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The Ultrastructure of Papillary Cystadenoma Lymphomatosum of the Parotid Gland*

By

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With 6 Figures in the Text

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Introduction

The original segregation by ALBRECHT and ARZT in 1910, the description in the American literature by WARTHIN in 1929 and the histogenetic and embryologic studies of THOMPSON and BRYANT in 1950 have been milestones in the history of papillary cystadenoma lymphomatosum (PCL). The tumor is benign and the clinical problem consists in differentiation of PCL from other neoplasmas. Studies of the epithelium of PCL have been limited to descriptions of oxphilic, granular, pseudostratified, non-ciliated, columnar epithelium (FOOTE and FRAZELL); classification of these cells as oncocytes and speculation regarding their degenerative or senescent nature (HAMPERL [1]). ROTH et al. in their survey of oxyphilic cells state that these cells are filled with mitochondria. It is the purpose of this paper to illustrate the ultrastructure of PCL and discuss these observations.

Material and Methods

Portions of three tumors, histologically characteristic of PCL were fixed in phosphate buffered osmium tetroxide (MILLONIG) and embedded in Epon 812 (LUFT). Unstained one-half-micron-thick sections were examined by phase microscopy or were stained with paraphenylenediamine (Estable-Puig) and examined by light microscopy. Sections showing silver to gold interference colors were stained with uranyl acetate or lead citrate (REYNOLDS) and examined in an RCA 3 F at 50 KV. Other portions of the tumors were fixed in neutral formalin or Regaud's fixative, processed into paraffin, cut at 6 microns and stained with hematoxylin and eosin and Bensley's acid analine fuchsin.

Observations

The characteristic gross and light microscopic appearance of PCL are well known. The granularity of the cytoplasm is due to the presence of many mitochondria. These are readily seen with phase contrast microscopy (Fig. 1) or in the sections from the tissue fixed in Regaud's fluid and stained with Bensley's acid analine fuchsin.

1. Basal cells. The polygonal basal cells are set upon a fine basement membrane that is occasionally thrown into folds. The lateral and apical portions of the cell wall form fine finger-liker projections that interdigitate with those of adjacent cells. Desmosomes are seen infrequently between basal cells that have

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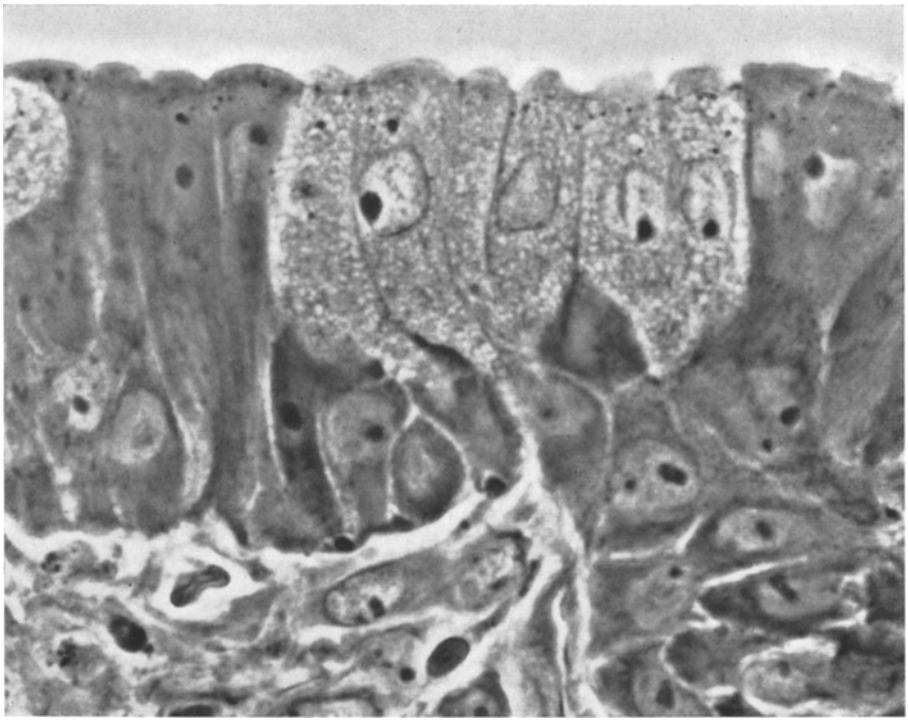

