THE CYTOLOGY OF THE NORMAL PARATHYROID GLANDS OF MAN AND VIRGINIA DEER

A Light and Electron Microscopic Study with Morphologic Evidence of Secretory Activity

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ABSTRACT

The normal parathyroids of six humans and a Virginia deer were studied by light and electron microscopy. The parenchyma of the deer parathyroid is composed of uniform chief cells, which contained 100 to 400 mμ electron-opaque, membrane-limited granules, presumed to be secretory granules, in addition to the usual cytoplasmic organelles. Desmosomes are present between adjacent cells, and rare cilia are observed protruding from the chief cells into the intercellular space. The human parathyroids contain chief cells in two phases—active and inactive—as well as oxyphil cells. Active chief cells have a large Golgi apparatus, sparse glycogen, numerous secretory granules, and rare cilia. Inactive chief cells contain a small Golgi apparatus, abundant glycogen, and few secretory granules. Both forms have the usual cytoplasmic organelles and, between adjacent cells, desmosomes. Oxyphil cell cytoplasm is composed of tightly packed mitochondria and glycogen granules, with rare secretory granules. Cells with cytoplasmic characteristics intermediate between chief and oxyphil cells, possibly representing transitional cells, have been observed. Secretory granules of both man and deer are composed of 100 to 200 A particles and short rods, and the granules develop from prosecretory granules in the Golgi region of the cell. The human secretory granules are smaller and more variable in shape than those of the deer. The granules are iron and chrome alum hematoxylin–positive, argyrophilic, and aldehyde fuchsin–positive, permitting light microscopic identification. They are also found in the capillary endothelial cells of the parathyroid and in its surrounding connective tissue. The secretory granules of the parathyroid cells can thus be followed from their formation in the Golgi apparatus almost to their extrusion into the blood stream.

INTRODUCTION

The parathyroid gland has been described by light microscopists as containing a confusing and contradictory variety of secretion granules (2, 27). Endocrine organs that secrete a protein or a polypeptide hormone generally form an easily demonstrable secretory granule. Examples of such cells are the alpha and beta cells of the pancreatic islets (13, 17–19, 28), the eosinophils and basophils of the anterior pituitary (11), and the neurosecretory cells of the hypothalamus (33). Parathyroid hormone has been characterized by Rasmussen (35, 36) as a small protein or polypeptide having a molecular weight of 9500; hence, one might expect the parathyroid gland to form secretory granules as do other protein-secreting endocrine organs.

Grafflin (14) has convincingly described numerous iron hematoxylin–positive bodies in chief cells of the deer parathyroid gland. These granules could not be directly implicated as secretory granules, since they did not vary in number during the year in male deer nor in female deer in various physiologic states (15). Similar granules are present in the plates accompanying Rosof's description (39) of the rat parathyroid gland.

Weymouth and Baker (45) found small Bodian protargol–positive granules in the cytoplasm of the chief cells of parathyroid glands in seven species (rat, hamster, guinea pig, rabbit, dog, monkey, and man). These argyrophilic granules were not depleted upon stimulation of the rat parathyroid gland following bilateral nephrectomy but, rather, large argyrophilic granules accumulated in the cytoplasm (44). Suppression of secretory activity accompanying parathyroid hormone administration induced an increase in the concentration of these granules. On the basis of these findings, Weymouth (44) was unable conclusively to link these granules to stored parathyroid hormone.

Electron microscopic studies have not entirely clarified the nature of the secretory product in the parathyroid gland. Lever (22, 23), describing the fine structure of the rat parathyroid glands, felt that the "light" and "dark" cells indicated different states of the secretory activity. Trier (42) studied the ultrastructure of the parathyroid gland of the monkey and described a PAS-positive, diastase-resistant granule by light microscopy and a 0.1 μ granule of low electron opacity which he considers as possibly representing a secretion granule. Davis and Enders (9) noted small, dense granules derived from smaller granules and vacuoles in the Golgi region in the parathyroid glands of the rat. They felt that these granules were the secretory droplets, and that the granules coalesced into multivesicular bodies. Lange (20), in a study of human parathyroid adenomas, described several cytoplasmic inclusions, including electron-opaque, membrane-limited granules. He did not, however, identify them as the secretory granules. None of these studies related the various granules identified in electron micrographs to the descriptions of secretory material as visualized with the light microscope.

The present study was undertaken to study the ultrastructure of the hematoxylin positive bodies of the deer described by Grafflin (14, 15), and the argyrophilic bodies described by Weymouth (44) and Weymouth and Baker (45), and to determine whether these bodies were the secretory products of the parathyroid gland.

MATERIALS AND METHODS

Two parathyroid glands, one at the upper pole of each lobe of the thyroid gland, were taken from a 1 year old male Virginia deer, anesthetized with intramuscular barbiturate. These glands, measuring about 2 mm in diameter, were white, firm, somewhat rubbery, and easily separable from the surrounding thyroid gland and connective tissue.

Biopsies of human parathyroid glands were obtained from six adults undergoing surgical exploration of the neck. The six patients were a 40 year old white woman with a thyroid adenoma, a 42 year old white woman with a thyroid nodule, a 36 year old white woman with thyroid adenoma, a 38 year old white man with bone disease of undetermined origin, a 58 year old colored male with a thyroid adenoma, and a 42 year old colored female with a thyroid nodule. Each patient had one gland biopsied, except the fourth, who had all four glands biopsied.

The human biopsies and the deer parathyroid glands were bisected, and half was placed in neutral buffered 4 per cent formaldehyde (25) for 12 hours, dehydrated in alcohols, and embedded in paraffin. Sections of the paraffin-embedded material were stained with hematoxylin and eosin; periodic acid–Schiff (PAS), with and without prior diastase digestion; iron hematoxylin; Gomori's chrome alum

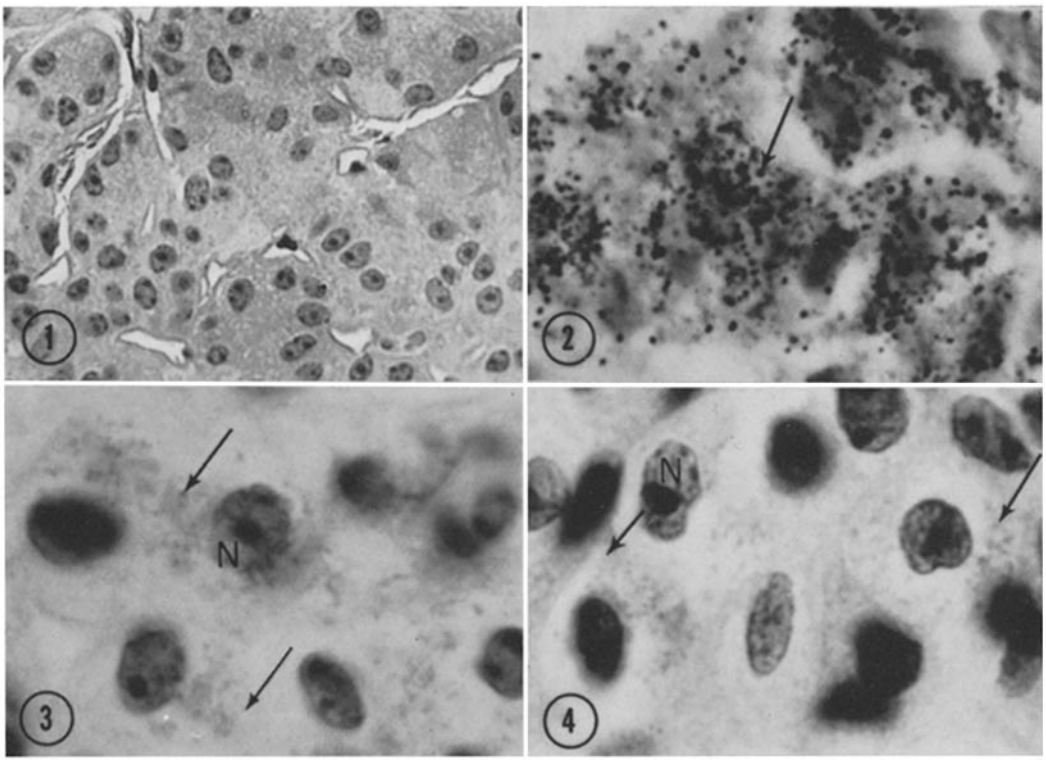


FIGURE 1

Deer parathyroid gland, osmium-fixed, H and P stained. Cords and sheets of chief cells are separated by small vascular and connective tissue spaces. × 650.

FIGURE 2

Deer parathyroid gland, formaldehyde-fixed paraffin sections stained with the Bodian protargol method. The individual granules (arrow) can be easily resolved and exhibit irregular outlines and variability in size (0.5 to 2 μ). × 1,800.

FIGURE 3

Deer parathyroid gland, formaldehyde-fixed, stained with iron hematoxylin. In the cytoplasm of the chief cells are numerous small hematoxylin-positive granules (arrows) which correspond in number to, but are somewhat smaller than, the argyrophilic bodies seen in Fig. 2. The density of the granules as seen in this micrograph is much less than in the Bodian-stained section. The nuclei (*N*) are also intensely stained with this technic. × 1,800.

FIGURE 4

Deer parathyroid gland, formaldehyde-fixed, stained with chrome alum hematoxylin. The cytoplasmic granules (arrows) of the chief cells are also stained with this technic and correspond to the Bodian-positive iron hematoxylin–positive bodies in Figs. 2 and 3. The nuclei (*N*) and granules (arrows) are stained blue-black in this preparation. × 1,800.

hematoxylin; Gomori's aldehyde fuchsin; Bensley's aniline–acid fuchsin following a 12 hour mordanting in Zenker's fluid for mitochondria (25), and Bodian's protargol (5).

The remaining tissue was placed in Dalton's chrome osmium fixative (7) or in a fixative developed by Bauer and Richardson (4, 38) containing 1 per cent osmium tetroxide in White's balanced salt

solution with extra calcium chloride. This latter fixative gave superior results. The osmium-fixed tissue was then dehydrated through alcohols. Half of the tissue was embedded in methacrylate, the other half in Epon 812 according to the method of Luft (26). One- to two-micron sections of the osmium-fixed, methacrylate-embedded tissues were stained with Ehrlich's hematoxylin and phloxine (H and P), PAS, iron hematoxylin, and chrome alum hematoxylin according to methods described in detail by Munger (29). Thin sections of Epon-embedded tissue were cut with glass knives on a Porter-Blum microtome and examined in RCA EMU 3C, 3D, and 3F electron microscopes. The sections were unstained, or were stained with uranyl acetate (43) or lead subacetate (8).

