

The Ultrastructure of Primary Water-Clear Cell Hyperplasia of the Parathyroid Glands

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WATER-CLEAR CELL HYPERPLASIA OF THE PARATHYROID GLANDS in patients with primary hyperparathyroidism was first described in 1934 by Albright *et al.*¹ The histology of these glands has been extensively described.²⁻⁴ Careful examination of well-fixed, water-clear cells at high magnification reveals that instead of an absence of cytoplasm²⁻⁴ the water-clear cell is filled with small vacuoles surrounded by an eosinophilic cytoplasm. The characteristic ultrastructure of the water-clear cells was first described by Holzmann and Lange⁵ and subsequently by Sheldon.⁶ Both studies^{5,6} described numerous vacuoles that were membrane limited, 1-5 μ in diameter and, except for an occasional dense body, contained a finely particulate thread-like material. Neither of these groups found secretory granules of the type described in normal human⁷⁻⁹ or other vertebrate¹⁰⁻²³ parathyroid glands, in adenomas,^{5,8,24-28} in primary chief cell hyperplasia,^{8,26} or in secondary hyperplasia.²⁹ This paper will describe the ultrastructure of a third case of primary water-clear cell hyperplasia and provide evidence regarding the pathogenesis of the characteristic, unusual vacuoles of these glands. It will also provide evidence as to the source of the parathyroid hormone from these glands.

Materials and Methods

A 61-year-old Caucasian housewife was admitted for evaluation of recurrent renal calculi. She had renal colic and passed a stone 7 years prior to admission. Urinary stones, gravel and renal colic recurred 2 years prior to admission and a bout of severe renal colic caused her to be hospitalized and referred for evaluation of her parathyroid function. Past history, family history and physical examination were unremarkable. Significant laboratory values were: serum calcium 11.0-13.4 mg%, serum phosphorus 2.8-2.4 mg% and alkaline phosphatase 8.6-10.0 Bodansky units. On the fourth hospital day a subtotal parathyroidectomy was performed. Postoperatively her serum calcium fell to 6.7 mg% and her serum phosphorus rose to 3.5 mg%. She

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developed mild tetany requiring intravenous calcium and was discharged 8 days after operation to be followed by her local physician.

Gross and microscopic examination were typical of clear cell hyperplasia of the parathyroid glands.^{3,4} At surgery, multiple 1-mm cubes of all four glands were fixed in buffered 2% osmium tetroxide at 4 C^{30,31} and glutaraldehyde-paraformaldehyde mixtures, at 22 C³² with postfixation in Caulfield's³⁰ osmium tetroxide. All of the tissue was embedded in flat molds³³ in Epon 812³⁴ after potassium permanganate staining.³⁵ Sections 600–800 Å thick were cut with diamond knives on a Porter-Blum MT-2 ultramicrotome; after lead citrate^{36,37} and/or uranyl acetate staining,³⁸ the sections on Formvar-coated or bare grids were examined at 100 kV in an RCA Emu 3 G electron microscope.

Formalin-fixed, paraffin-embedded sections stained with hematoxylin and eosin and 1- μ Epon sections stained with 1% alkaline toluidine blue³⁹ were examined with the light microscope.

Results

By light microscopy the majority of cells are typical water-clear cells. These are 10–20 μ in diameter, arranged in cords and sheets but often forming acini (Fig 1). The nuclei are basally located and, in this case in contrast to the usual case, large and pleomorphic with numerous giant forms. Nuclear palisading is common. The cytoplasm is composed of numerous clear vacuoles (Fig 2) surrounded by eosinophilic wisps of cytoplasm. In 1- μ sections stained with toluidine blue, the cytoplasmic vacuoles are easily identified (Fig 3) and range from 0.1 to 4 μ in diameter. Asymmetric dense areas can be seen in some vacuoles. In several areas the cells are much smaller, resembling the typical parathyroid chief cells, measuring 6–8 μ in diameter containing a faintly eosinophilic cytoplasm with minimal vacuolization (Fig 4).

By electron microscopy the cells vary between two extremes. The vast majority have an ultrastructure similar to that described by Holzmänn and Lange⁵ and by Sheldon.⁶ In these, the cytoplasm is largely filled with spherical membrane-limited vacuoles 0.2–2 μ in diameter (Fig 5). The membranes of the vacuole are composed of the typical trilaminar unit membranes with no ribosomes attached to the surface of any vacuoles (Fig 6 inset). Frequently vacuoles are incomplete and show fusion with adjacent vacuoles (Fig 5 and 6). Most vacuoles appear empty or contain dispersed thread-like or granular material. In some vacuoles there are electron-dense areas up to 200 m μ in diameter. These are irregular in diameter, with an irregular or dumbbell shape. Most vacuolar inclusions are uniformly dense, resembling lipid. Other inclusions are a series of concentric reduplications of membranes, similar to myelin figures. They are usually asymmetrically located in the vacuoles. Other vacuolar densities have an appearance similar to the

centers of parathyroid secretory granules, and rare ones have a striated appearance with a center-to-center distance of striations of $10\text{ m}\mu$ (Fig 7). These densities appear to correspond to those seen with the light microscope. Numerous small vesicles resembling Golgi vesicles are often mixed with mature secretory granules (Fig 8).

Between the vacuoles are the usual cytoplasmic contents and organelles, mitochondria and free ribosomes, both aggregated and dispersed. There are dispersed flattened sacs of granular endoplasmic reticulum. The mitochondria are tubular with loosely interdigitating cristae. Mitochondrial granules are rarely present on the cristae. There are small arrays of agranular membranes resembling Golgi vesicles. Very few Golgi vacuoles are present in these areas (Fig 7). Occasional cells contain, near their periphery, numerous membrane-limited spherical granules $0.2\text{--}0.4\ \mu$ in diameter (Fig 5, 7 and 8). These have markedly electron-dense granular centers surrounded by a clear halo, and they resemble the secretory granules of the parathyroid glands.⁷⁻²⁸

A few cells are present that contain only rare vacuoles characteristic of water-clear cell hyperplasia (Fig 9). These cells have the ultrastructural features of chief the parathyroid glands. They contain the usual cell organelles. The Golgi apparatuses are both large with numerous vacuoles and vesicles and small with few vacuoles and vesicles. The granular endoplasmic reticulum in most of these cells is dispersed but in a few the lamellae are aggregated (Fig 6 and 9), forming the bodies of Papenheimer and Wilens.⁷ Parathyroid secretory granules (Fig 9 and 10) are frequent, while centrioles and cilia are rarely present (Fig 10) in these cells. Approximately equal in number to the chief cells are cells that, on section, contain from 10 to 20 vacuoles similar to those described in water-clear cell hyperplasia. The fully developed vacuoles are scattered throughout the cell and measure up to $1\ \mu$ in diameter. Smaller vacuoles are frequently present in the region of the larger Golgi apparatuses (Fig 11) and the contents of these vacuoles resemble those of the Golgi vacuoles. Ribosomes are never seen attached to the surface of these vacuoles. The granular endoplasmic reticulum either dispersed or aggregated is often seen in close proximity to these vacuoles but is never seen connecting with them.

