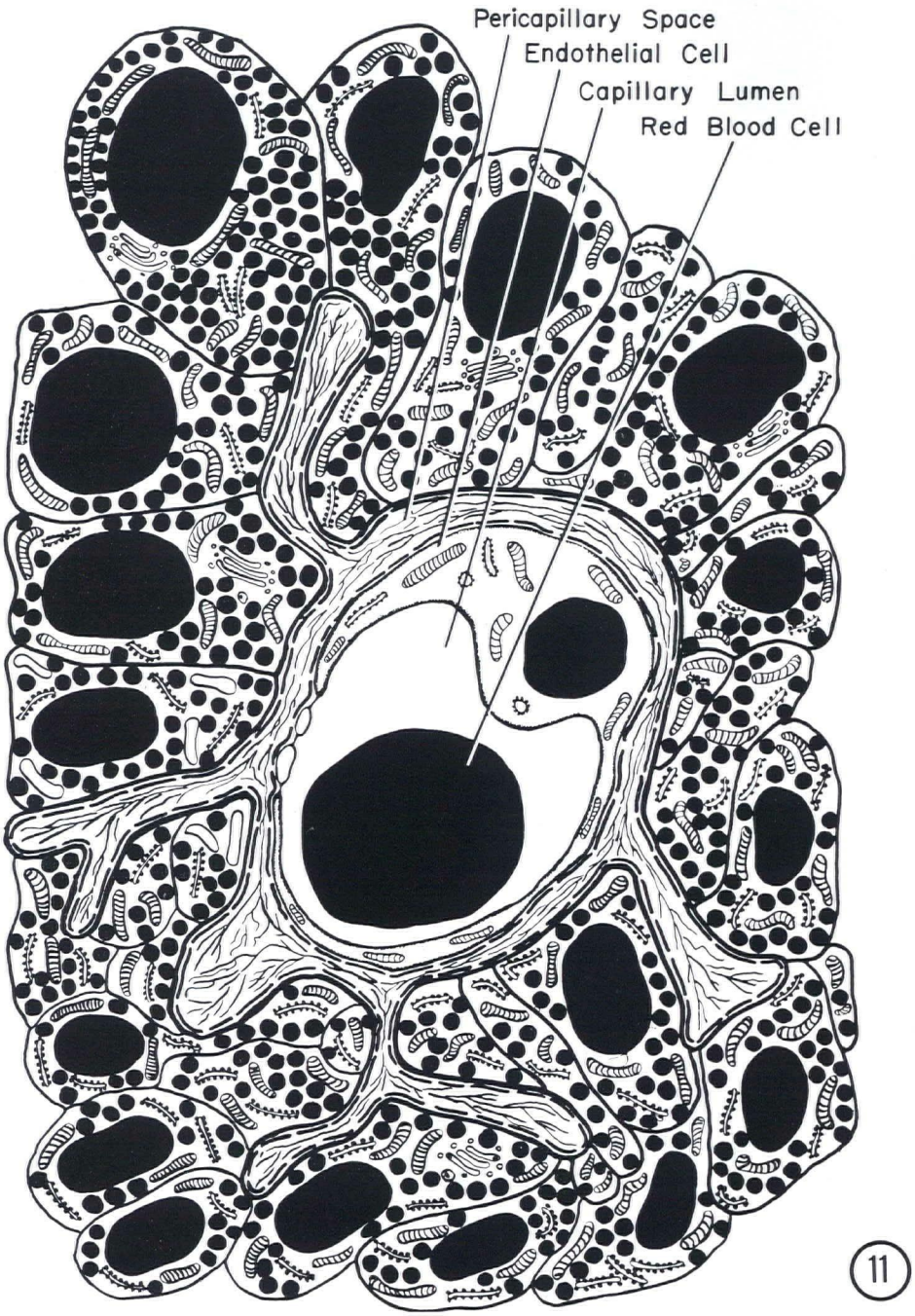

Figure 11

A diagramatic summary of the capillary and pericapillary organization in the pituitary gland.