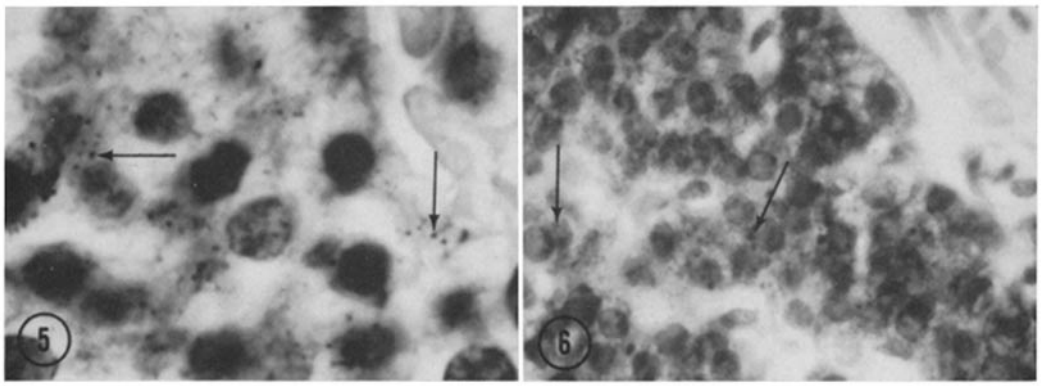


FIGURE 5

Human parathyroid gland, formaldehyde-fixed, stained with the Bodian silver method. In the cytoplasm of the chief cells are small, dense argyrophilic bodies (arrows) that correspond in general to those of the deer parathyroid gland in Fig. 2. The cytoplasmic granules in the human are smaller than in the deer, and more sparse. They tend to be located near the cell membranes. × 1,800.

FIGURE 6

Human parathyroid gland, formaldehyde-fixed, stained with the PAS method after diastase digestion. Within the cytoplasm of many cells are small PAS-positive, diastase-resistant bodies (arrows). These are larger and more irregular in outline than the Bodian-positive granules seen in Fig. 5. × 650.

OBSERVATIONS

Deer Parathyroid Gland

LIGHT MICROSCOPY: A single cell type, the chief cell, comprises the deer parathyroid gland. These chief cells have a moderate amount of acidophilic cytoplasm, and their nuclei are slightly irregular and stain intensely (Fig. 1). Small areas of cytoplasmic basophilia are scattered throughout the cell. A moderate amount of PAS-positive, diastase-digestible material, presumed to be glycogen, is present in the cytoplasm of the chief cells. Only scant PAS-positive, diastase-resistant material can be identified.

Small dense granules approximately 0.5 μ in diameter are scattered throughout the cytoplasm of chief cells when stained with Bodian protargol (Fig. 2), iron hematoxylin (Fig. 3), and chrome alum hematoxylin (Fig. 4). Each of these stains reveals similar distribution and number of these granules. Following the Bodian stain, individual granules (Fig. 2) are larger and more distinct (up to 2 μ in diameter), perhaps owing to precipitation of silver on the granules. These granules also stain faintly with aldehyde fuchsin. On the basis of arguments presented in the discussion, these granules are considered to represent secretory granules, and will be so designated in the remainder of the report.

The cells are arranged in cords and sheets separated by a prominent connective tissue stroma in which capillaries are embedded.

ELECTRON MICROSCOPY: Individual chief cells are polygonal and have nuclei of irregular

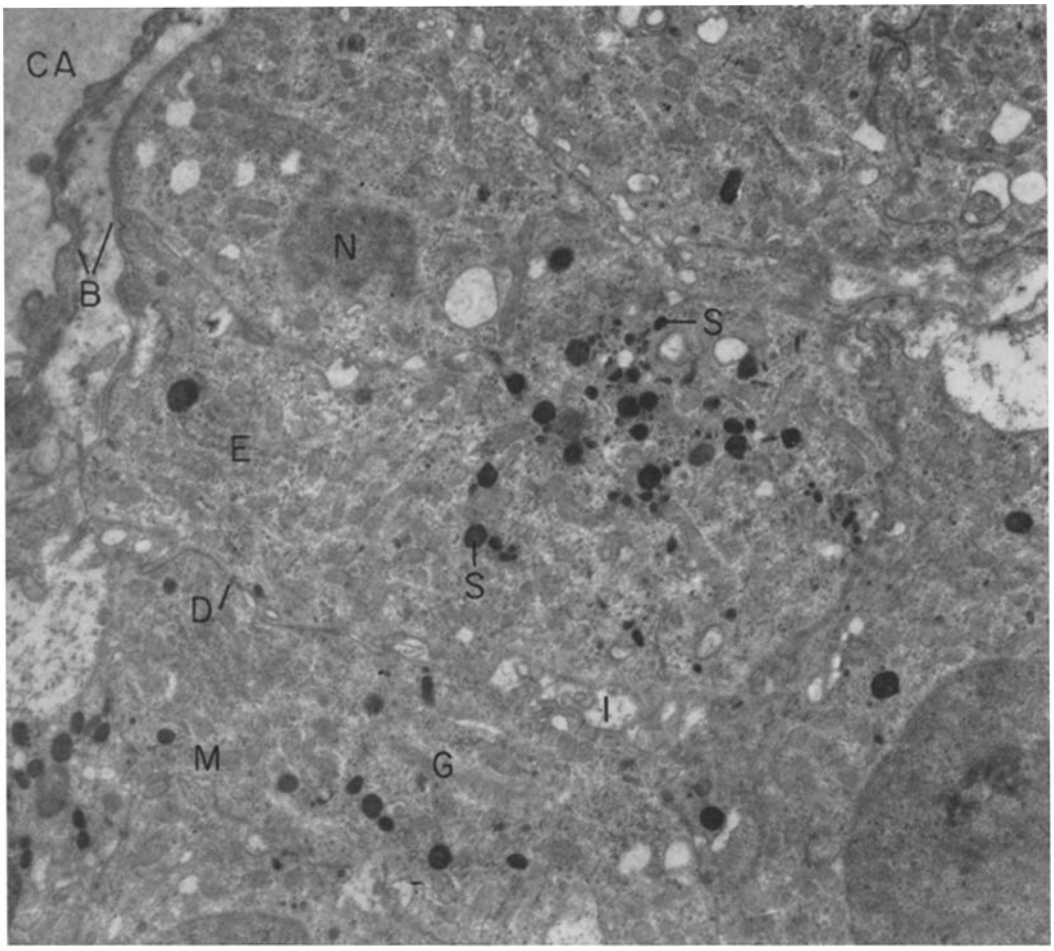
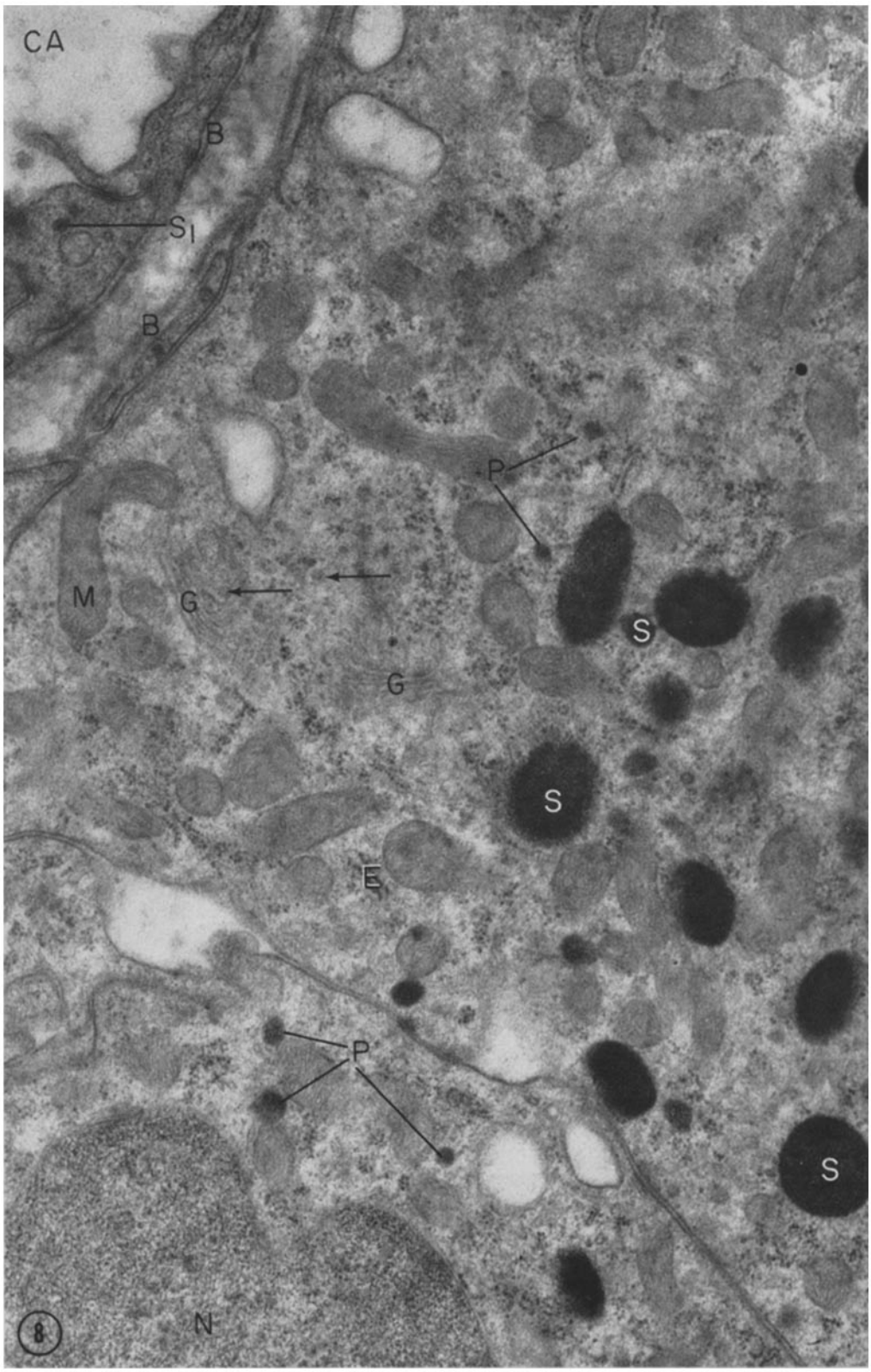


FIGURE 7

Deer parathyroid gland, uranyl acetate-stained. A group of chief cells abuts onto a small capillary (*CA*). Basement membrane (*B*) is applied to the plasma membranes of chief cells and capillary endothelial cells as they abut on the stroma. Within the cytoplasm of the chief cells are numerous dense secretion granules (*S*), which correspond in size and distribution to the Bodian-positive and the hematoxylin-positive granules seen in Figs. 2 to 4. Scattered mitochondria (*M*) and flattened ergastoplasmic sacs (*E*) are present in the cytoplasm which appears to be predominantly granular. A small intercellular space (*I*) is present between adjacent chief cells. A prominent Golgi apparatus (*G*) can be seen in most cells. A desmosome (*D*) is present between adjacent cells. A nucleus (*N*) cut in tangential section produces a small density in the upper cells. × 4,800.

outline (Fig. 7). The cytoplasm is granular, containing numerous ergastoplasmic sacs, 150 A particles presumed to be ribonucleoprotein granules, and rod-shaped mitochondria with loosely packed cristae (Figs. 7 and 8).