The nuclei of all the cells are roughly spherical with irregular chromatin and one or more prominent nucleoli. The nuclear envelopes frequently have ribosomes on their cytoplasmic surfaces. A basement membrane separates the cells from the extracellular space. Another basement membrane lies against the capillary endothelium. The endothelial cells are often swollen, occasionally vacuolated and contain nu-

merous fenestrae. Secretory granules and clear-cell vacuoles are not seen in the capillary endothelial cells.

Discussion

This study clearly demonstrates that the cells of primary water-clear cell hyperplasia are quite different ultrastructurally from both the dark and light chief cells seen in normal parathyroid glands,⁷⁻²³ in parathyroid adenomas,^{5,8,24-28} in primary chief cell hyperplasias,^{8,26} in carcinomas,⁴⁰ or in the vacuolated or chronically stimulated chief cells described in secondary hyperplasia.²⁹ Only the cells of primary water-clear cell hyperplasia contain the distinctive spherical vacuoles in their cytoplasm. These vacuoles are ultrastructurally quite distinct from the protein- or polypeptide-containing secretory granules of the light cells of the thyroid,⁴¹ the ultimobranchial bodies,⁴²⁻⁴⁴ the parathyroid glands of all species thus far studied,^{7,23} or any other endocrine organ.

Five theoretical origins of the vacuoles appear possible: (1) they are dilated Golgi or other agranular cytomembrane systems possibly related to the secretory granules; (2) they are dilated sacs of granular endoplasmic reticulum; (3) they are swollen mitochondria, (4) they are unrelated to any other cell organelle and are produced *de novo* by the cell; or (5) they are pinocytotic or lysosomal vesicles.

Since ribosomes are never seen on the vacuoles, cristae-like structures are not seen in the vacuoles, and there is no evidence or precedent for their formation *de novo*, it appears most likely that the vacuoles are related to the cytomembranes of the Golgi region or, possibly, to the secretory granules. Their contents are often similar to those of the Golgi vesicles, and small vacuoles, larger than the usual Golgi vesicles are often present in the Golgi region. Further, it has been postulated⁴⁵ that one of the functions of the Golgi complex is the production of intracellular cytomembranes. The relation of these vacuoles to secretory granules, which are also produced in the Golgi region, is not clear;⁷ however, some vacuoles do contain asymmetric dense bodies resembling the central core of a secretory granule, but secretory granules with a normal appearance are present in cells filled with vacuoles. The vacuoles do not ultrastructurally resemble pinocytotic or lysosomal vesicles.

Numerous, mature, membrane-limited dense granules resembling the parathyroid secretory granules are present in cells both with and without clear-cell vacuoles. The number of these granules is quite consistent with that seen in both normal human glands,^{7-9,28} in adenomas,^{5,8,24-27} and chief cell hyperplasias.^{26,46} Thus, it is not necessary to speculate that the vacuoles of the clear-cell hyperplasia contains any parathyroid

hormone, though this possibility can not be excluded. This finding of secretory granules in clear-cell hyperplasia is in contrast to their previously reported absence.^{5,6}

It has been shown here that the water-clear cells of primary hyperparathyroidism are, by virtue of their characteristic vacuoles, distinct from the chronically stimulated chief cells of secondary hyperplasia²⁹ and from the light chief cells seen in normal parathyroid glands, adenomas, and chief cell hyperplasias. For this reason the term *water-clear cell* should only be used for the cells in this disease.

The other organelles in incompletely developed water-clear cells appear similar to those of normal and pathologic chief cells, and the cells appear to have the same cyclic changes in structure.⁷⁻⁹ The Golgi region shows its characteristic variation in size and complexity and the granular endoplasmic reticulum its variation in aggregation and dispersal. It is not clear, due to the limited sample available, whether the cycle is normal⁷⁻⁹ or abnormal, as in adenomas and primary hyperplasias.^{5,8,24-28,46}

It is also apparent that, as postulated by Roth⁴ and by Roth and Marshall,²⁹ the water-clear cells are derived from the chief cells. While Roth and Marshall²⁹ do not indicate a transformation from hyperplastic chief cells to water-clear cells, this study indicates that such a transition is a possibility. Further, ultrastructural studies on cases of primary chief cell hyperplasia may discover a few water-clear cells in glands where the majority of the cells are chief cells, indicating that the two forms of hyperplasia are simply variations of the same disease.

References

1. Albright F, Bloomberg E, Castleman B, Churchill ED: Hyperparathyroidism due to diffuse hyperplasia of all parathyroid glands rather than adenoma of one. *Arch Intern Med* 54:315-329, 1934
2. Castleman B, Cope O: Primary parathyroid hypertrophy and hyperplasia. A review of 11 cases at the Massachusetts General Hospital. *Bull Hosp Joint Dis* 12:368-378, 1951
3. Castleman B: Tumors of the parathyroid glands, *Atlas of Tumor Pathology*, Washington, DC, Armed Forces Institute of Pathology, 1952, pp 1-74
4. Roth SI: Pathology of the parathyroids in hyperparathyroidism. Discussion of recent advances in the anatomy and pathology of the parathyroid glands. *Arch Path* 73:495-510, 1962
5. Holzmann K, Lange R: Zur Zytologie der glandula Parathyreoidea des menschen. Weitere Untersuchungen an Epithelkorperadenomen. *Z Zellforsch* 58:759-789, 1963
6. Sheldon H: On the water-clear cell in the human parathyroid gland. *J Ultrastruct Res* 10:377-383, 1964
7. Munger BL, Roth SI: The cytology of the normal parathyroid glands of man and Virginia deer: a light and electron microscopic study with morphologic evidence of secretory activity. *J Cell Biol* 16:379-400, 1963

