Virchows Arch. path. Anat. 341, 317—326 (1966)

Fine Structure of Oncocytes in Human Salivary Glands*

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Received July 29, 1966

Oncocytes, epithelial cells displaying marked cytoplasmic acidophilia and granularity, are found in a variety of organs (HAMPERL, 1931, 1933, 1962a; ZIPPEL, 1942). Normally, they occur singly or in small clusters and appear to increase in frequency with age of the individual (HAMPERL, 1962a). Scattered foci of oncocytes are also found in many tumors, and several salivary gland tumors (Warthin's tumor and oncocytoma) consist primarily of oncocytes (HAMPERL, 1962a, b, c). Electron microscopic examination of such tumors has revealed that these cells contain unusually high numbers of mitochondria (TANDLER and SHIPKEY, 1964; MCGAVRAN, 1965; BALOGH and ROTH, 1965; TANDLER, 1966).

While the morphology of oncocytes in neoplasms of the salivary glands has been characterized, nothing is known of the ultrastructure of such cells in the normal salivary glands. In this study, the fine structure of oncocytes found in acini and in intercalated and striated ducts of normal human salivary glands has been examined, and the possible mode of development of these cells is discussed.

Materials and Methods

Specimens of normal human salivary glands were obtained by surgery from patients ranging in age from 8 to 90 years. Some of the tissue was fixed directly in osmium tetroxide buffered with veronal-acetate (PALADE, 1952) or phosphate (MILLONIG, 1961a), while other tissue was fixed in glutaraldehyde buffered with phosphate (SABATINI, BENSCH, and BARRNETT, 1963) or in a mixture of acrolein and glutaraldehyde (SANDBORN et al., 1964) buffered with veronal-acetate. Specimens fixed in these aldehydes were postfixed in osmium. The specimens were embedded in Maraglas-Dow Epoxy Resin 732, (ERLANDSON, 1964) or in methacrylate. Sections were cut with a Porter-Blum ultramicrotome, and were stained with lead hydroxide (MILLONIG, 1961b) prior to examination in a Siemens Elmiskop I electron microscope.

Observations

Oncocytes were observed in 5 of 25 specimens of submaxillary gland, in 1 of 2 specimens of sublingual gland, and in 1 of 56 specimens of minor labial mucous salivary gland. While these cells occurred only in small numbers, they could be readily distinguished from normal cells. Regardless of their location in any of the various salivary glands, oncocytes contain numerous mitochondria and lack the characteristic specialized structures of neighboring cells.

Most of the oncocytes were observed in the glandular acini, and are of several types, which may represent stages in the development of these cells. The most common type contains mitochondria of relatively normal morphology (Figs. 1, 2 and 3). These organelles, however, are packed so tightly that only a few wisps

* This work was supported in part by grants from the Max C. Fleischmann Foundation of Nevada and the Henry Spenadel Trust, and by N. C. I. grant CA 08748.