Fig. 1. This phase photomicrograph illustrates two variants of the epithelial cells. In the six centra apical cells the cytoplasm has a fine vesicular appearance. The apical cells laterally and the basal cells have a dense, almost uniform texture in which slightly darker dots, rods, and commas are seen. The larger, apically located, black dots are lipid. Magnification, 1,700 × (reduced to $^{19}/_{20}$)

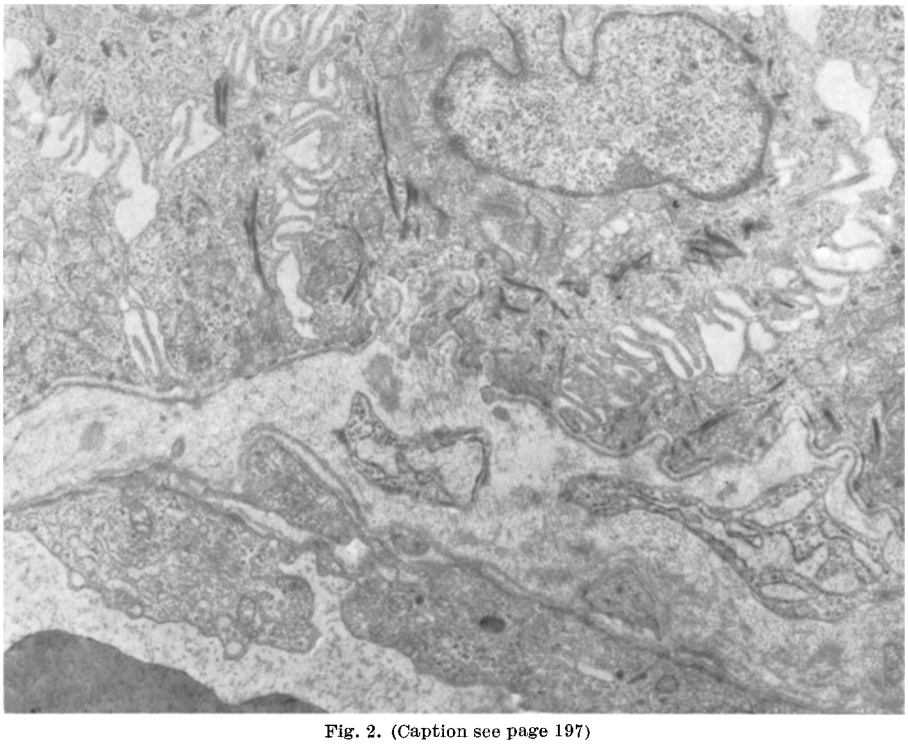

Fig. 2. (Caption see page 197)