8. Weymouth RJ, Sheridan MN: Fine structure of human parathyroid glands: Normal and pathological. *Acta Endocrin* 53:529-546, 1966
9. Mazzocchi G, Meneghelli, Frasson F: The human parathyroid glands: an optical and electron microscopic study. *Sperimentale* 117:383-447, 1967
10. Davis R, Enders AC: Light and electron microscope studies on the parathyroid gland, *The Parathyroids*. Edited by RO Greep RV Talmage. Springfield, Ill, Charles C Thomas. 1961, pp 76-92
11. Kayser C, Petrovic A, Porte A: Variations ultrastructurales de la parathyroïde du hamster ordinaire (*Cricetus cricetus*) au cours du cycle saisonnier. *CR Soc Biol* 155:2178-2183, 1961
12. Montsko T, Tigyí A, Benedeczky I, Lissak K: Electron microscopy of parathyroid secretion in *Rana esculenta*. *Acta Biol Acad Sci Hung* 14:81-94, 1963
13. Roth SI, Raisz LG: Effect of calcium concentration on the ultrastructure of rat parathyroid in organ culture. *Lab Invest* 13:331-345, 1964
14. Capen CC, Rowland GN: The ultrastructure and histochemistry of normal parathyroid glands of pregnant and non-pregnant cows. *Lab Invest* 14:1673-1690, 1965
15. Lange R, Von Brehm H: On the fine structure of the parathyroid gland in the toad and frog, *The Parathyroid Glands: Ultrastructure Secretion, and Function*. Edited by PJ Gaillard, RV Talmage, AM Budy. Chicago, The University of Chicago Press 1965, pp 19-26
16. Rogers DC: An electron microscope study of the parathyroid gland of the frog (*Rana clamitans*). *J Ultrastruct Res* 13:478-499, 1965
17. Nakagami K: Comparative electron microscopic studies of the parathyroid gland. I. Fine structure of monkey and dog parathyroid glands. *Arch Histolog Jap* 25:435-465, 1965
18. Stoeckel ME, Porte A: Observations ultrastructurales sur la parathyroïde de souris. I. Etude chez la souris normale. *Z Zellforsch* 73:488-502, 1966
19. Cortelyou JR, McWhinnie DJ: Parathyroid glands of amphibians. I. Parathyroid structure and function in the amphibian, with emphasis on regulation of mineral ions in body fluids. *Amer Zool* 7:843-855, 1967
20. Mazzocchi G: L'ultrastruttura delle ghiandole paratiroïdi di Scimmia (*Erythrocebus patas*). *Atti Soc Med Chir Padova* 42:3-23, 1967
21. Nakagami K: Comparative electron microscopic studies of the parathyroid glands. II. Fine structure of the parathyroid gland of the normal and the calcium chloride treated mouse. *Arch Histol Jap* 28:185-205, 1967
22. Hara J, Nagatsu I: Ultrastructural changes in the parathyroid glands by the injection of parahormone in rats. *Okajima Folia Anat Jap* 44:99-133, 1968
23. Capen CC, Rowland GN: The ultrastructure of the parathyroid glands of young cats. *Anat Rec* 162:327-339, 1968
24. Engfeldt B, Hellstrom J, Ivemark B, Rhodin J: Elektron mikroskopisk och histokemi vid parathyreoïdeadenom. *Nord Med* 61:558-559, 1959
25. Lange R: Zur Histologie und Zytologie der glandula Parathyreoïdea des Menschen. Licht- und elektronenmikroskopische Untersuchungen an Epithelkörperadenomen. *Z Zellforsch* 53:765-828, 1961
26. Roth SI, Munger BL: The cytology of the adenomatous, atrophic, and

- hyperplastic parathyroid glands of man: a light and electron microscopic study. *Virchow Arch Path Anat* 335:389-410, 1962
27. Marshall RB, Roberts DK, Turner RA: Adenomas of the human parathyroid: light and electron microscopic studies following selenium 75 methionine scan. *Cancer* 20:512-524, 1967
 28. Weymouth RJ, Seibel HR: An electron microscopic study of the parathyroid glands in man: Evidence of secretory material. *Acta Endocrin* 61:334-342, 1969
 29. Roth SI, Marshall RB: Pathology and ultrastructure of the human parathyroid glands in chronic renal failure. *Arch Intern Med* 124:397-407, 1969
 30. Caulfield JB: Effects of varying the vehicle for OsO₄ in tissue fixation. *J Biophys Biochem Cytol* 3:827-830, 1957
 31. Palade GE: A study of fixation for electron microscopy. *J Exp Med* 95:285-298, 1952
 32. Karnovsky MJ: A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J Cell Biol* 27:137a, 1965, abstr
 33. Rockwell AF, Norton P, Caulfield JB, Roth SI: A silicone rubber mold for embedding tissue in epoxy resins. *Science Tools* 13:9-10, 1966
 34. Luft JH: Improvements in epoxy resin embedding methods. *J Biochem Biophys Cytol* 9:409-414, 1961
 35. Parsons DF: A simple method for obtaining increased contrast in araldite sections by using postfixation staining of tissues with potassium permanganate. *J Biophys Biochem Cytol* 11:492-497, 1961
 36. Reynolds ES: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J Cell Biol* 17:208-212, 1963
 37. Venable JH, Coggeshall RA: A simplified lead citrate stain for use as in electron microscopy. *J Cell Biol* 25:407-408, 1965
 38. Watson ML: Staining of tissue sections for electron microscopy with heavy metals. *J Biophys Biochem Cytol* 4:475-478, 1958
 39. Trump BF, Smuckler EA, Benditt EP: A method for staining epoxy sections for light microscopy. *J Ultrastruct Res* 5:343-348, 1961
 40. Marshall RB, Roth SI: Unpublished data
 41. Pearse AGE: The cytochemistry of the thyroid C-cells and their relationship to calcitonin. *Proc Roy Soc (Biol)* 164:478-487, 1966
 42. Robertson DR, Bell AL: The ultimobranchial body in *Rana pipiens*. I. The fine structure. *Z Zellforsch* 66:118-129, 1965
 43. Stoeckel ME, Porte A: Localisation ultimobranchiale et thyroïdienne des cellules C (cellules a calcitonine) chez deux columbidae: le pigeon et le tourtereau. Etude au microscope electronique. *Z Zellforsch.* 102:376-386, 1969
 44. Capen CC, Young DM: Fine structural alterations in thyroid parafollicular cells of cows in response to experimental hypercalcemia induced by vitamin D. *Amer J Path* 57:365-382, 1969
 45. Ross R: The connective tissue fiber forming cell, *Biology of Collagen*. Edited

by BS Gould. Vol 2, Part A of Treatise on Collagen. Edited by GN Ramachendran. New York, Academic Press, 1968, pp 2-82