Large numbers of very dense, round to oval bodies 100 to 400 mμ in diameter are scattered throughout the cytoplasm (Figs. 7 and 8). These electron-opaque bodies correspond in location, size, and distribution to the secretory granules identified by light microscopy in Bodian- or hematoxylin-stained sections (Figs. 2 to 4). The individual secretory granules are surrounded by a delicate and closely applied limiting membrane (Fig. 9). This membrane is not always visualized, probably owing to the plane of sectioning. The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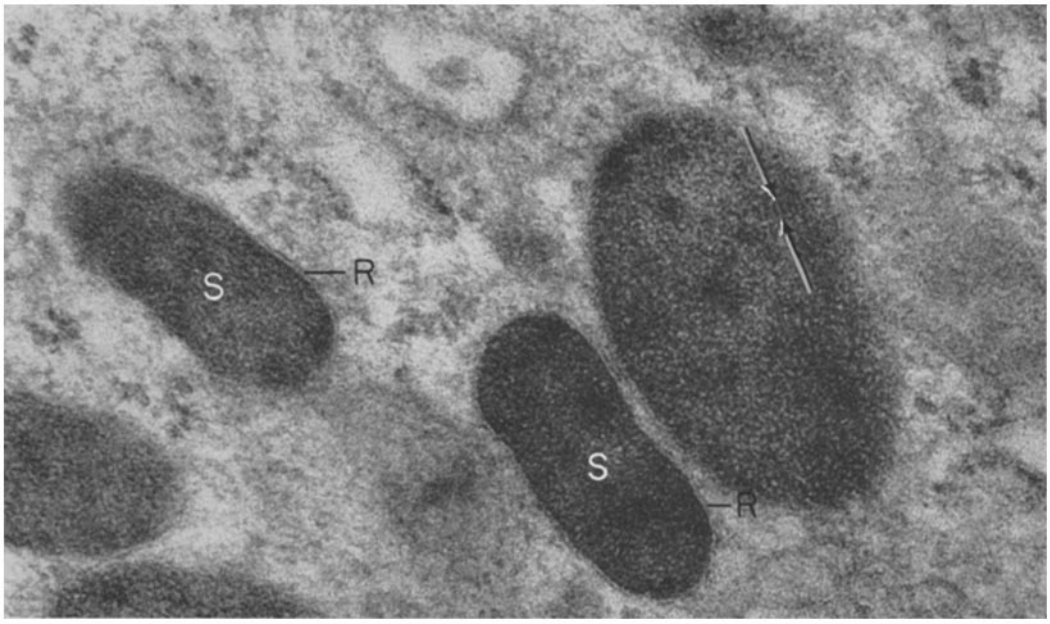


FIGURE 9

Secretion granules of the deer parathyroid, uranyl acetate–stained. The individual secretion granules (*S*) are bounded by a delicate limiting membrane (*R*), which cannot be resolved in some areas owing to tangential sectioning. Within the dense portion of a secretion granule, irregularly disposed dense rods (between the arrows) and small round, dense particles can be resolved. There is little suggestion of a crystalline packing of these densities. × 85,000.

dense internal portion of the granules contains closely packed short rods and round profiles (approximately 60 A in diameter) (Fig. 9), which are not grouped in any definable crystalline array.

The Golgi apparatus is prominent in most cells and is associated with numerous small vesicles, some of which appear to have an increased internal electron opacity. The increased density of these vesicles appears diffuse throughout; the vesicles are bounded by an agranular membrane (Fig. 8). These bodies might possibly represent prosecretory granules, that is, secretory granules in the process of formation. A morphologic sequence can be traced between these prosecretory granules and mature secretory granules (Fig. 8).

FIGURE 8

Deer parathyroid gland, uranyl acetate–stained. The cytoplasm of the chief cells contains numerous secretion granules (*S*) of varying size and shape. A prominent Golgi apparatus (*G*) is composed of flattened agranular membranous sacs associated with small vesicles of varying density (arrows) which might be interpreted as secretion granules in the process of formation (prosecretory granules). Other granules of small diameter and intermediate density (*P*) might also be prosecretory granules in a later state of maturation. Rod-shaped mitochondrial profiles (*M*) are scattered throughout the cytoplasm. Fattened ergastoplasmic sacs (*E*) as well as free ribonucleoprotein granules are present. A portion of one nucleus (*N*) is present in the lower left. Basement membrane (*B*) is applied to the cords of parathyroid chief cells as well as to the capillary (*CA*). Within the endothelial cells are occasional dense secretory granules (*S*) similar in appearance to those seen within the chief cell cytoplasm. × 44,000.

The plasma membranes of adjacent cells are usually straight and parallel, though there are areas of interdigitation. In some zones there is expansion of the intercellular spaces (Figs. 7 and 8). Adjacent cell membranes are occasionally connected by desmosomes (Figs. 7, 8, and 10). The structure of the desmosomes (Fig. 10) is identical with that seen in the desmosomes of instance depicted only eight external filaments can be counted. Only rare perfect cross-sections of cilia were observed[1] and the frequency of this occurrence could not be determined.

A prominent basement membrane is applied to the base of the chief cells as they abut on the large connective tissue space (Fig. 7). This space contains collagen, processes of fibroblasts, bundles of

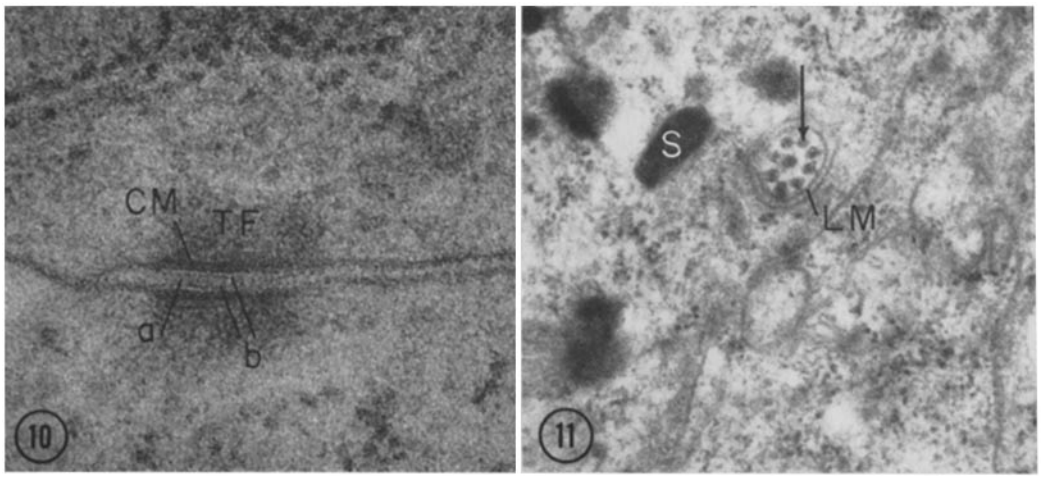


FIGURE 10

A desmosome from the adjacent cell membranes of two chief cells in the deer parathyroid gland, uranyl acetate–stained. This desmosome is identical with that seen in squamous epithelia (31). The apposed cell membranes (*CM*) are thickened, and each of the subunits of the individual cell membranes is more dense than usual. On either side of the cell membranes is a dense zone (*TF*) representing an area corresponding to tonofilaments. Between the cell membranes are three dense lines—the intermediate dense lines (*b*) and the intercellular contact layer (*a*). × 110,000.

FIGURE 11

A cross-section of a cilium between two chief cells of the deer parathyroid gland, uranyl acetate–stained. The cilium is bounded by a definite limiting membrane (*LM*) and within the cilium are eight peripheral filaments (arrow) and one central filament. A few secretion granules (*S*) are present adjacent to the cilium. × 35,000.

stratified squamous epithelium (31). Each unit membrane of the apposed plasma membranes is thickened, and there are three lines of increased density between the plasma membranes. There is a zone of increased density in the cell cytoplasm on each side of the desmosomes similar to the area of the attachment of tonofilaments in the epidermis (31), although no distinct bands of tonofilaments are present in the parathyroid cell cytoplasm.

Within occasional chief cells cilia have been observed protruding from the plasma membrane into the intercellular space (Fig. 11). In the unmyelinated nerve fibers within Schwann cells, and capillaries. This space is similarly prominent in the parathyroid glands of other species (2, 22, 42), but not in other endocrine organs (10, 11, 28, 30). Occasionally 100 mμ dense granules are present in the stroma and also within endothelial cells of adjoining capillaries (Fig. 8). Such granules within endothelial cells are more prominent in the human.

[1] Recently, in our laboratory, Dr. H. Salazar has observed eight external filaments repeatedly in cells of the rabbit anterior pituitary.