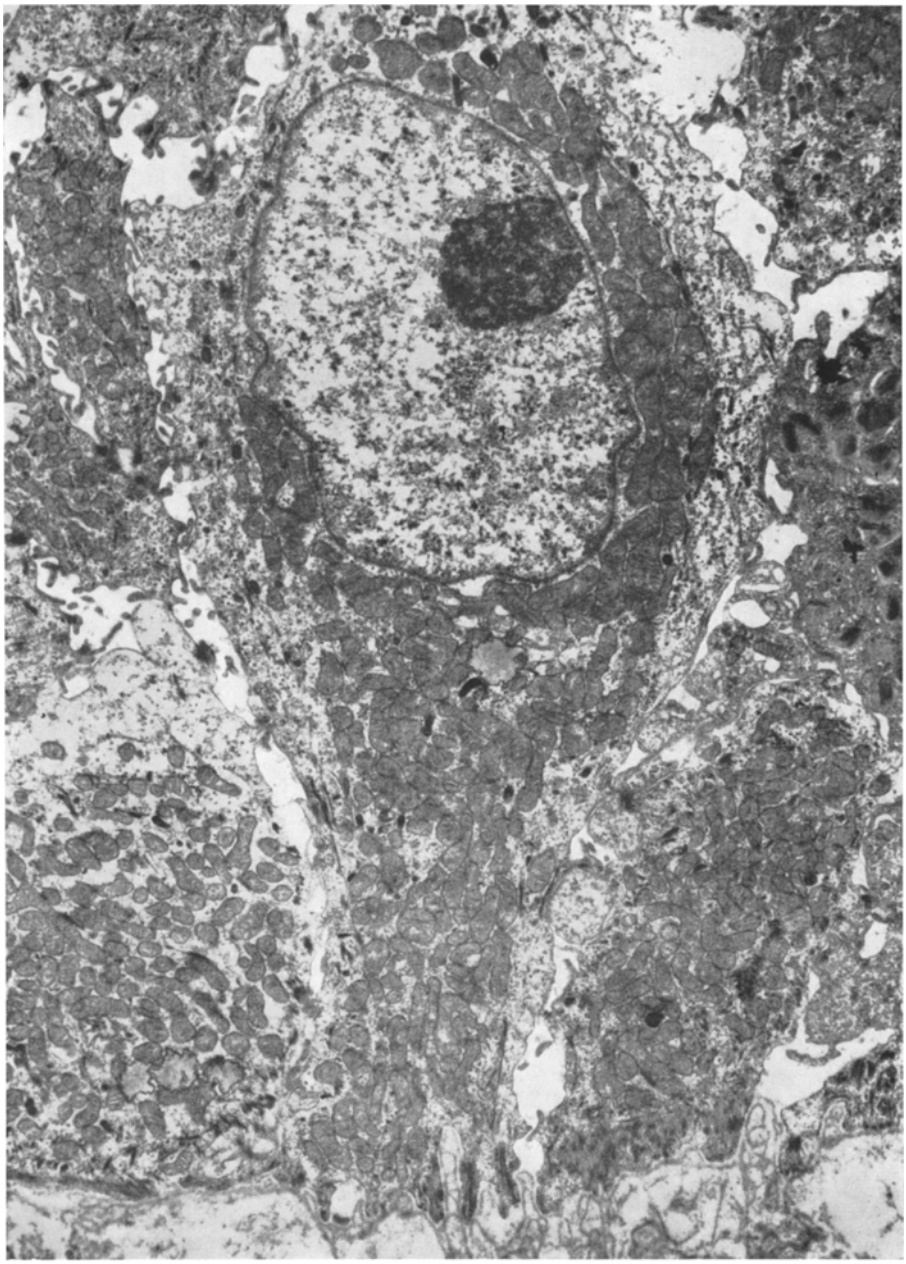

Fig. 3

Fig. 2. Characteristics of the basal epithelial cells are shown in this low power electron micrograph. Occasionally, a desmosome is interspersed between the interdigitating lateral cytoplasmic fingers. Clusters of tonofilaments, mitochondria and ribosomes fill the remaining cytoplasm. The connective tissue space is bounded by basement membrane, above that of the epithelium and below that of the capillary endothelium. Magnification, 9,500 × (reduced to $^{19}/_{20}$)

Fig. 3. A tangential section of the epithelium in which an apical cells is seen extending to the basement membrane between two basal cells. The numerous mitochondria are apparent. In addition a few lipid vacuoles, irregularly shaped dense bodies of unknown nature and ribosomes are present within the cytoplasm. A portionof a polymorphonuclear leukocyte is seen on the right. Magnification, 8,000 × (reduced to $^{19}/_{20}$)

few tonofilaments. In cells having bundles or sheaves of tonofilaments, an ultrastructural manifestation of squamous metaplasia, desmosomes are more frequent. The cytoplasm contains many mitochondria, ocasional oval to club-shaped membrane limited dense bodies, scattered lipid vacuoles, a few strands of ergastoplasm and scattered ribosomes (Figs. 2 and 3). Polymorphonuclear leukocytes are seen on occasion migrating through the epithelium, between the cells (Fig. 3).