46. Black WC III: Correlative light and electron microscopy in primary hyperparathyroidism. Arch Path 88:225-241, 1969

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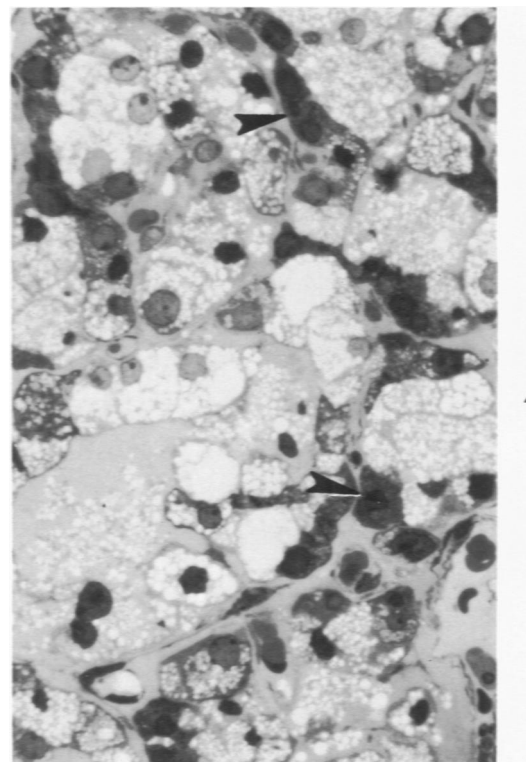
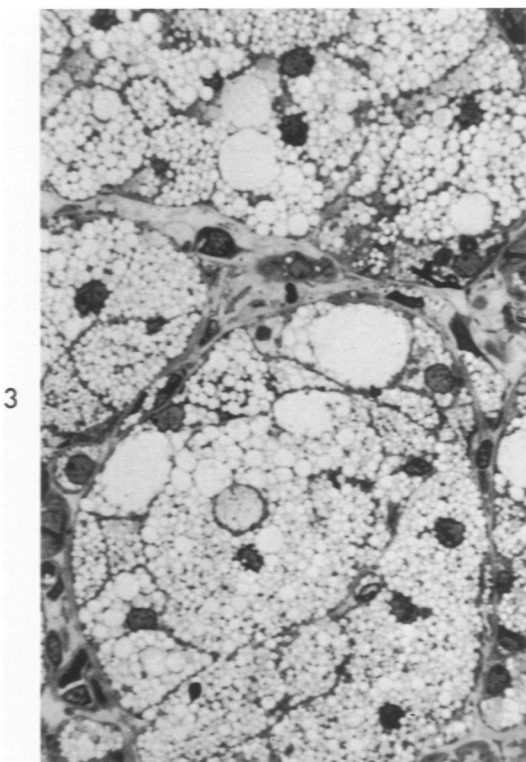
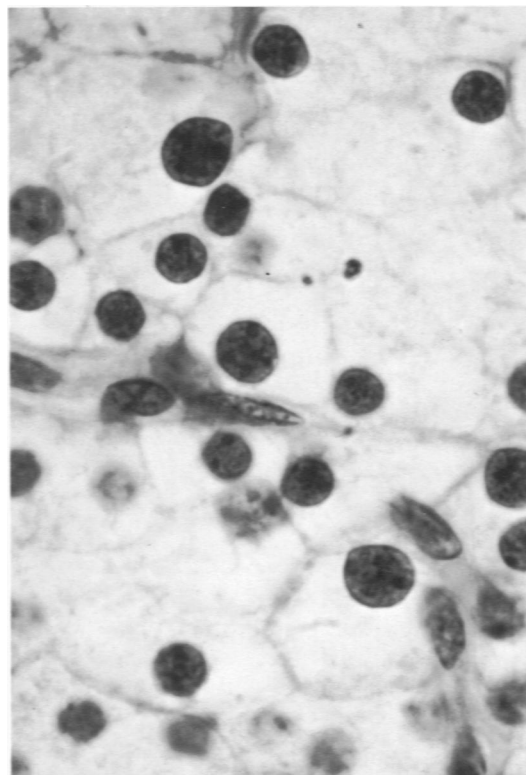
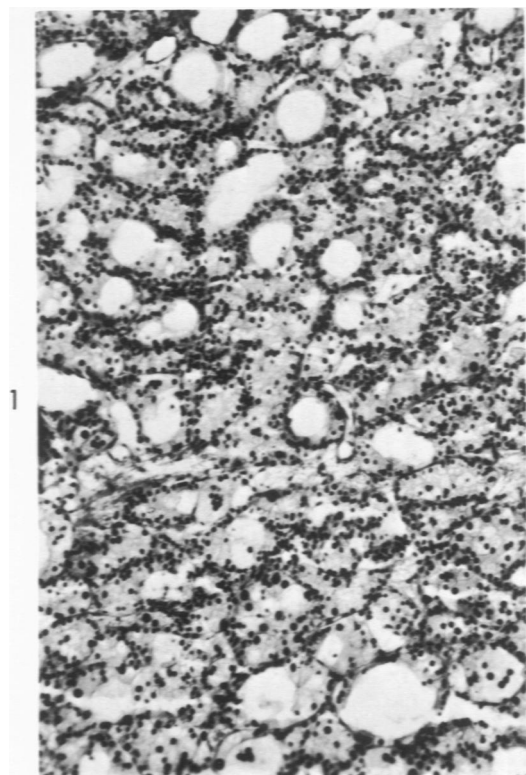
Legends for Figures

Fig 1.—Light micrograph of water-clear cells showing large cells with relatively clear cytoplasm and basally oriented nuclei. There is moderate variation in nuclear size. Cells are arranged in cords, sheets and acini. Paraffin-embedded, H&E. $\times 37$.

Fig 2.—Light micrograph of water-clear cells showing faint vacuolization of cells. Thin wisps of cytoplasm are visible in cells. Paraffin-embedded, H&E. $\times 1000$.

Fig 3.—Light micrograph of water-clear cells with large numbers of vacuoles in their cytoplasm. Epon-embedded, toluidine blue. $\times 500$.

Fig 4.—Light micrograph of region of gland where there are chief cells (arrows), containing minimal number of vacuoles in midst of water-clear cells. Other cells contain intermediate numbers of vacuoles. Epon-embedded, toluidine blue. $\times 500$.