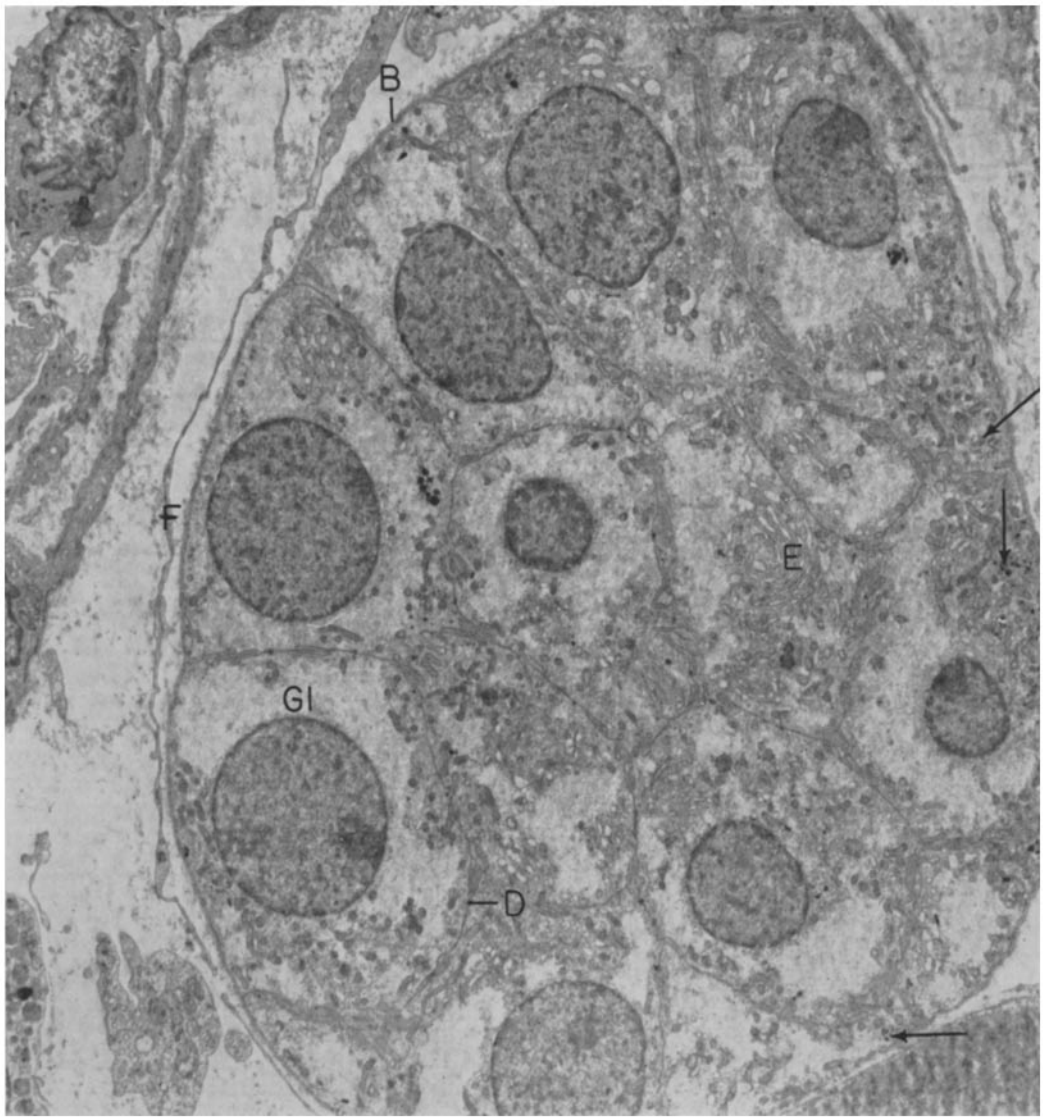


FIGURE 12

Human parathyroid gland, uranyl acetate–stained. A lobule of chief cells is surrounded by a definite basement membrane (*B*). The connective tissue stroma contains capillaries such as those at the upper left, delicate processes of fibroblasts (*F*), collagen, and occasional fat cells, a portion of one appearing at the lower right. Within individual chief cells are prominent accumulations of glycogen (*Gl*). Clumps of ergastoplasm (*E*) and mitochondria are scattered throughout the cytoplasm. Dense secretion granules (arrows) can be identified even at this very low magnification. Desmosomes (*D*) are present between adjacent chief cells. × 3,800.

Human Parathyroid Gland

LIGHT MICROSCOPY: The normal human parathyroid is composed of sheets, cords, and rare acini of chief cells, and of occasional oxyphil cells. There is a prominent fibrous stroma with numerous fat cells. Chief cells are polygonal, 7 to 10 μ in diameter, with a slightly acidophilic cytoplasm. The oxyphil cells, found either singly

or in groups, have a brightly acidophilic cytoplasm and are larger than the chief cells. Many of the chief cells contain large amounts of PAS-positive, diastase-digestible material, presumably glycogen. There are also numerous globules of diastase-resistant, PAS-positive material (Fig. 6), most likely corresponding to the fluorescent pigment described by Hamperl (16). The oxyphils contain large numbers of mitochondria, as indicated by the aniline–acid fuchsin stain for mitochondria.

A moderate number of small iron hematoxylin–positive, chrome alum hematoxylin–positive, and argyrophilic (Fig. 5) granules are present in the cytoplasm of human chief cells. These granules are especially concentrated near the plasma membrane. The granules are smaller and less numerous in man than in the deer, but have identical staining properties. The distribution and numbers of the granules are the same with the Bodian and hematoxylin stains. In occasional chief cells no granules can be identified. These granules could not be found in oxyphils.

ELECTRON MICROSCOPY: Within the lobules of parathyroid tissue (Fig. 12) two types of chief cells (Fig. 13) can be identified, based on the presence of secretory granules, the prominence of the Golgi apparatus, and the amount of glycogen present in the cell. These will be referred to as "active" and "inactive" on the basis of arguments presented in the discussion.

Active chief cells are characterized by the presence of a prominent Golgi apparatus, scant cytoplasmic glycogen, numerous secretory granules (Fig. 13), and occasional arrays of flattened ergastoplasmic sacs (Fig. 14). The Golgi apparatus is present in the perinuclear region of the cell. Intimately associated with the Golgi apparatus are numerous small vesicles and small granules (Figs. 13 and 15), some of which may represent prosecretory granules.

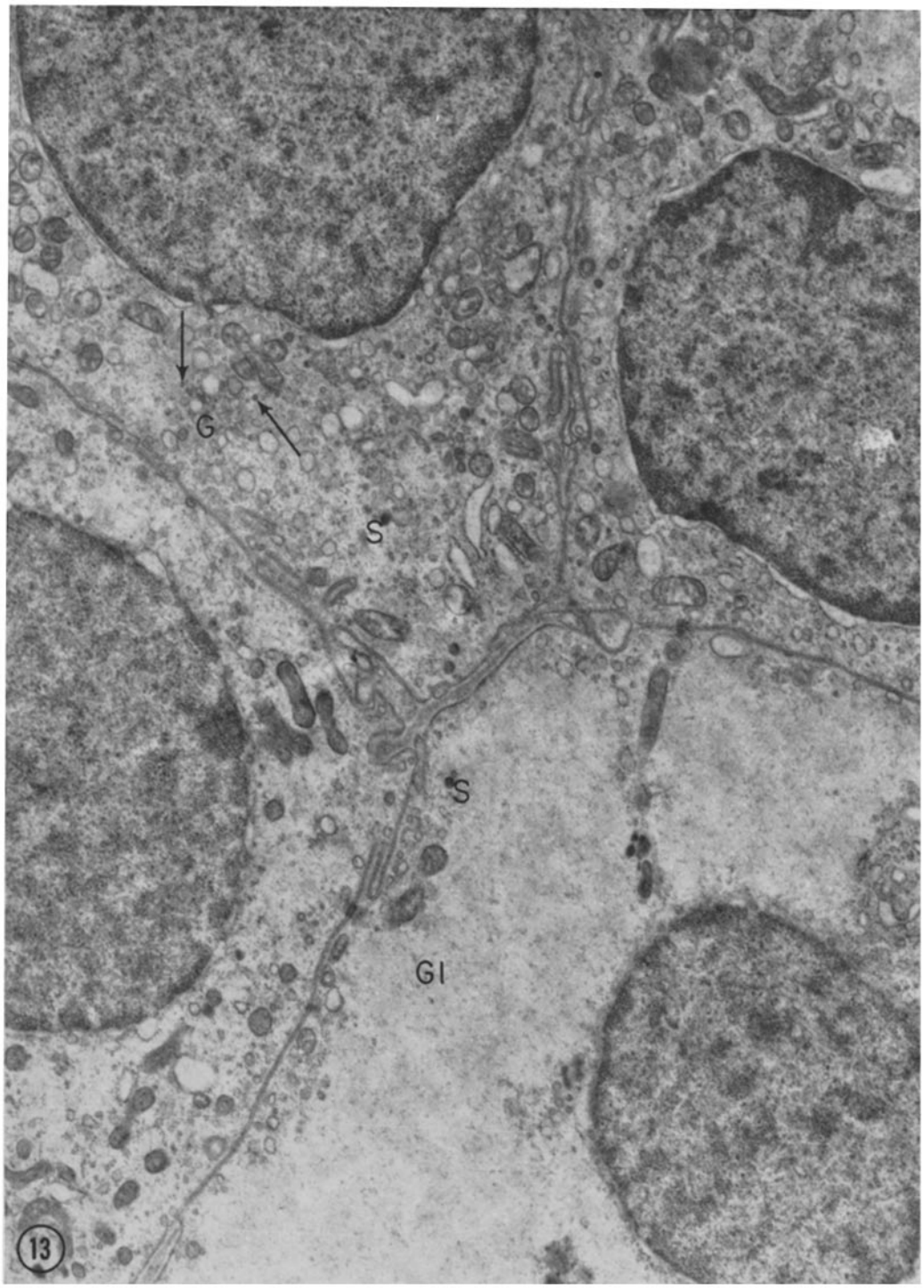
Secretory granules are relatively numerous in active chief cells and correspond in distribution to the argyrophilic and hematoxylin-positive granules seen with the light microscope. Frequently secretory granules are concentrated near the plasma membrane, especially subjacent to a capillary or connective tissue space (Fig. 12). Both prosecretory granules and mature secretory granules have irregular outlines; *i.e.*, some are oval, others are dumbbell-shaped or are of bizarre outline (Fig. 16). In some cells the secretory granules have areas of increased density within the substance of the granule (Fig. 16). Internally these secretory granules are composed of small granules and rods, similar in appearance to those of the secretory granules of the deer. The granules are bounded by a limiting membrane.

The cytoplasm of active chief cells is finely granular and contains rod-shaped and filamentous mitochondria, and in some specimens large, round, often multilocular electron-opaque bodies with central portions of low density (Fig. 17). These bodies, presumably lipofuscin, correspond in size and distribution to the PAS-positive, diastase-resistant globules seen in light microscopic sections. They probably represent the very prominent autofluorescent "wear and tear pigment" identified by Hamperl (16) in the human parathyroid gland. Scattered sacs of ergastoplasm and ribonucleoprotein particles are also present. The occasional parallel arrays of flattened ergastoplasmic sacs (Fig. 14) correspond to the "basophilic body" described by light microscopists (2), and this identification agrees with that of Trier (42) in the monkey parathyroid gland. Cilia have been observed in an occasional active chief cell. These cilia appear identical with those seen in the deer parathyroid cells.

Resting or inactive chief cells, which are the most numerous cells of the normal human parathyroid, are characterized by a granular cytoplasm containing large lakes of glycogen (Figs. 12 and 13). Lipid bodies are also prominent in many of