2. Apical cells. These cells, with their apically located nuclei, extend between the basal cells to abut on the basement membrane. The cytoplasmic constituents are similar to those of the basal cells with the dominant organelle being the mitochondrion. Tonofilaments and desmosomes are seen infrequently. Apically the cells are joined by terminal bars. A thin rim of apical cytoplasm becomes free of mitochondria and forms a 'terminal web' that contains smooth-walled vesicles, tubules and a few ribosomes. Electron-dense-membrane-limited vacuoles are found in this zone. The luminal surface is studded with short irregularly spaced microvilli (Fig. 4).

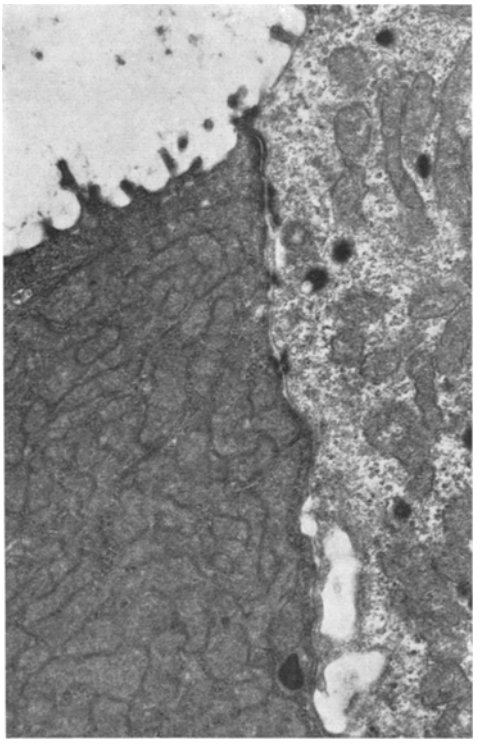

Fig. 4. The variation in the packing of mitochondria seen in Fig. 1 is illustrated here. In the cell on the left the mitochondria are packed one immediately next to another, whereas in the cell to the right the mitochondria though increased in number, are somewhat separated. The terminal bars are readily seen. Magnification, 12,000 ×

3. Mitochondria. Two types of mitochondria are found in these epithelial cells. The predominant one is the generally observed type of mitochondrion (Fig. 5). The less frequent type of mitochondria are 2—3 times larger and the cristae form closely packed sheaves or lamallae (Fig. 6). The mitochondrial matrix is moderately electron opaque. Dense bodies are not observed.

4. Stroma and Lymphoid Tissue. Beneath the basement membrane is a connective tissue space containing collagen, fibrocytes, and occasional imflammatory cells. This space is traversed by capillaries that have an endothelial lining of the fenestrated type found at sites of active fluid transport (BENNETT, LUFT, and HAMPTON) and an occasional lymphatic (Fig. 2). The lymphoid tissue conforms in structure to that of a lymph node with a peripheral sinus lined by reticular cells and the underlying cortex and medulla (CLARK [1]).

Discussion

These observations confirm the absence of cilia noted by THOMPSON and BRYANT. The absence of cilia supports the thesis that these tumors are not derived