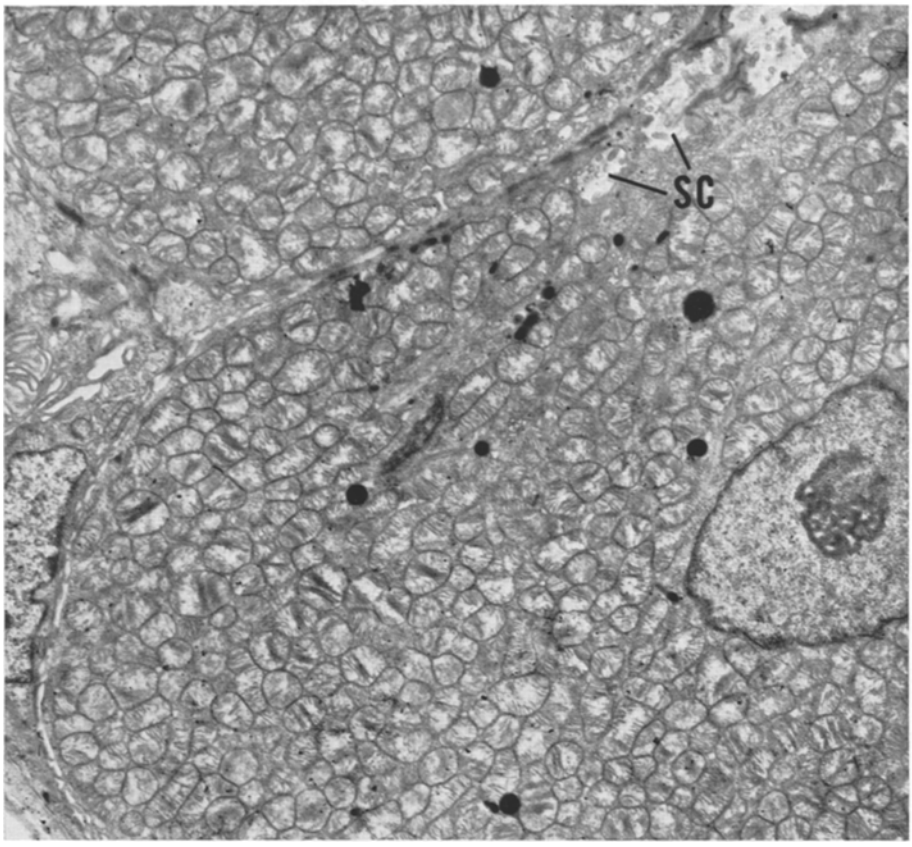

Fig. 1. A portion of two oncocytes within an acinus of the sublingual gland. The mitochondria are of normal size, but are extremely abundant. A few of the mitochondria show increased numbers of cristae. *SC*, secretory capillary. ×5,000

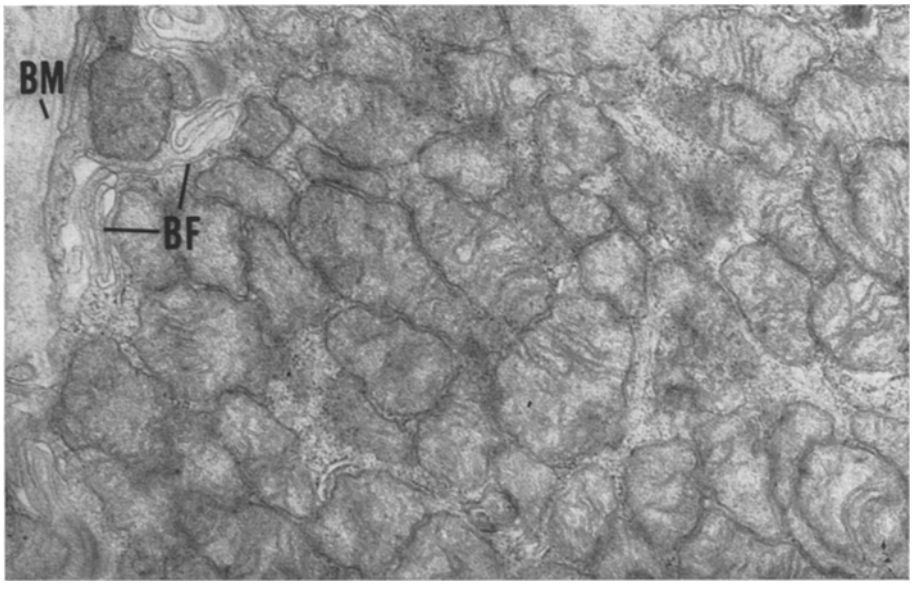

Fig. 2. The basal portion of an acinar oncocyte in the submaxillary gland. Mitochondria of relatively normal appearance are very numerous. Vestiges of basal folds (*BF*) are present adjacent to the basement membrane (*BM*). ×18,000