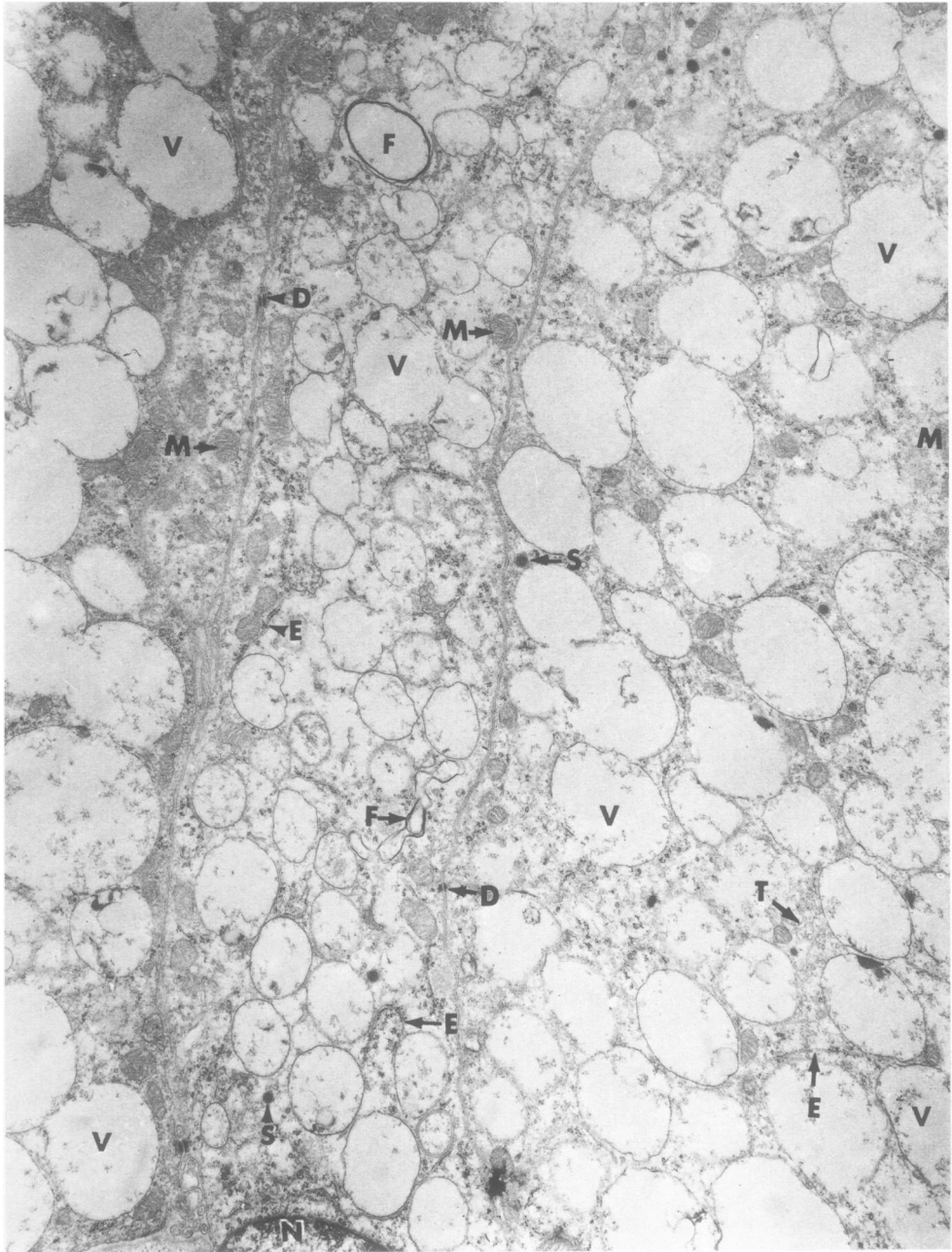


Fig 5.—Electron micrograph of regions of three adjacent water-clear cells. Cytoplasm of all three cells is largely filled with numerous membrane-limited vacuoles (V), 0.5–2 μ in diameter. Many of these contain thread-like material. Some of vacuoles appear to be breaking down, while others appear to be coalescing. Myelin figures (F) are present. A rare small (0.1 μ) secretory granule (S) is present. Mitochondria (M), nucleus (N), granular ergastoplasm (E) and small vesicles (T) are identifiable between vacuoles. Desmosomes (D) are present along adjacent cell membranes. Lead citrate and uranyl acetate. \times 12,800.

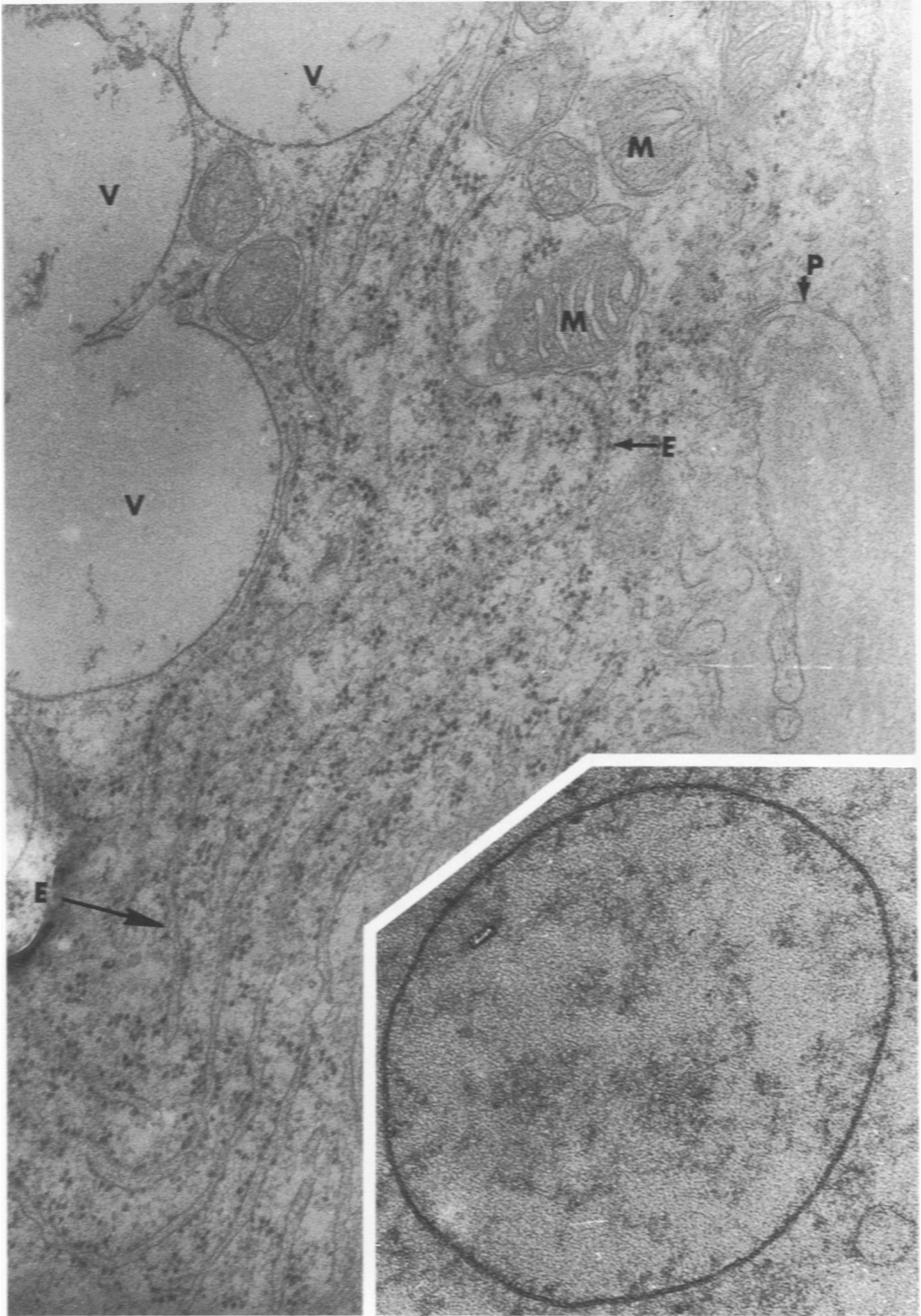


Fig 6.—Electron micrograph of edge of a clear cell showing large aggregate of granular endoplasmic reticulum (*E*) near cell membrane (*P*). Parts of three vacuoles (*V*) and trilaminar structure of vacuole membrane (*inset*) can be seen. Two lower vacuoles are coalescing. Mitochondria (*M*). Lead citrate and uranyl acetate. $\times 23,400$. Inset, $\times 77,900$.