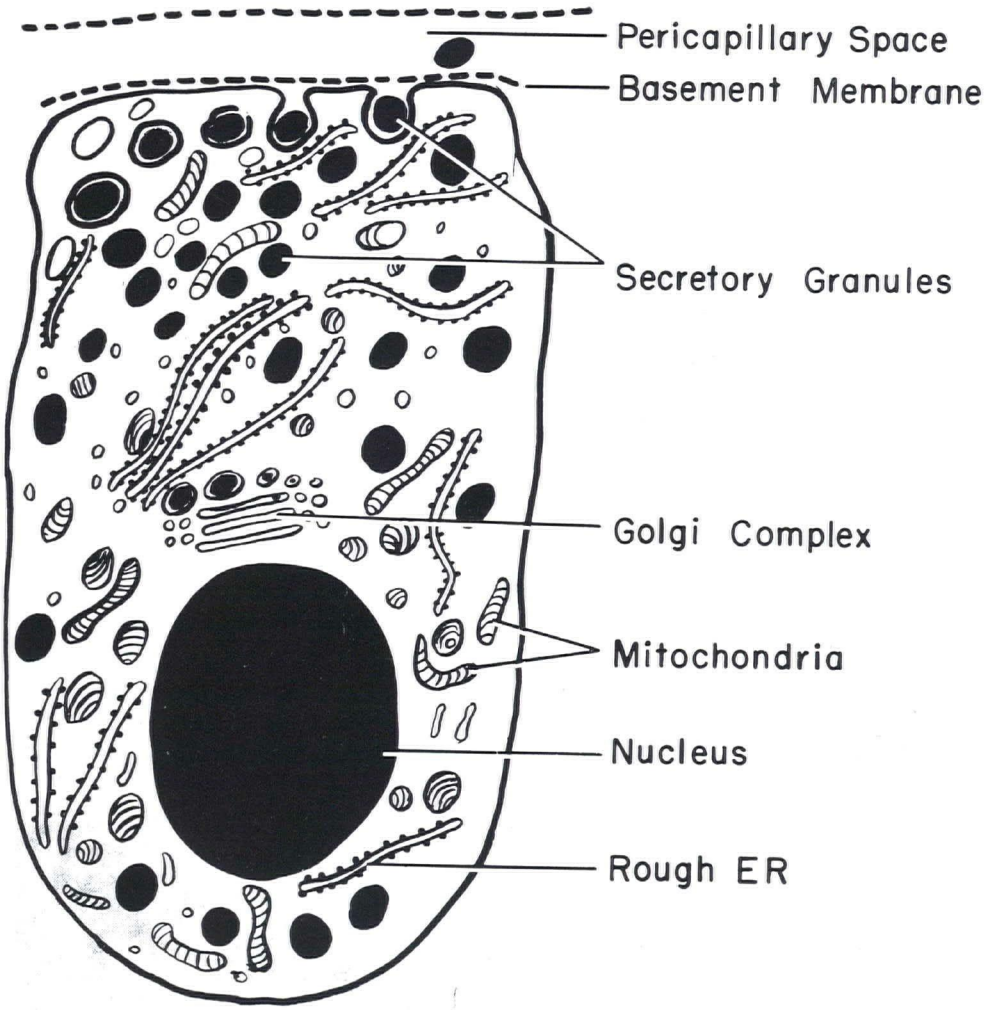

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Figure 12

A drawing which illustrates the stages in the formation and release of the secretory granules in a pituitary secretory cell. Other details of the cell are included.