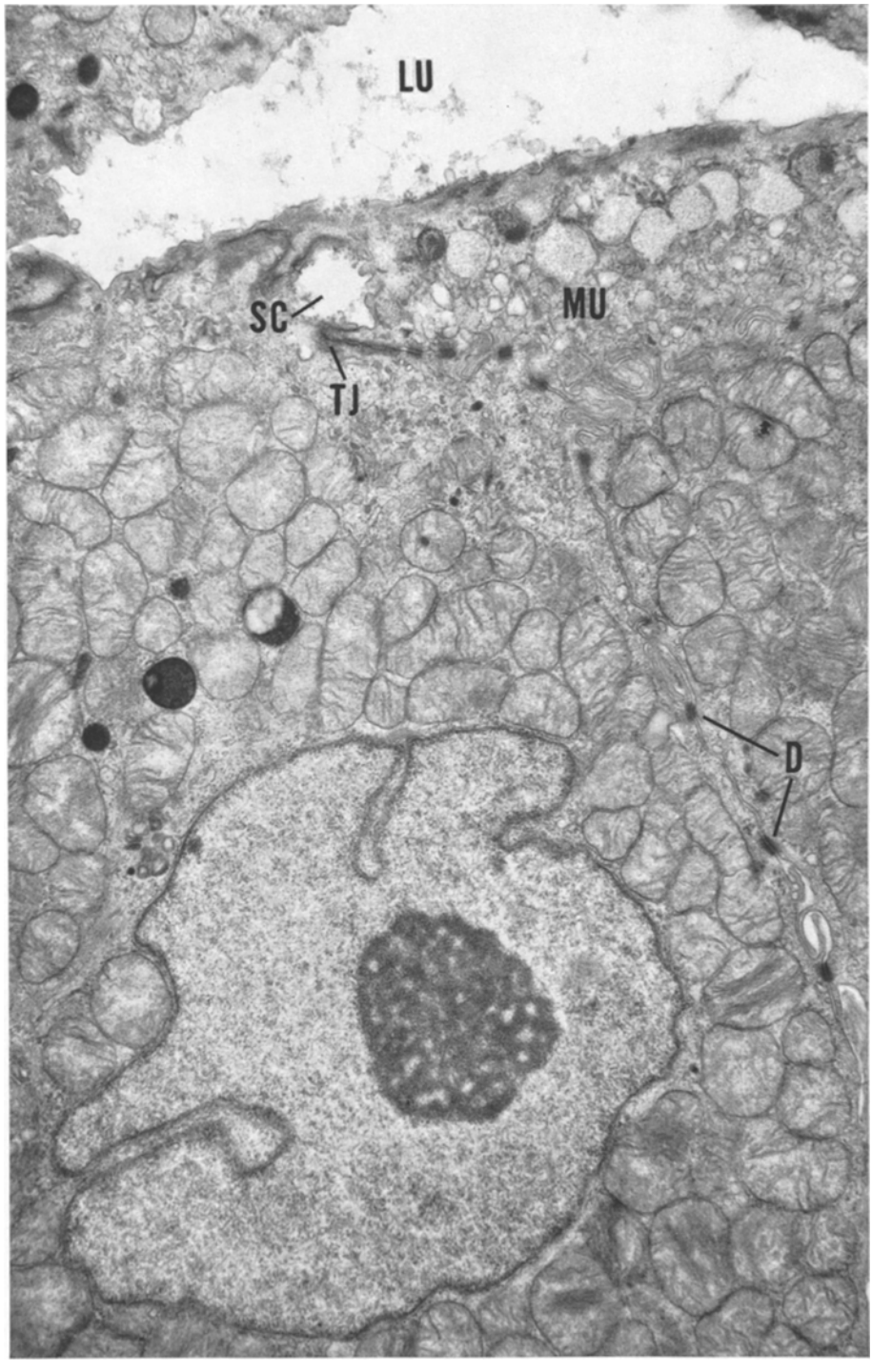

Fig. 3. Oncocytes within an acinus of the sublingual gland. The mitochondria are greatly increased in number, and a few display tightly packed cristae. The nucleus is deeply notched and contains a prominent nucleolus. The oncocytes are joined to each other and to adjacent mucous cells (*MU*) by desmosomes (*D*) and tight junctions (*TJ*). A transversely sectioned secretory capillary (*SC*) is seen in proximity to the acinar lumen (*LU*). ×13,000