FIGURE 13

Human parathyroid chief cells, uranyl acetate–stained. The cell to the lower right is an inactive chief cell containing a large amount of glycogen (*Gl*) in the cytoplasm, with only scant cytoplasmic organelles and secretory granules (*S*). The cell to the upper left (active chief cell) has scant glycogen, more numerous secretory granules and a prominent Golgi apparatus (*G*) in which area prosecretory granules (arrows) can be seen. The cell to the lower left is intermediate in type. × 12,000.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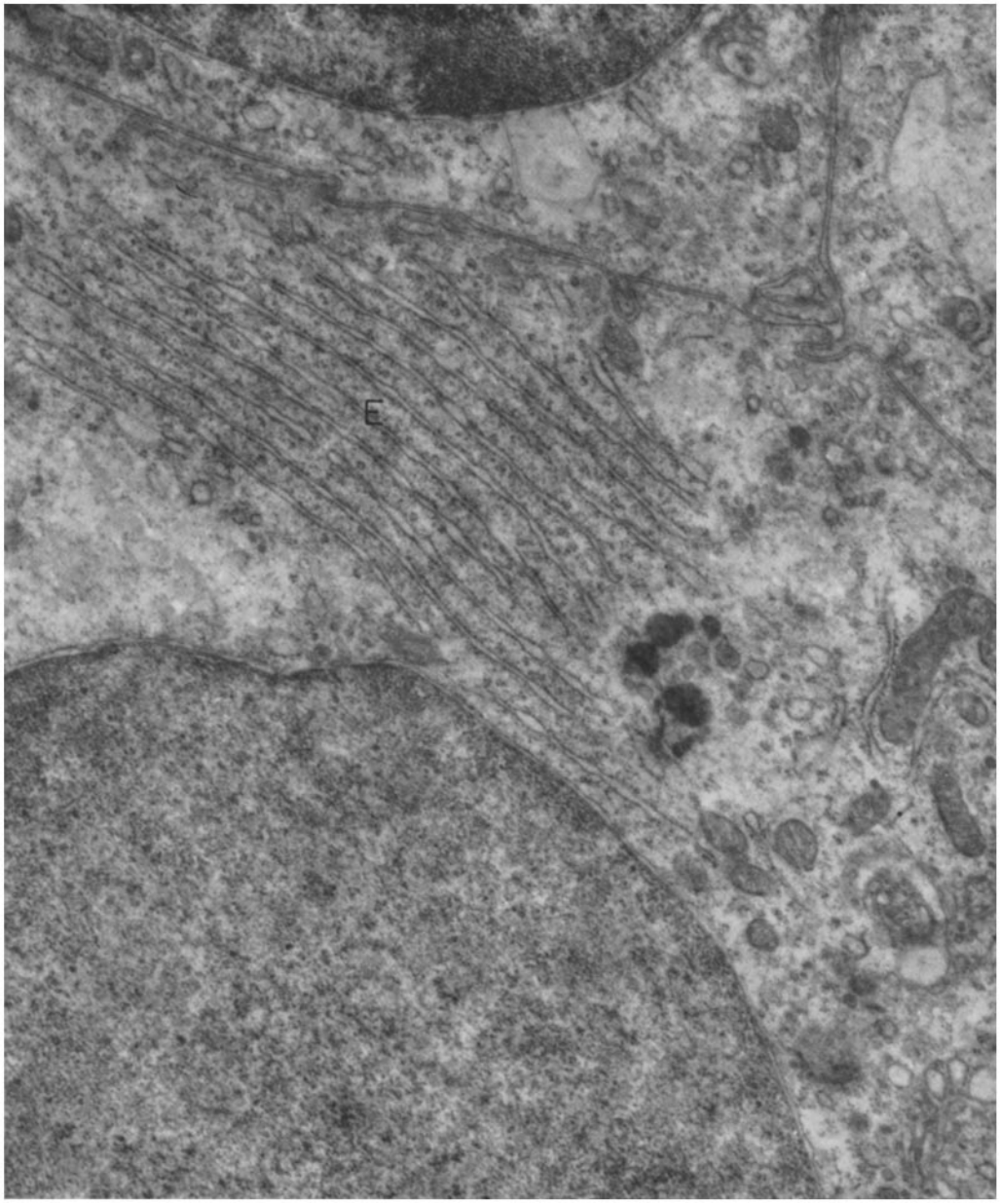


FIGURE 14

Human parathyroid gland chief cell, uranyl acetate–stained. A parallel array of flattened ergastoplasmic sacs (*E*) is present. Such an accumulation of ergastoplasmic sacs would produce a distinct area of cytoplasmic basophilia by light microscopy, that is, a basophilic body. A portion of a small Golgi apparatus of this cell is present at the lower right. × 14,500.

these inactive cells. The Golgi apparatus of the resting chief cells is small. There are only rare accumulations of secretory granules; these are identical with those of the active cells, and they are usually seen near the plasma membrane (Fig. 12). The ergastoplasm of inactive chief cells is characteristically distributed as localized lamellar arrays of flattened granular sacs (Fig.

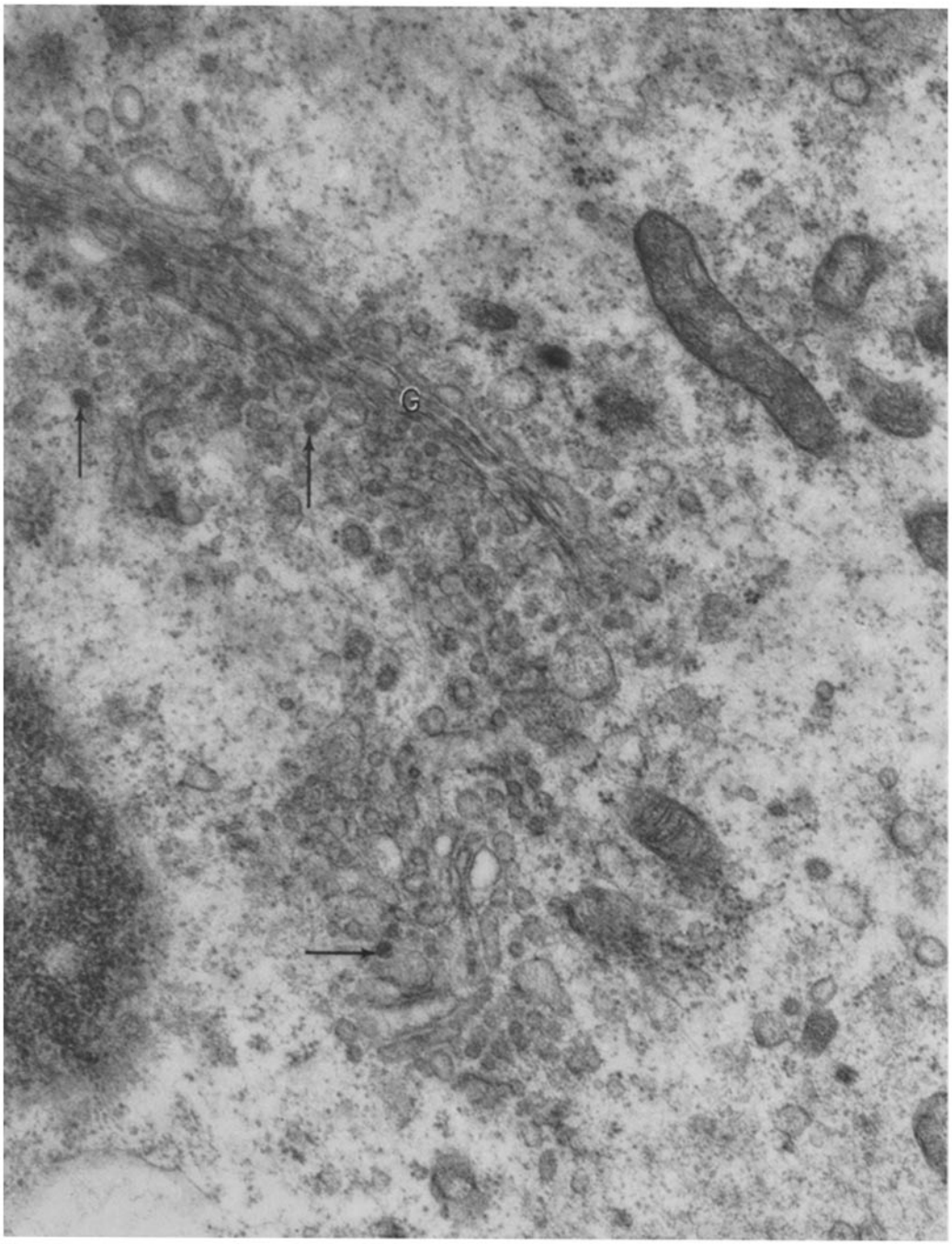


FIGURE 15

Human parathyroid gland, Golgi region of an active chief cell, uranyl acetate–stained. A prominent Golgi apparatus (*G*) is present associated with numerous small vesicles and granules of varying density (arrows) which appear to represent secretory granules in the process of formation, that is, prosecretory granules. × 37,000.

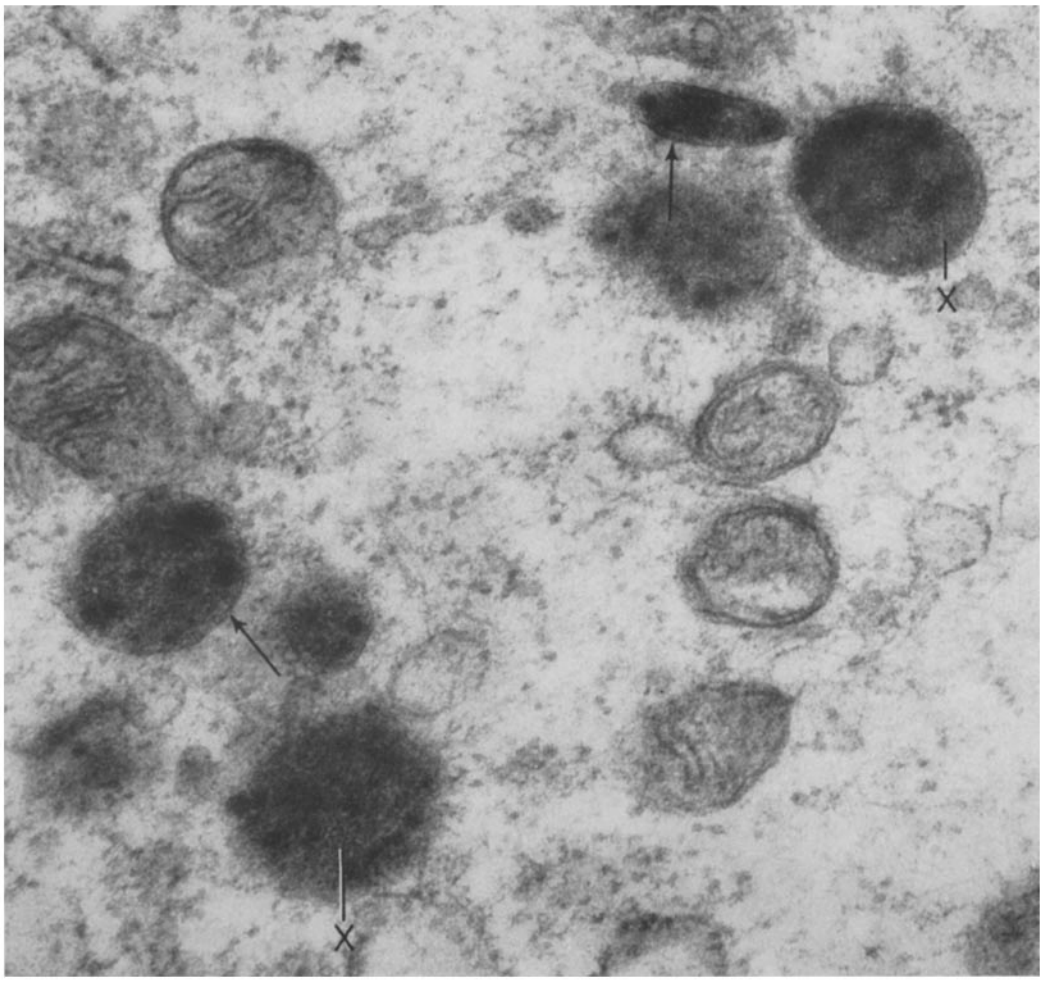


FIGURE 16

Human parathyroid gland, secretory granules in a chief cell, uranyl acetate–stained. Secretory granules of varying size are bounded by a definite limiting membrane (arrows). The internal portion of the granule is composed of short, dense, rod-like profiles (*X*), which in some areas assume an array suggesting crystalline packing. Areas of varying density are present within the secretory granules, as if the dense rods and granules were packed more closely. × 85,000.

12). Cilia have not been observed in resting chief cells.

Connecting the adjacent plasma membranes of both the active and inactive chief cells are occasional desmosomes identical in fine structure with those seen in the deer parathyroid gland and similar to those of the epidermis (31).