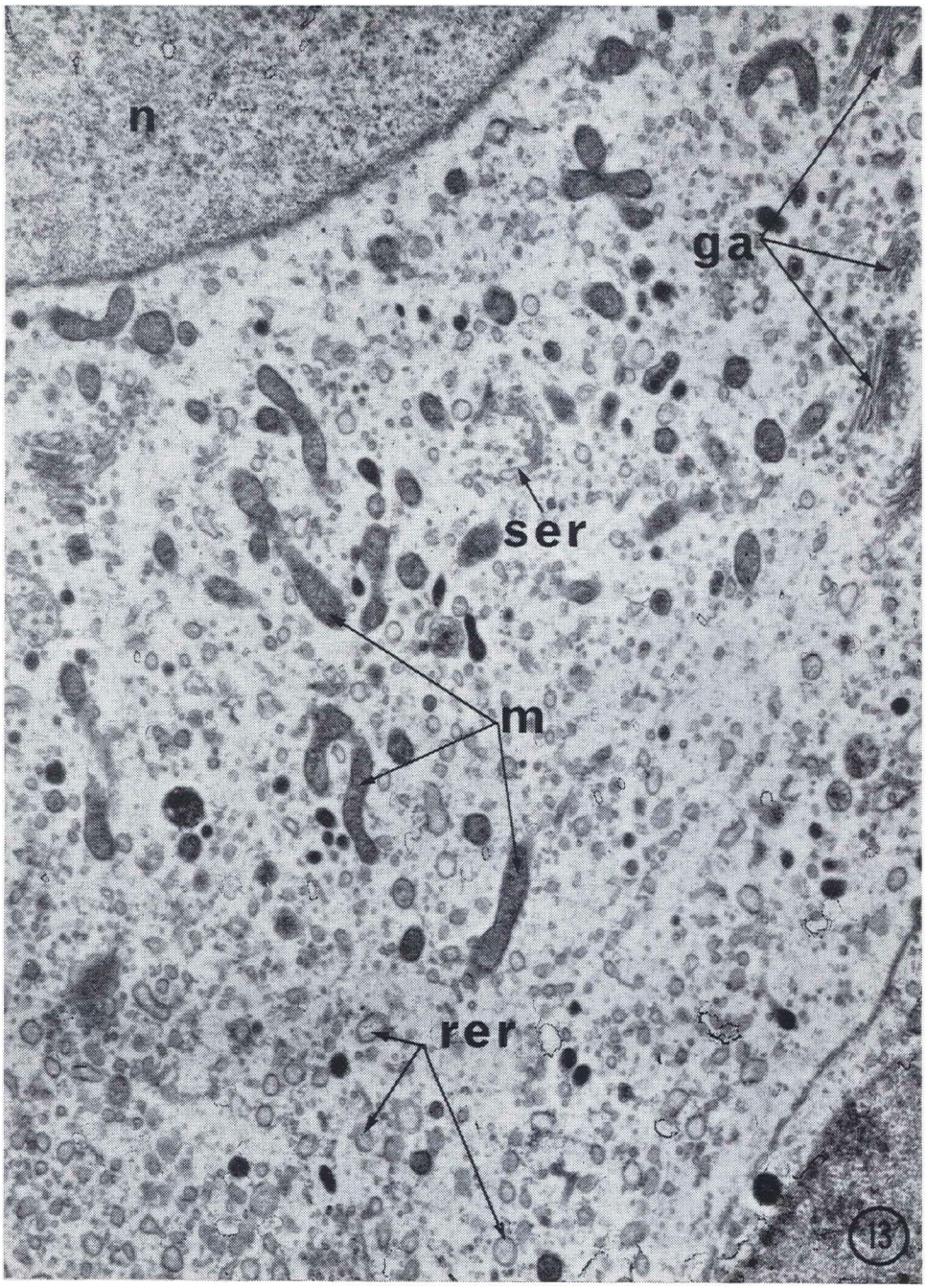

Figure 13

A portion of a pituitary secretory cell of the salamander which has been stimulated to secrete TSH by thyroidectomy. Magnification 18,000X.