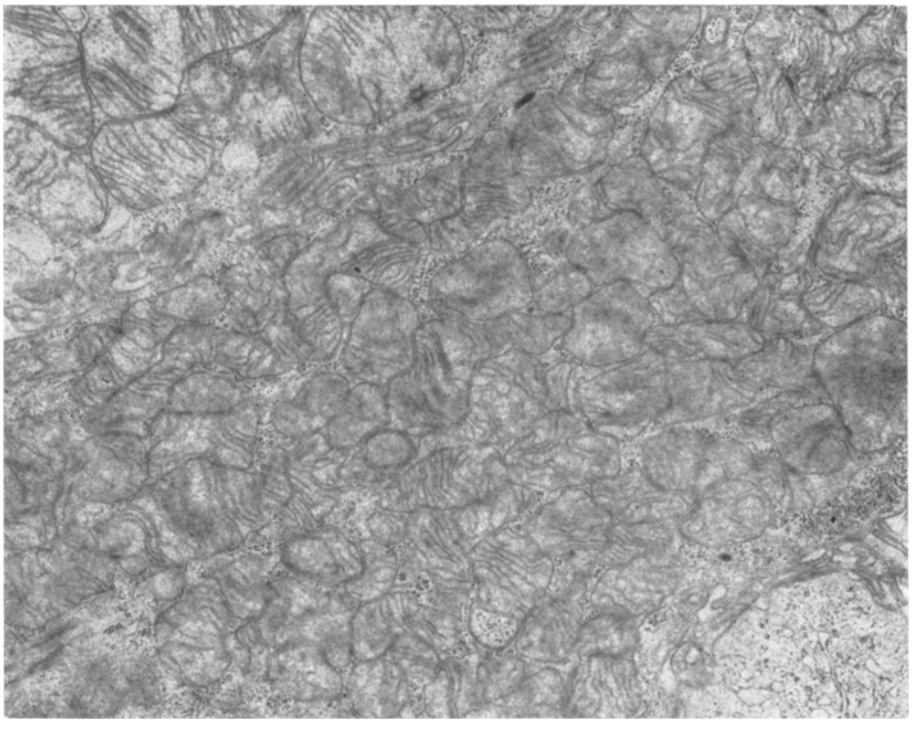

Fig. 5. The closely packed, relatively uniform mitochondria in the midportion of an epithelial cell are seen in the electron-micrograph. At the upper left, in another cell, the mitochondria are swollen. Magnification, 11,500 × (reduced to $^{19}/_{20}$)

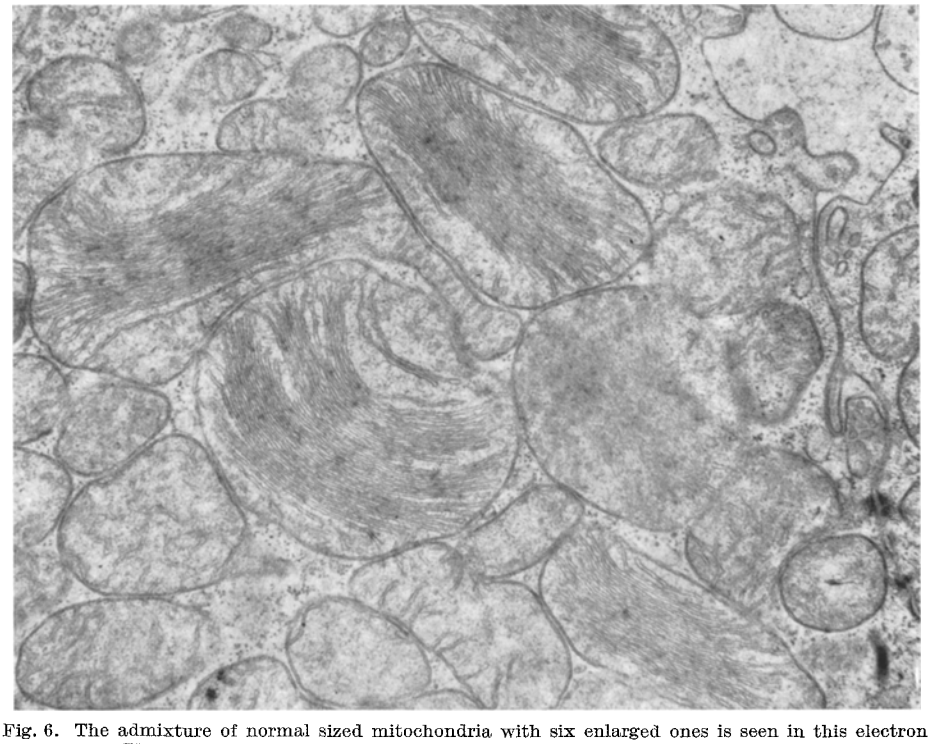

Fig. 6. The admixture of normal sized mitochondria with six enlarged ones is seen in this electron micrograph. The cristae mitochondrales form lamellar arrays in the enlarged mitochondria and when cut horizontally the matrix appears blurred as in the mitochondrion just right of center. Magnification, 24,000 × (reduced to $^{19}/_{20}$)

from the branchial apparatus of the foregut, in that structures derived therefrom contain ciliated cells; e.g., thyroid (BAUER and MCGRAVAN), thymus [CLARK (2)], parathyroid (ROTH and MUNGER) , branchial cysts and sinuses, and thyroglossal duct cysts.