Chief cells are occasionally grouped into acinar units (Fig. 17). Such acinar units are characterized by microvilli projecting from the apical surface of the cell into the lumen and by terminal bars present between adjacent plasma membranes at the luminal surface. Desmosomes also connect adjacent acinar cells. The internal structure of the acinar chief cells is identical with that of other chief cells—both inactive and active cells being present and containing scattered secretory granules. The acinar lumen contains only a faintly granular material of low electron opacity.

The oxyphil cells (Fig. 18) are polygonal and

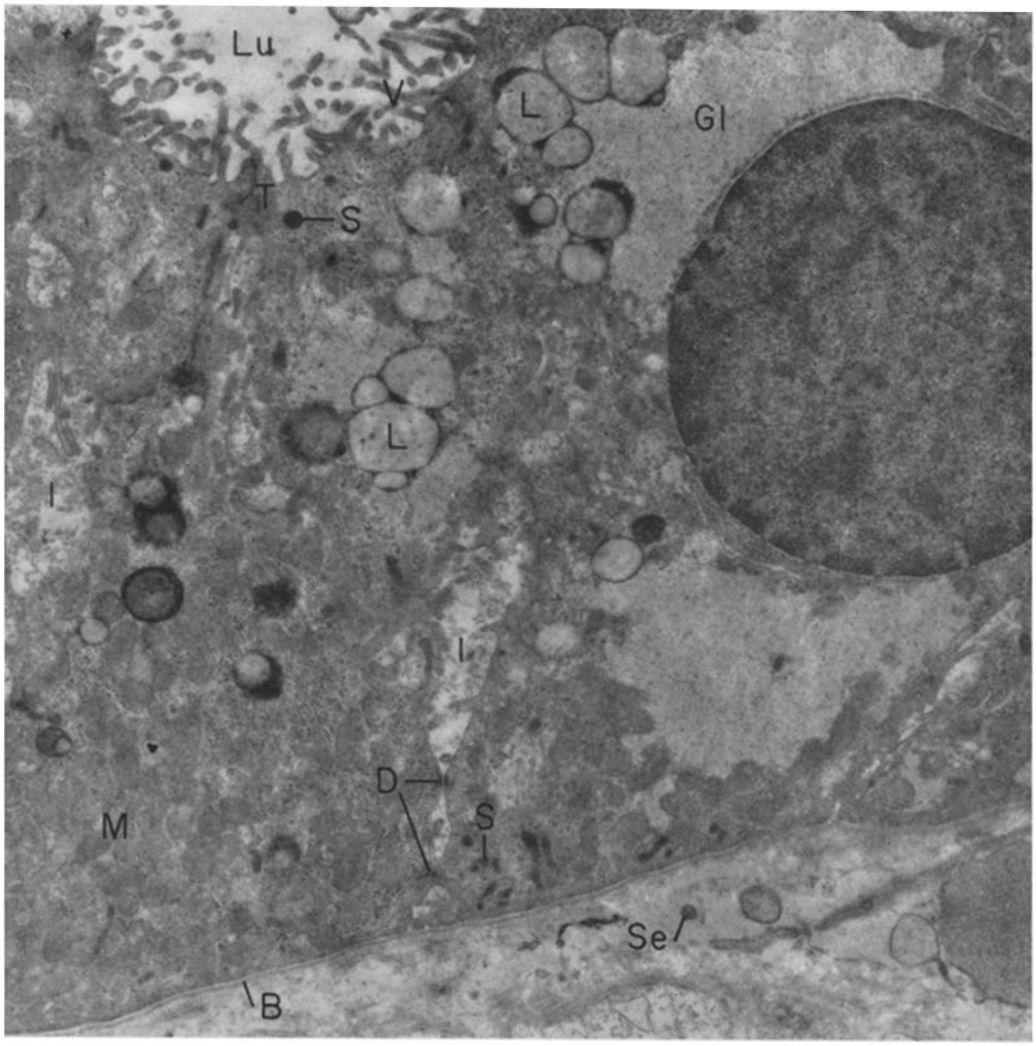


FIGURE 17

Human parathyroid gland, an acinar configuration of several chief cells, uranyl acetate–stained. The chief cells surround a distinct lumen (*Lu*), into which microvilli (*V*) project from the apical cytoplasm of the cells. The cell membranes of the adjacent cells are thickened as they abut at the lumen to form terminal bars (*T*), and desmosomes (*D*) also are present between the apposed cell membranes, but are not related to the lumen. Within the cytoplasm of the cells are large accumulations of glycogen (*Gl*), lipid (*L*), and scattered secretion granules (*S*). The large number of mitochondria (*M*) present to the lower left is reminiscent of the appearance of an oxyphil cell (Fig. 19) and suggesting that this could represent a transitional state between chief cell and oxyphil. The intercellular space (*I*) between adjacent cells is prominent. A distinct basement membrane (*B*) surrounds the group of cells. Within the connective tissue is a rounded dense body (*Se*) which might be interpreted as an extracellular secretion granule. × 9,600.

stellate in outline. Their cytoplasm is largely filled with tightly packed, rod-shaped mitochondria having closely packed interdigitating cristae. Occasionally mitochondrial granules are present. Between the mitochondria the cytoplasm is filled with granules 300 to 500 A in diameter whose electron opacity increases after staining with lead salts. This material is presumed to

represent glycogen (37). In the oxyphil cells, ergastoplasm and Golgi membranes have not been observed. Rare secretory granules, similar to those found in chief cells, can be found in oxyphil cells.

Occasional cells in the normal human parathyroid gland have cytoplasmic characteristics intermediate between chief and oxyphil cells (Fig. 19). Mitochondria are numerous, but Golgi and ergastoplasmic membranes are present unlike a classical oxyphil cell.

Surrounding the cords and sheets of human parathyroid cells is a distinct basement membrane (Figs. 12, 17, 19, and 20) into which collagen fibers insert themselves. Between the basement membrane and surrounding capillaries is a prominent connective tissue space containing numerous fibroblasts, collagen fibers, and unmyelinated nerve fibers enmeshed in Schwann cell cytoplasm. This space is larger and more prominent in the human than in the deer parathyroid gland; this finding is in agreement with the light microscopic descriptions of human parathyroid glands (34). Occasional dense granules (Fig. 17) are present in the connective tissue space, similar in structure to the secretory granules within the chief cell cytoplasm. Such granules can also be rarely seen within Schwann cell cytoplasm (Fig. 20) associated with unmyelinated nerves in the gland, but granules have never been observed within the axoplasm.

Secretory granules are frequently present within the endothelial cell cytoplasm of capillaries associated with the parathyroid gland (Fig. 21). These granules in the capillaries are more numerous in the human parathyroid than in the deer parathyroid. The internal structure and size of the granules in the capillaries in man appear to be similar to those observed in the chief cells of the parathyroid gland. The secretory granules within the endothelial cells vary in number from capillary to capillary, but they can be identified within all specimens of human and deer parathyroid examined. In rare instances (Fig. 21) a granule was observed in a cytoplasmic bulge protruding from the surface of an endothelial cell into the capillary lumen, suggesting release of the granule into the blood stream.

DISCUSSION

The hematoxylin-positive bodies (14, 15) and the argyrophilic bodies (44, 45) present in the chief cells of the human and deer parathyroid glands have thus been identified as electron-opaque, membrane-limited cytoplasmic granules. Positive identification of these bodies as secretory granules awaits successful isolation coupled with structural, chemical, and physiologic studies. On the basis of circumstantial evidence, however, these granules can be considered to be secretory granules.

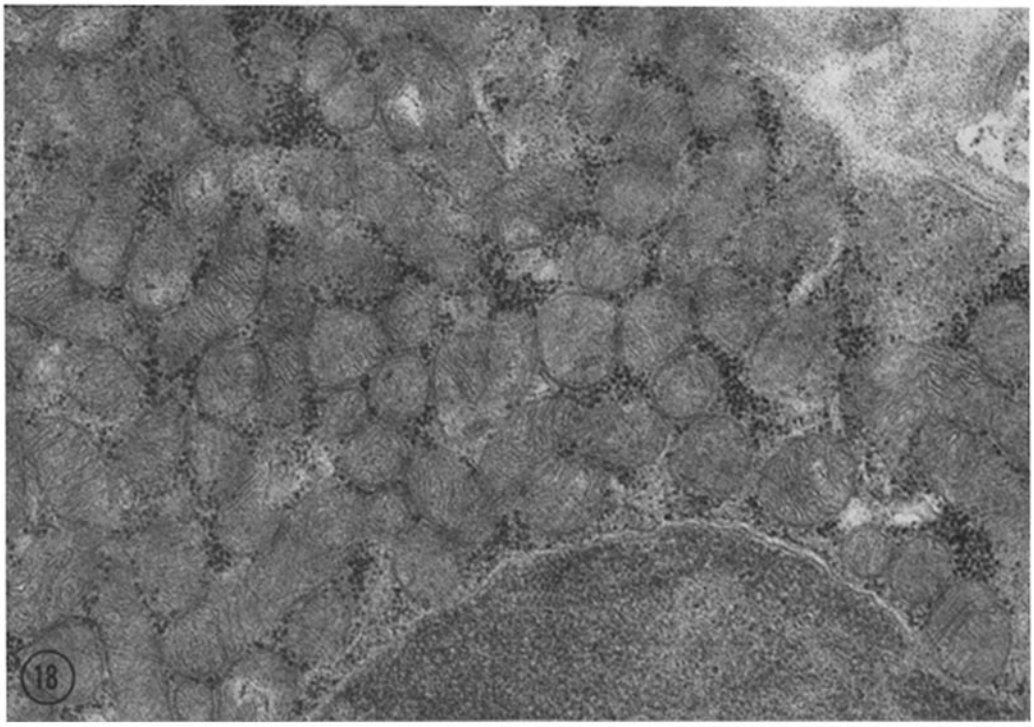
First, the ultrastructure of these granules resembles that of secretory granules of other endocrine organs secreting protein and polypeptide substances. These include the hypothalamus (33), the anterior pituitary (11) and the alpha and beta cells of the pancreatic islets (13, 18, 28, 30). In the second place, the staining of these granules with chrome alum hematoxylin and aldehyde fuchsin is similar to that of the insulin-containing granules (17, 19, 28) in the beta cells of the pancreatic islets and to that of the various cells of the anterior (11) and posterior (3) pituitary and hypothalamus (33). The granules of the parathyroid cells differ from those of other glands in that they are argyrophilic with the