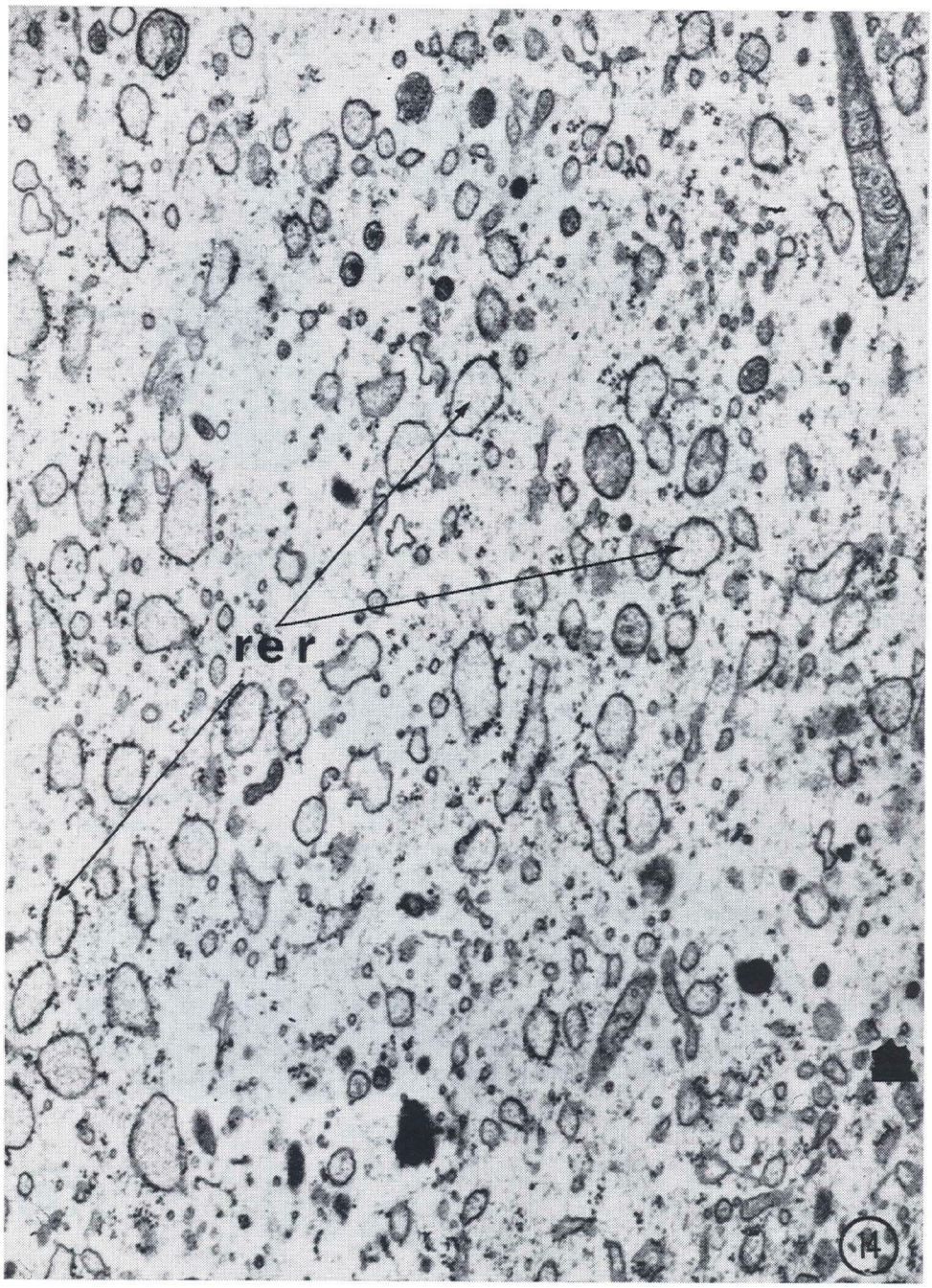

Figure 14

An electronmicrograph of a salamander thyroidectomy cell which demonstrates the enlargement of the rough-surfaced endoplasmic reticulum (rer). Magnification 28,000X.