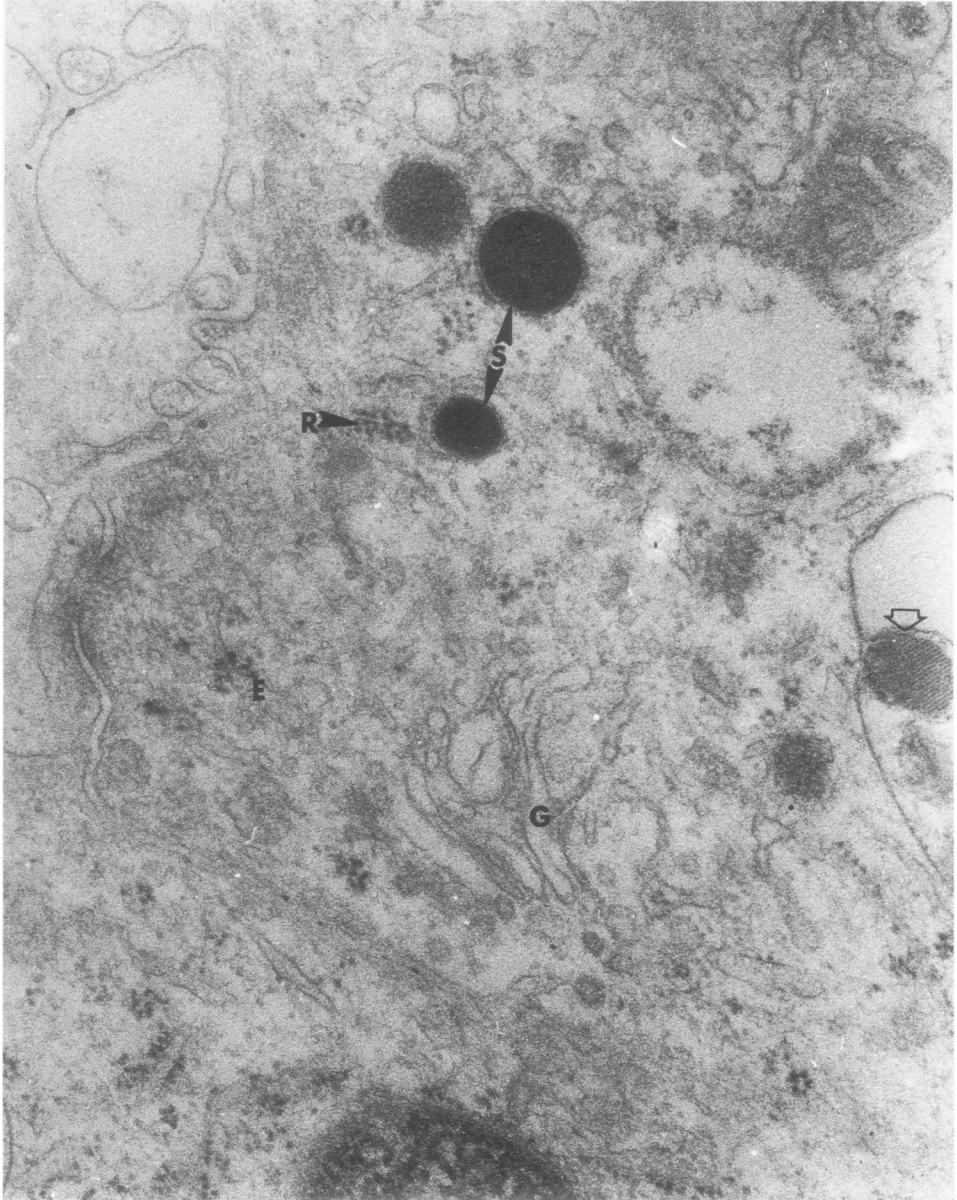


Fig 7.—Electron micrograph of Golgi region (G) of water-clear cell. Golgi is small with few vacuoles. Edge of vacuole containing striated dense region (*arrow*) is visible. These striations have center-to-center distance of 10 m μ . Membrane-limited parathyroid type secretory droplets (S), free ribosomes (R), and granular endoplasmic reticulum (E) are present. Lead citrate and uranyl acetate. $\times 49,300$.

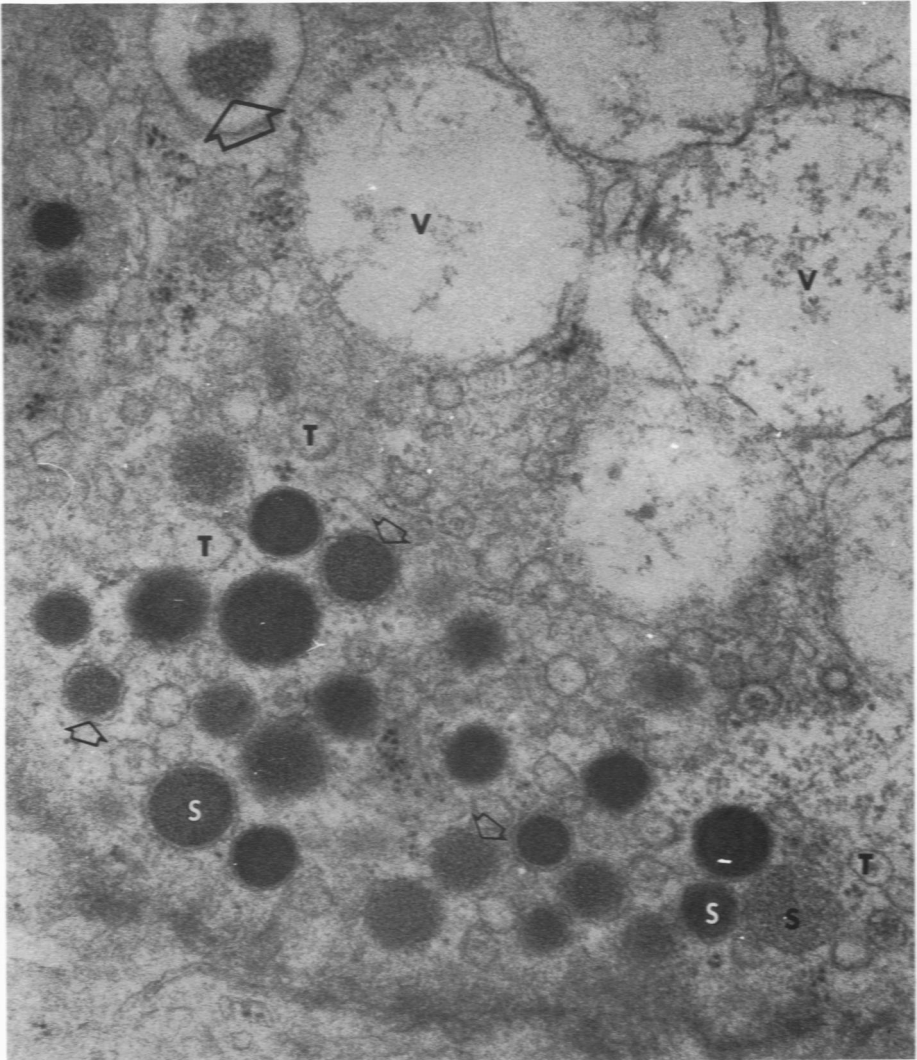


Fig 8.—Electron micrograph of edge of water-clear cell. In addition to characteristic vacuoles (V) containing threadlike and particulate material, there are numerous small vesicles (T) and secretory granules of varying density. Centers of some granules (arrows) resemble contents of one of vacuoles (large arrow). Lead citrate and uranyl acetate. $\times 42,600$.