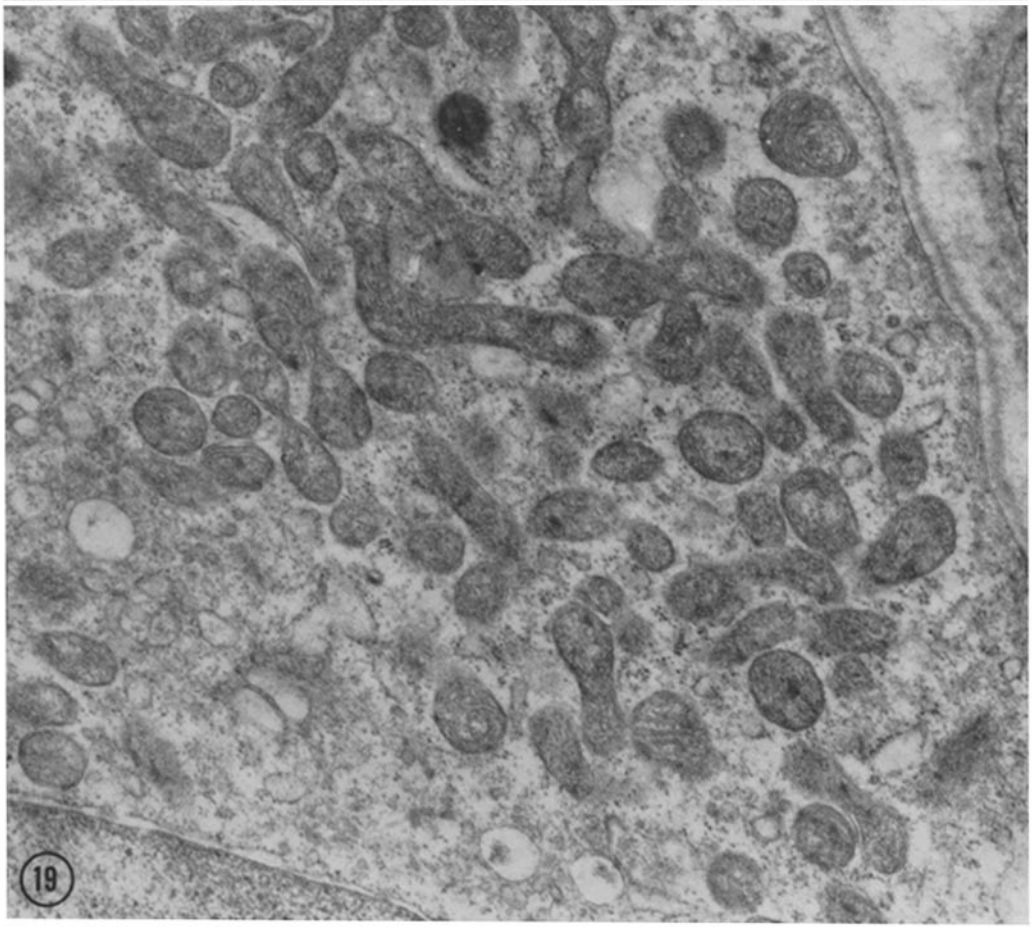
FIGURE 18

Human parathyroid gland oxyphil cell, stained with lead subacetate. The cristae of the mitochondria are very closely packed. Between the mitochondria are numerous glycogen granules. × 42,000.

FIGURE 19

Human parathyroid gland, transitional oxyphil, uranyl acetate-stained. This cell appears to be intermediate in characteristics between the oxyphil depicted in Fig. 18 and the chief cells of the previous figures. Many mitochondria are present, but they are separated by a generous amount of background cytoplasm containing scattered ergastoplasmic sacs and agranular membranes of the Golgi apparatus. The cell is bounded on the right by basement membrane and connective tissue space. × 25,000.


18


19

Bodian protargol stain. Third, we have shown that these granules may develop sequentially from small vacuoles and granules in the region of the Golgi apparatus. This apparatus is thought to function as an organelle for packaging of cell secretory products (28, 30, 32, 33). In the human parathyroid chief cells with numerous secretory granules, the Golgi apparatus is large and prominent, while in those chief cells with few secretory granules, the Golgi apparatus is small and relatively inconspicuous. Fourth, studies on functional human parathyroid adenomas (20, 41) have indicated large numbers of such granules in parathyroid cells. Similar relationships are present in parathyroid cells of patients found to have primary chief cell hyperplasia (41) as a cause of their hyperparathyroidism. Fifth, surrounding parathyroid adenomas in the rims of "atrophic normal" tissue, there are only rare secretory granules (41). Lastly, differential centrifugation studies by L'Heureux and Melius (24), though not controlled by electron or phase microscopic studies of the fractions, indicate that most of the parathyroid hormone activity of the bovine gland is associated with a particulate fraction.

Thus, on the basis of this indirect evidence, we can presume that the electron-opaque, membrane-limited granules seen in the parathyroid glands represent secretory granules. A structural basis for the existence of a second parathyroid hormone postulated by Copp *et al.* (6) could not be determined in so far as the structure of the secretory granules is concerned.

These granules are present in the parathyroid glands of all species so far studied with the electron microscope. Davis and Enders (9) considered similar dense granules in the parathyroid gland of the rat to possibly represent secretion product, and they described the coalescence of the granules into multivesicular bodies, a finding which we were unable to confirm in human and deer parathyroids. Trier (42), in the monkey, did not describe secretory granules of the type discussed in this paper, but in the plates accompanying his paper one can identify granules similar to those seen in human parathyroids. This would be consistent with the finding of argyrophilic granules in monkey parathyroid glands by Weymouth and Baker (45).

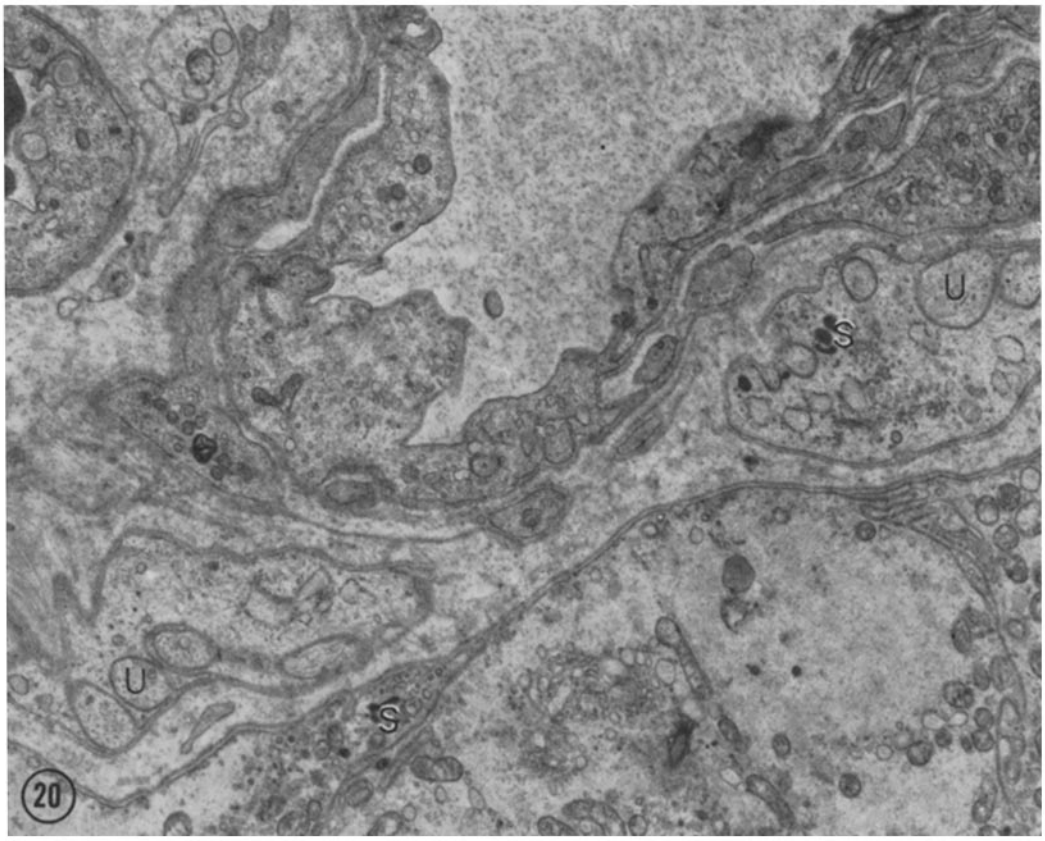
The presence of an occasional secretory granule in an oxyphil cell would seem to indicate that these cells are capable of some production of parathyroid hormone. But, cytologically, these cells in the normal parathyroid do not appear to be secretory, since they lack prominent ergastoplasm and Golgi apparatus. Yet these cells do have a considerable amount of histochemically demonstrable oxidative enzyme activity (1). The oxyphil cells of the monkey parathyroid (42), the human parathyroid adenoma (20, 41), and the hyperplastic human parathyroid (41) are essentially the same as those of the normal parathyroid, except that in one parathyroid adenoma and one case of primary chief cell hyperplasia (41) the oxyphil cells contained large numbers of secretory granules, a prominent ergastoplasm, and a prominent Golgi apparatus. These findings

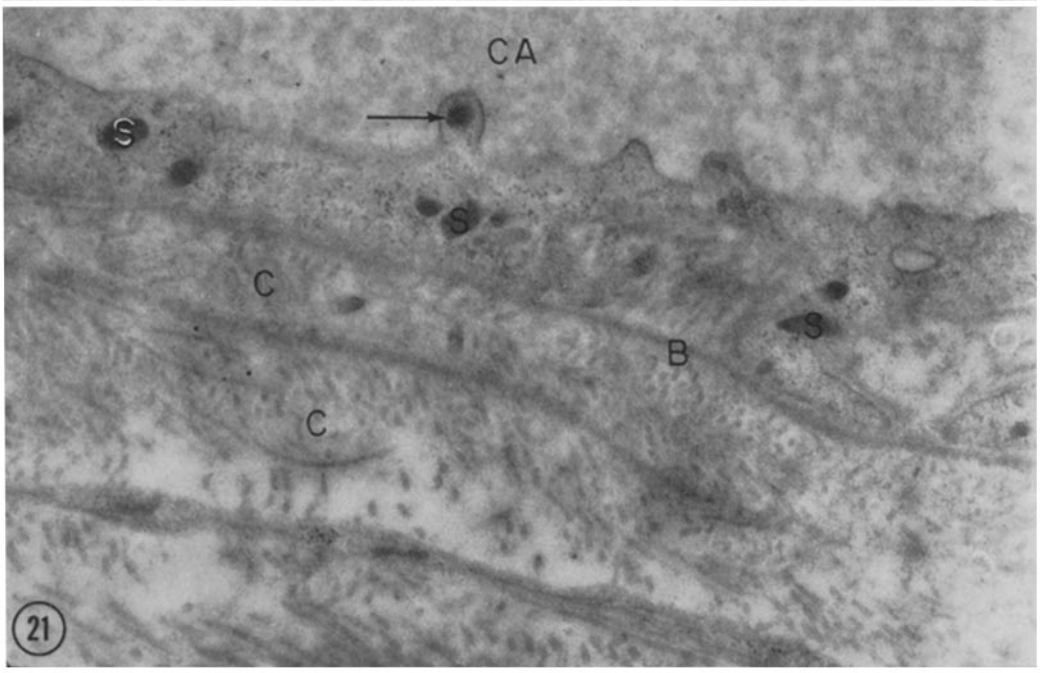
FIGURE 20

Human parathyroid gland, uranyl acetate–stained. A capillary is present in the center of the micrograph. Two portions of a bundle of unmyelinated nerves (*U*) embedded in Schwann cell cytoplasm are present in the connective tissue space between the capillary and the parathyroid glandular tissue. Dense secretory granules (*S*) are present in the cytoplasm of the Schwann cell identical in appearance to those present in the chief cells. No such granules are ever seen within the axons of such unmyelinated nerves. × 9,600.