The light or dark appearance of certain epithelial cells (Figs. 1 and 4) is due to variation in the packing of mitochondria. In the dark cells the mitochondria are packed tightly and their matrices are opaque. In the light cells the mitochondria are separated and their matrices are less opaque.

The presence of many mitochondria in these cells poses questions to which answers are not readily apparent. The structurally most closely related normal cells are those in the striated ducts of the parotid (BAUER and MCGAVRAN). These cells have the structural specialization, numerous infoldings of the basalar plasma membrane enclosing mitochondria, associated with fluid transport and the underlying capillaries have fenestrated endothelium. That there be mitochondrial proliferation in the epithelial cells of PCL, in the absence of obvious functional need, is contradictory to the thesis that structural differentiation accompanies functional specialization. Thus, it may be assumed that these mitochondria are abnormal. The abnormality (ies) may be in a particular enzymatic function or more probably an imbalance in feedback control mechanisms, as suggested by LUFT and coworkers.

TREMBLAY and TREMBLAY and PEARSE have shown, *in vitro*, that the Askenazy cells of the thyroid contain oxidative enzymes and 'high levels' of cytochrome oxidase and adenosine triphosphatase activity. Assuming comparable *in vivo* capacities an hypothesis that the oxyphils have escaped from the interdependent controls of aerobic and anaerobic oxidation is tenable.

Some generally applicable theory is requisite to explain the distribution of oxyphils (oncocytes) in various epithalia (HAMPERL [2]). Clinical correlations with altered function are limited. Hashimoto's thyroiditis is generally accompanied by hypothyroidism. ROTH and MUNGER found little or no correlation between the number of oxyphils and the functional states of the parathyroid. In fact, they describe a case of hyperparathyroidism in which the adenoma was composed of oncocytes.

Within the limited sampling of this study variation has been found in the extent of the mitochondrial hyperplasia. This observation is supported by the light microscopic studies, in which a gradual transition to the oncocytes is noted. This may reflect the extent or duration of the requisite stimuli. The significance of the two types of mitochondria is unknown. They do not appear attributable to fixation artifact. The absence of mitochondrial granules is not surprising considering the duration of hypoxia during the meticulous dissection of these tumors (TRUMP, GOLDBLATT, and STOWELL).

Though our observations on oncocytes in other sites are limited certain differences between the oncocytes in PCL and those of an oncocytoma (oxyphil adenoma) of the submandibular gland have been noted. These are, briefly, the absence of two structural forms of mitochondria and the presence of intramitochondrial accumulations of glycogen in the oncocytoma.

Summary

The ultrastructure of papillary cystadenoma lymphomatosum (PCL), Warthin's tumor, of the parotid salivary gland has been described. The oncocytic cells are filled with mitochondria. Two morphologic forms of mitochondria are seen. The cells of the striate ducts in the normal parotid, have specializations associated with fluid transport and are structurally most nearly akin to the oxyphils. The subcellular hyperplasia of mitochondria may be a manifestation of aberrant function.

Die Ultrastruktur des papillären Cystadenoma lymphomatosum der Parotisdrüse

Zusammenfassung

Die onkocytären Zellen sind voll von Mitochondrien. Zwei Formen von Mitochondrien sind nachweisbar. Die Zellen der Streifenstücke (Speichelrohre) der normalen Parotis besitzen besondere Einrichtungen, die mit dem Flüssigkeitstransport in Zusammenhang gebracht werden, und sind gestaltlich am nächsten mit den oxyphilen Zellen verwandt. Die subcelluläre Hyperplasie der Mitochondrien könnte Ausdruck einer abwegigen Funktion darstellen.

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