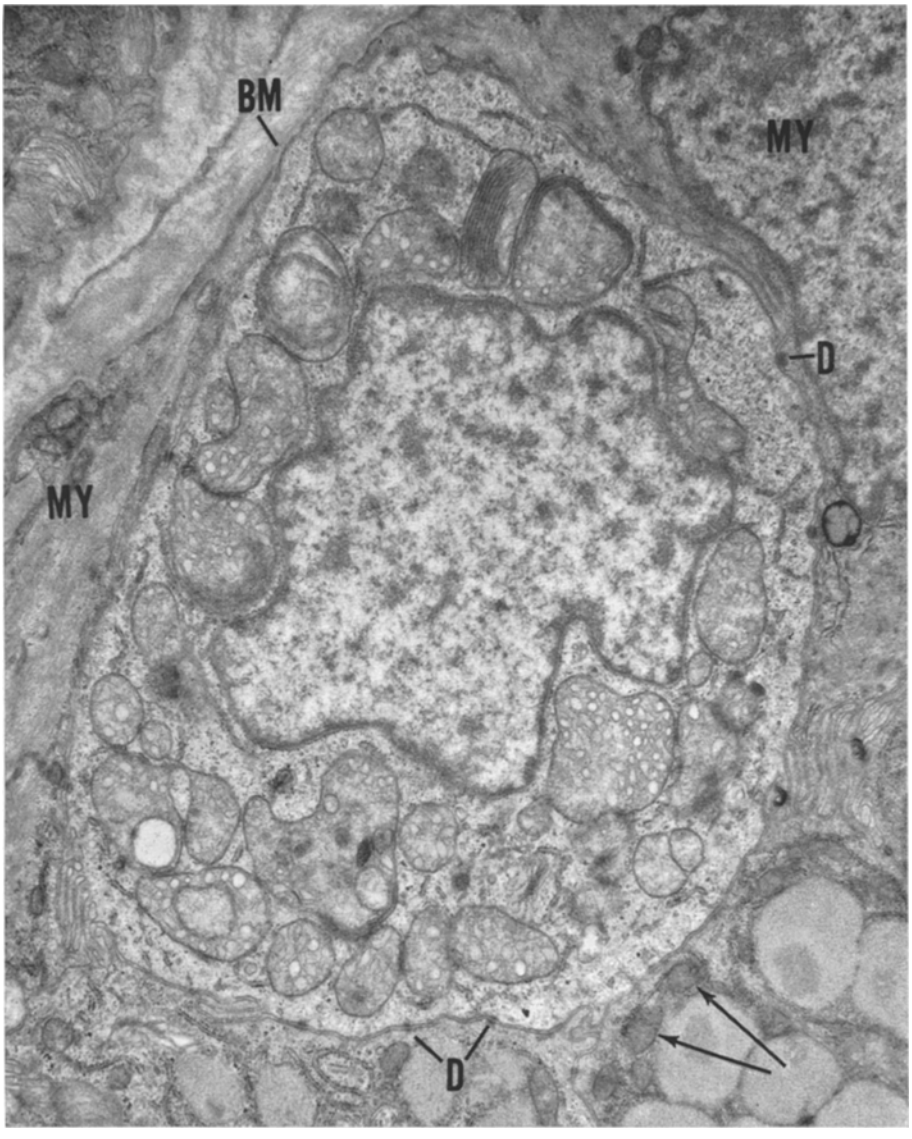

Fig. 4. An oncocyte within an acinus of the submaxillary gland. The oncocyte rests on a basement membrane (*BM*), and is surrounded by myoepithelial cells (*MY*) and seromucous cells. It is joined to both cell types by small desmosomes (*D*). The nucleus is irregular, and the cytoplasm contains many bizarre mitochondria. The size of these mitochondria should be compared with that of the organelles (↑) in the adjoining cell. Secretory granules are completely absent from the oncocyte. ×13,500

of the cytoplasmic matrix are evident. Oncocytes of this type frequently display rudimentary basal folds similar to those found in normal acinar cells (TANDLER, 1962) (Fig. 2). In the other types of oncocyte, the mitochondria are also unusually abundant, but many are enlarged and show striking changes in morphology (Figs. 4, 5 and 6). The mitochondrial cristae are increased in number and extent, and are disposed parallel to the long axis of the organelle, or are vesicular in form. Occasionally, enlarged, flattened mitochondria are observed in rouleaux-like

aggregates similar to those seen in oncocytes of Warthin's tumor (TANDLER and SHIPKEY, 1964) and oncocytoma (HÜBNER, KLEIN and SCHÜMMELFELDER, 1965) (Fig. 6). The identical morphology and close juxtaposition of organelles in such aggregates suggests that these groupings represent mitochondrial clones. In many of the flattened mitochondria, the cristae are villiform, rather than lamellar. Viewed in profile, such cristae are seen to be polygonal.