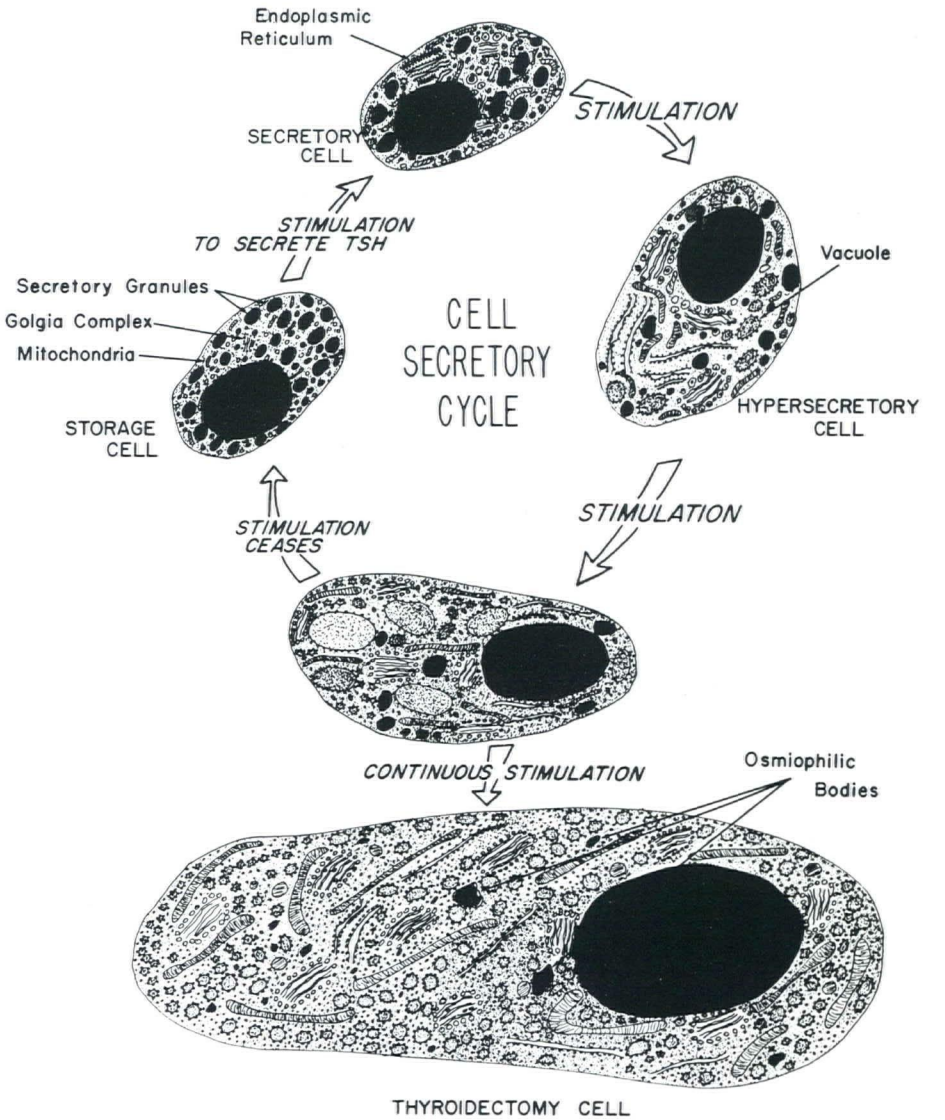

Figure 15

A proposed secretory cycle for the cells of the anterior pituitary gland.