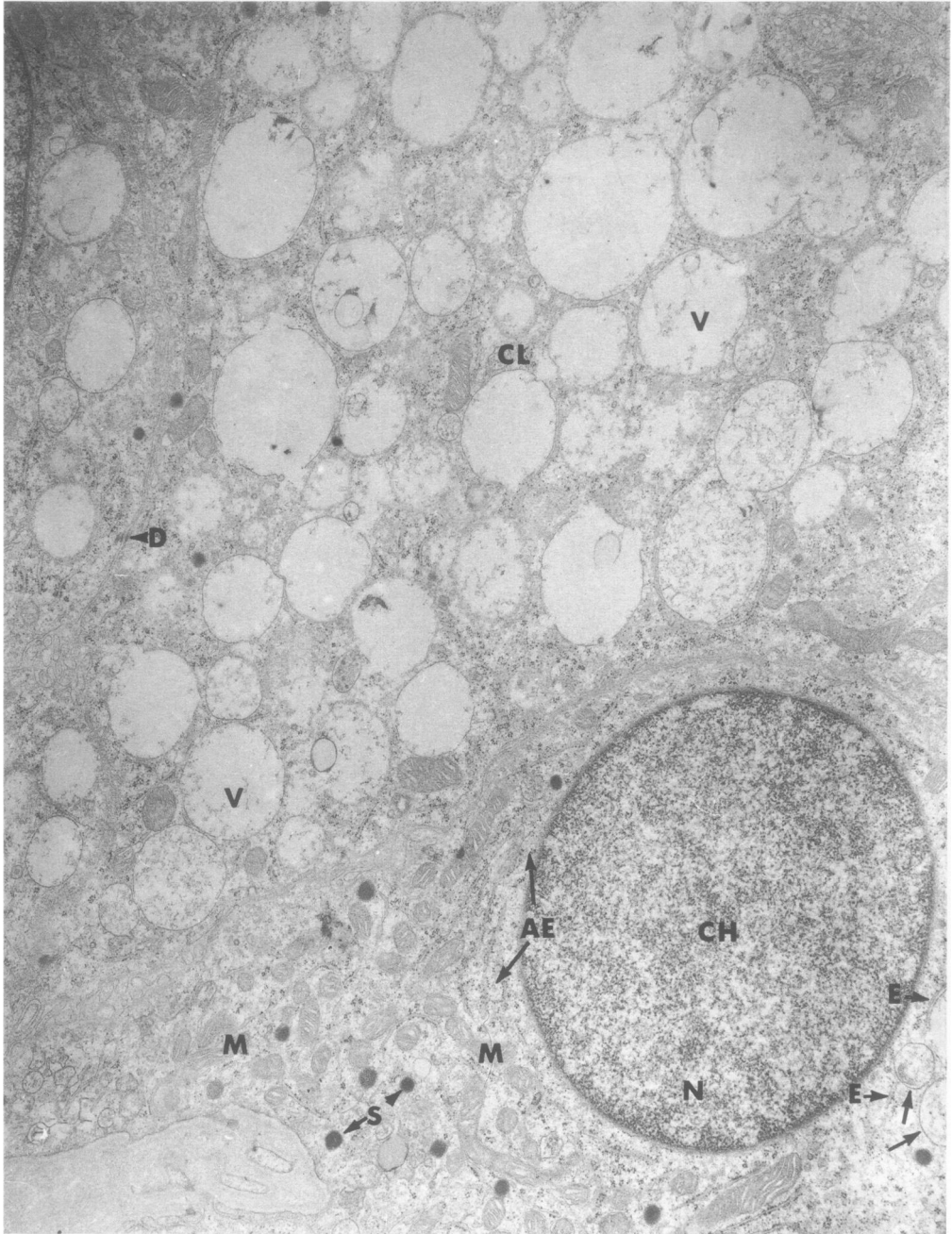


Fig 9.—Electron micrograph of three adjacent cells. Central cell (CL) contains numerous typical clear-cell vacuoles (V). The cell in upper left corner contains three vacuoles in portion showing in micrograph. Cell in lower left corner (CH) resembles chief cell with numerous secretory droplets (S), partially aggregated sacs of granular endoplasmic reticulum (AE) and two clear-cell vacuoles (arrows). Secretory granules are present in other cells. Mitochondria (M), dispersed sacs of granular endoplasmic reticulum (E), nucleus (N) and free ribosomes, identical in all the cells. A desmosome (D) is present. Lead citrate and uranyl acetate. $\times 12,000$.