FIGURE 21

Human parathyroid gland, a portion of an endothelial cell, uranyl acetate–stained. The capillary lumen (*CA*) is present at the top of the micrograph. Numerous parathyroid secretory granules (*S*) are present in the cytoplasm, and one is contained in a protuberance (arrow) of endothelial cell cytoplasm projecting into the capillary lumen. The collagen fibers (*C*) and basement membrane (*B*) surrounding the capillary are well defined. × 26,500.


U
S
U
S
20


CA
S
S
C
B
S
C
21

raise the possibility that some oxyphil cells are probably capable of production of parathyroid hormone.

Cells intermediate in cytoplasmic characteristics between chief and oxyphil cells are occasionally seen in electron micrographs. Such transitional cells have been described by light microscopy (40), but even by electron microscopy positive identification as transitional oxyphil cells is uncertain. The importance of such tentatively identified transitional cells awaits further study.

The problem of classification of cell types in the parathyroids has not been completely solved. Roth (40) recently proposed a classification of the cell types based on light microscopic examination of hematoxylin and eosin preparations of normal and pathologic parathyroid glands. In this classification the chief cells were divided into a light and a dark type. Based on the ultrastructural appearance of the active and inactive chief cells, it is felt that the "light chief cell" represents the relatively glycogen-rich, slightly larger, inactive chief cell, and that the "dark chief cell" is the smaller, relatively glycogen-poor active chief cell. It is presumed that individual cells undergo periods of active secretion followed by periods of physiologic rest. True water-clear cells could not be identified in any of the preparations of normal human parathyroid glands we studied. Neither did we find transitional water-clear or vacuolated chief cells in our electron micrographs.

The presence of parathyroid secretory granules in the capillary endothelial cells of both the normal human and deer parathyroid is an interesting finding. In human parathyroid adenomas and primary chief cell hyperplasias (41), numerous parathyroid secretory granules have also been found in the capillary endothelial cells. A rare granule has been found in the extracellular perivascular space. It is thus presumed that the intact secretory granule leaves the parathyroid chief cell, traverses the epithelial basement membrane, the perivascular extracellular space, and the capillary basement membrane to enter the capillary endothelial cell. Granules then appear to be accumulated in the endothelial cells and released into the blood stream. The mode of this passage and the method of uptake and release of the granules by the endothelial cells are not clear. Neither is it clear whether this storage of granules in endothelial cells plays a role in the control of the rate of parathyroid hormone secretion. A somewhat similar secretory mechanism has been found in the alpha cells of the pancreatic islets, where the secretory granule breaks up into small particles within the cytoplasm which are transported intact into the capillaries (30).

The importance of the cilia found in the active chief cells of the human parathyroid gland and in the cells of the deer parathyroid gland is unknown. Similar findings have been obtained in the islet cells of the pancreas (28, 30) and the adrenal medulla (10) and the secretory cells of the hypophysis (3). The interesting feature of the cilia found in the parathyroids is that there were only eight external fibers and one internal fiber in instances where cross-sections were obtained. This is in contrast with the almost universal finding in other organs and cells of nine pairs of external fibers and two internal fibers (12) or nine pairs of external fibers and no internal fibers (3).

The human parathyroid gland has been shown to have large amounts of autofluorescent lipid (16) in the chief cell cytoplasm. This lipid, which corresponds in distribution to the PAS-positive, diastase-resistant material and to the "lipid" bodies in the electron micrographs, has been considered by Hamperl (16) to be "wear and tear" pigment. This could account for its relative sparsity in the cells of the young deer parathyroid which we examined, and in the cells of the young monkey parathyroid examined by Trier (42). Similar lipid inclusions are quite abundant in the cells of parathyroid adenomas (20, 41) and in those of the rim of normal parathyroid tissue surrounding human parathyroid adenomas (41).

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REFERENCES

1. BALOGH, K., and COHEN, R. B., Oxidative enzymes in the epithelial cells of normal and pathological human parathyroid glands. A histochemical study, *Lab. Inv.*, 1961, **10**, 354.
2. BARGMANN, W., Die Epithelkorperchen *in* Handbuch der mikroskopischen Anatomie des Menschen, (W. v. Mollendorff, editor), Berlin, Julius Springer, 1939, **6**, pt. 2, 137.
3. BARNES, B. G., Ciliated secretory cells in the pars distalis of the mouse hypophysis, *J. Ultrastruct. Research*, 1961, **5**, 453.
4. BAUER, W. C., personal communication.
5. BODIAN, D. B., A new method for staining nerve fibers and nerve endings in mounted paraffin sections, *Anat. Rec.*, 1936, **65**, 89.
6. COPP, D. H., CHENEY, B. A., DAVIDSON, A. G. F., AND DUBE, W. J., Hypercalcemia after parathyroidectomy—The homeostatic role of calcitonin, *Fed. Proc.*, 1962, **21**, 206.
7. DALTON, A. J., A chrome-osmium fixative for electron microscopy, *Anat. Rec.*, 1955, **121**, 281.
8. DALTON, A. J., AND ZEIGEL, R. F., A simplified method of staining thin sections of biological material with lead hydroxide for electron microscopy. *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 409.
9. DAVIS, R., and ENDERS, A. C., Light and electron microscope studies on the parathyroid gland, (R. O. Greep and R. V. Talmage, editors), *in* The Parathyroids, Springfield, Illinois, Charles C. Thomas, 1961, 76.
10. DE ROBERTIS, E. D. P., and SABATINI, D. D., Submicroscopic analysis of the secretory process in the adrenal medulla, *Fed. Proc.*, 1960, **19**, suppl. 5, 70.
11. FARQUHAR, M. G., Origin and fate of secretory granules in cells of the anterior pituitary gland, *Tr. New York Acad. Sc.*, 1961, **23**, 346.
12. FAWCETT, D. W., and PORTER, K. R., A study of the fine structure of ciliated epithelia, *J. Morphol.*, 1954, **94**, 221.
13. FERREIRA, D., L'ultrastructure des cellules du pancreas endocrine chez l'embryon et al rat nouveau-ne, *J. Ultrastruct. Research*, 1957, **1**, 14.
14. GRAFFLIN, A. L., Cytological evidence of secretory activity in the mammalian parathyroid, *Endocrinology*, 1940, **26**, 857.
15. GRAFFLIN, A. L., Further observations upon the parathyroid gland of the Virginia deer, in specimens taken at intervals throughout the year, *Endocrinology*, 1942, **30**, 571.
16. HAMPERL, H., Die fluorescenzmikroskopie menschlicher Gewebe, *Virchows Arch. path. Anat.*, 1934, **292**, 1.
17. LACY, P. E., Electron microscopy of normal islets of Langerhans, Studies with dog, rabbit, guinea pig, and rat, *Diabetes*, 1957, **6**, 498.
18. LACY, P. E., Electron microscopic identification of different cell types in the islets of Langerhans of the guinea pig, rat, rabbit, and dog, *Anat. Rec.*, 1957, **128**, 255.
19. LACY, P. E., Electron microscopy of the beta cell of the pancreas, *Am. J. Med.*, 1961, **31**, 851.
20. LANGE, R., Zur Histologie und Zytologie der Glandula Parathyreoidea des Menschen. Licht und elektronenmikroskopische Untersuchungen an Epithelkorperadenomen, *Z. Zellforsch., u. Mikroskop. Anat.*, 1961, **53**, 765.
21. LEVER, J. D., The subendothelial space in certain endocrine tissues, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 293.
22. LEVER, J. D., Fine structural appearances in the rat parathyroid, *J. Anat.*, 1957, **91**, 73.
23. LEVER, J. D., Cytological appearances in the normal and activated parathyroid of the rat. A combined study by electron and light microscopy with certain quantitative assessments, *J. Endocrinol.*, 1958, **17**, 210.
24. L'HEUREUX, M. V., and MELIUS, P., Differential centrifugation of bovine parathyroid tissue, *Biochim. et Biophysica Acta*, 1956, **20**, 447.
25. LILLIE, R. D., Histopathologic Technic and Practical Histochemistry, New York, McGraw-Hill Book Co., 1954.
26. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
27. MARINE, D., The thyroid, parathyroid and thymus, *in* Special Cytology, (E. V. Cowdry, editor), New York, Paul B. Hoeber, Inc., 1932, **2**, 82.
28. MUNGER, B. L., A light and electron microscopic study of cellular differentiation in the pancreatic islets of the mouse, *Am. J. Anat.*, 1958, **103**, 275.
29. MUNGER, B. L., Staining methods applicable to sections of osmium-fixed tissue for light microscopy, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 502.
30. MUNGER, B. L., The secretory cycle of the pancreatic islet alpha cells. An electron microscopic study of normal and synthalin-treated rabbits, *Lab. Inv.*, 1962, **11**, 885.
31. ODLAND, G. F., The fine structure of the interrelationship of cells in the human epidermis, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 529.
32. PALAY, S. L., The morphology of secretion, *in*

Frontiers in Cytology, (S. L. Palay, editor), New Haven, Yale University Press, 1958, 305.
33. PALAY, S. L., The fine structure of secretory neurons in the preoptic nucleus of the goldfish, (*Carassius auratus*), *Anat. Rec.*, 1960, **138,** 417.
34. PETERSEN, H., Anatomische Studie über die Glandulae Parathyreoideae des Menschen, *Virchows Arch. path. Anat.*, 1903, **174,** 413.
35. RASMUSSEN, H., Parathyroid hormone. Nature and mechanism of action, *Am. J. Med.*, 1960, **30,** 112.
36. RASMUSSEN, H., and CRAIG, L. C., Isolation and characterization of bovine parathyroid hormone, *J. Biol. Chem.*, 1961, **236,** 759.
37. REVEL, J. P., NAPOLITANO, L., and FAWCETT, D. W., Identification of glycogen in electron micrographs of thin tissue sections, *J. Biophysic. and Biochem. Cytol.*, 1960, **8,** 575.
38. RICHARDSON, K. C., personal communication.
39. ROSOF, J. A., An experimental study of the histology and cytology of the parathyroid glands in the albino rat, *J. Exp. Zool.*, 1934, **68,** 121.
40. ROTH, S. I., Pathology of the parathyroids in hyperparathyroidism, with a discussion of recent advances in the anatomy and pathology of the parathyroid glands, *Arch. Path.*, 1962, **73,** 495.
41. ROTH, S. I., and MUNGER, B. L., The cytology of the adenomatous, atrophic, and hyperplastic parathyroid glands of man: A light and electron microscopic study, *Virchows Arch. path. Anat.*, 1962, **335,** 389.
42. TRIER, J. S., The fine structure of the parathyroid gland, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 13.
43. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 475.
44. WEYMOUTH, R. J., The cytology of the parathyroid glands of the rat after bilateral nephrectomy, administration of parathyroid hormone, and hypophysectomy, *Anat. Rec.*, 1957, **127,** 509.
45. WEYMOUTH, R. J., and BAKER, B. L., The presence of argyrophilic granules in the parenchymal cells of the parathyroid glands, *Anat. Rec.*, 1954, **119,** 519.