Table I*

HORMONE	LIGHT MICROSCOPY	GRANULE SIZES FROM ELECTRON MICROSCOPY				
		Human	*Rat*	*Mouse*	*Rabbit*	*Salamander*
Somatotrophin (STH)	Orange G. Acidophil	350-400	350	350-400	150-350	360
Luteotrophin (LTH)	Azocarmine Acidophil	Unknown	600	100-200 × 200-400	250-900	240
Thyrotrophin (TSH)	AF+Basophil	Unknown	150	50-100	50-100	One cell type with two sized granules. Small - 120×320 Large - Up to 3 u
Gonadotrophin (FSH&LH)**	AF−Basophil	Unknown	200	100-200	100-200	
Corticotrophin	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown

*Data obtained from the literature but this does not include all reported granule sizes.

**No attempt has been made to separate these although data is available on two distinct cell types for the production of FSH and LH.

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REFERENCES

1. Farquhar, M. G.: Origin and fate of secretory granules in cells of the anterior pituitary gland, Trans. N. Y. Acad. Sci. 23:346, 1961.

2. Cardell, R. R.: Observations on the ultrastructure of the par distalis of the pituitary gland of the salamander *(Triturus viridescens),* J. Appl. Physics 32:1628, 1961.

3. .....: The origin of the thyroidectomy cell in the salamander *(Triturus viridescens)* Fifth International Congress for Electron Microscopy 2:WW-3, 1962.

4. .....: Observations on the cell types of the salamander pituitary gland: an electron microscopic study, J. Ultrastruct. Res. (In press), 1963.

5. Purves, H. D., and Griesbach, W. E.: The site of thyrotrophin and gonadotrophin production in the rat pituitary studied by McManus-Hotchkiss staining for glycoprotein, Endocrinology 49:244, 1951.

6. Fernandez-Moran, H., and Luft, R.: Submicroscopic cytoplasmic granules in the anterior lobe cells of the rat hypophysis as revealed by electron microscopy, Acta. Endocr. 2:199, 1949.

7. Farquhar, M. G., and Rinehart, J. F.: Electron microscopic studies of the anterior pituitary gland of castrate rats, Endocrinology 54:516, 1954.

8. .....: Cytologic alterations in the anterior pituitary gland following thyroidectomy, Endocrinology 55:857, 1954.

9. Farquhar, M. G.: Electron microscopy of the rat anterior pituitary gland. A cytophysiologic study, Ph.D. Thesis Univ. of Calif., Berkeley, Calif., 1955.

10. Yamada, K., and Sano, M.: Electron microscopic observations of the anterior pituitary of the mouse, Okajmas Folia Anatomica Japonica. 34:449, 1960.

11. Barnes, B. G.: Electron microscopic studies on the secretory cytology of the mouse anterior pituitary, Endocrinology 71:618, 1962.

12. Salazar, H.: Electron microscopy of the female rabbit adenohypophysis, Fifth International Congress for Electron Microscopy, 2:WW-9, 1962.

13. Doerr-Schott, J.: Evolution des cellules gonadotropes bau cours du cycle annual chez la Grenouille Rousse *Rana temporaria,* L. Etude au microscope electronique; observations Histochemiques et cytophysiologiques, Gen. Comp. Endo. 2:541, 1963.

14. Follenius, E., et Porte, A.: Etude des differents lobes de l'hypophyse de la perche *Perca Fluvia lis* lo au microscope electronique, Comp.. Rend. Soc. Biol. 155:128, 1961.

15. Hartley, M. W., McShan, W. H., and Ris, H.: Isolation of cytoplasmic pituitary granules with gonadotrophic activity, J. Biophys. Biochem. Cytol. 7:209, 1960.

16. Dalton, A. J.: Golgi appartus and secretion granules. *In* "The Cell", (J. Brachet and A. E. Mirsky, Eds.) New York, Academic Press, 2:603, 1961.

17. Rinehart, J. F., and Farguhar, M. G.: The fine vascular organization of the anterior pituitary gland. An electron microscopic study with histochemical correlations, Anat. Rec. 121: 207, 1955.

18. Cardell, R. R., and Knighton, R. J.: The ultrastructure of a human pituitary tumor, J. Appl. Physics (In press), 1963.