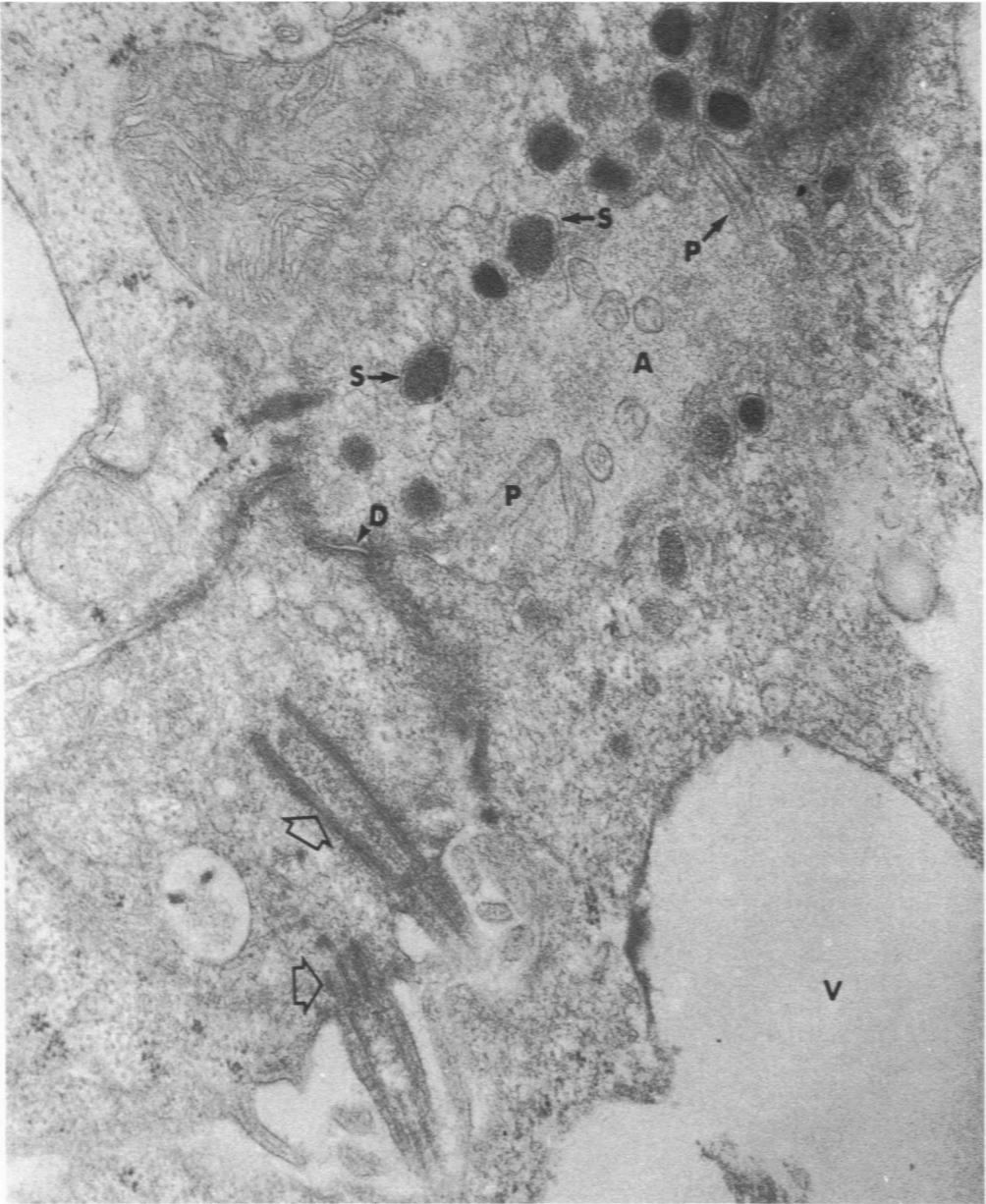


Fig 10.—Electron micrograph of group of transitional water-clear cells. Upper cell contains numerous secretory granules (S), and centriole. Surrounding central lumen (A) plasma membranes are thrown up in microvilli (P). Desmosomes (D) are present along cell membrane. Two cilia (arrows) are present in lower cell. Clear-cell vacuoles (V). Lead citrate and uranyl acetate. $\times 40,000$.

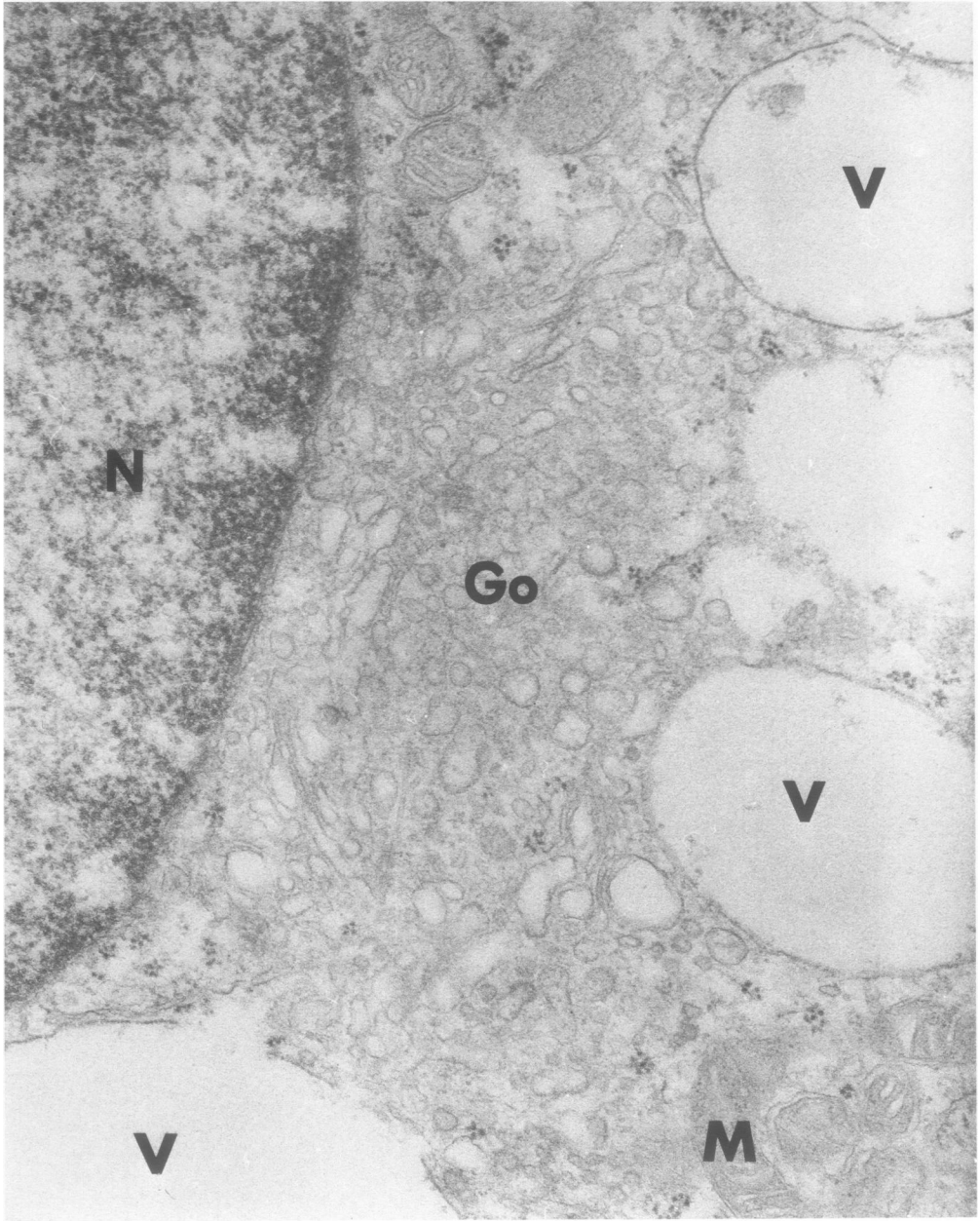


Fig 11.—Electron micrograph of Golgi region of clear cell. Golgi vesicles range from 0.045 to 0.250 μ . Most of Golgi vesicles are empty as are larger clear-cell vacuoles (V) in Golgi region. No secretory-type densities or prosecretory granules are present in Golgi. No definite evidence of fusion of Golgi vesicles into clear-cell vacuoles is present. Lead citrate and uranyl acetate. $\times 44,500$.