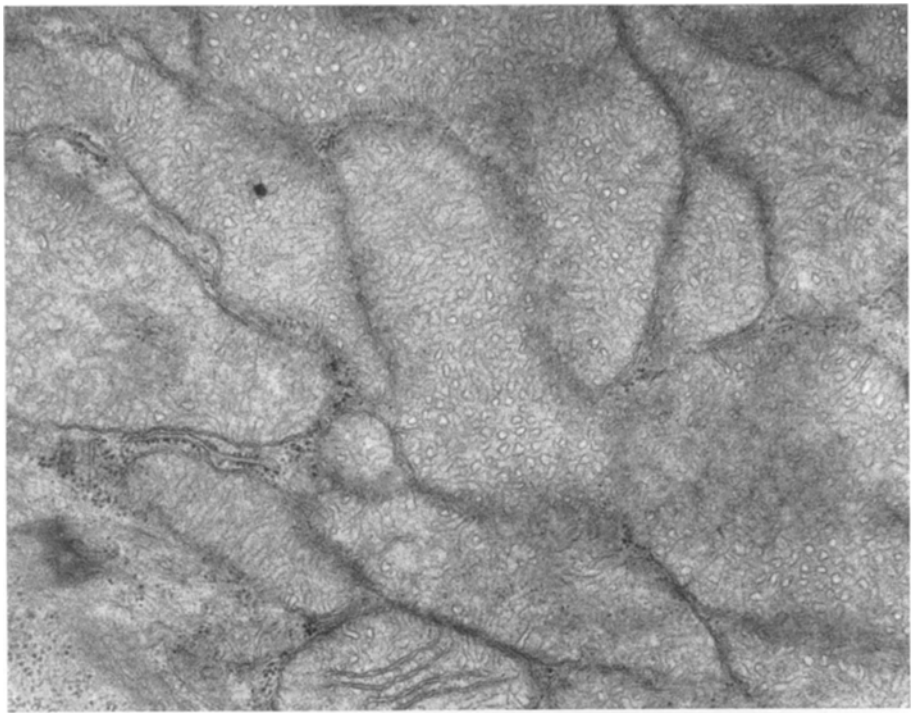

Fig. 5. Enlarged mitochondria within an acinar oncocyte in a labial salivary gland. The finger-like cristae are triangular or polygonal in cross-section. ×32,000

All acinar oncocytes lack the extensive and highly organized endoplasmic reticulum usually found in acinar secretory cells, but have a few, isolated ergastoplasmic cisternae and abundant free ribosomes. Their luminal surfaces bear a few short microvilli, and their lateral walls sometimes participate in the formation of a secretory capillary (intercellular canaliculus) (Figs. 1 and 3). The oncocytes are attached to adjacent normal cells by tight junctions and small desmosomes (Figs. 3, 4 and 6).

Like the oncocytes in the acini, oncocytes in the intercalated ducts possess a great many mitochondria. In this respect, they differ from normal intercalated duct cells, which contain only a few small mitochondria (TANDLER, 1965). The mitochondria in these oncocytes are increased in size, and are spherical to ovoid in form. The abundant cristae are usually closely packed.

The oncocytes of the striated ducts also differ from the adjacent normal cells. In normal striated duct cells, mitochondria are quite numerous in the basal cytoplasm. They are rod-shaped, with transverse cristae, and are lodged vertically

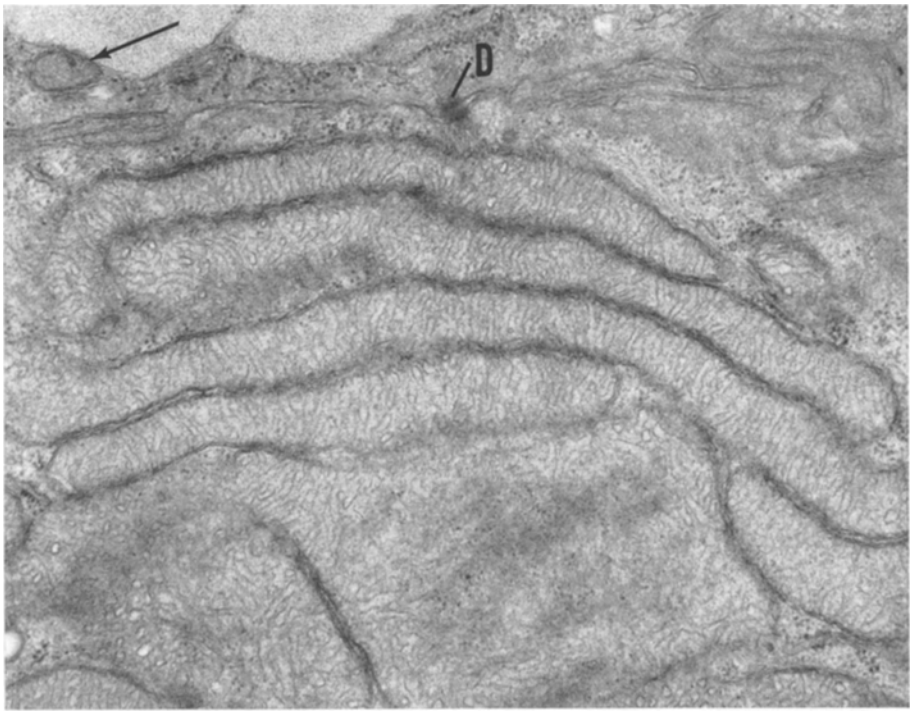

Fig. 6. Oncocyte mitochondria in an acinus of a labial salivary gland. The degree of enlargement of these organelles is indicated by comparison with a normal mitochondrion in an adjacent mucous cell (↑). The flattened mitochondria are arranged in a stack and possess villiform cristae. Some of the mitochondria contain dense, 100 Å particles in their matrix. ×32,000

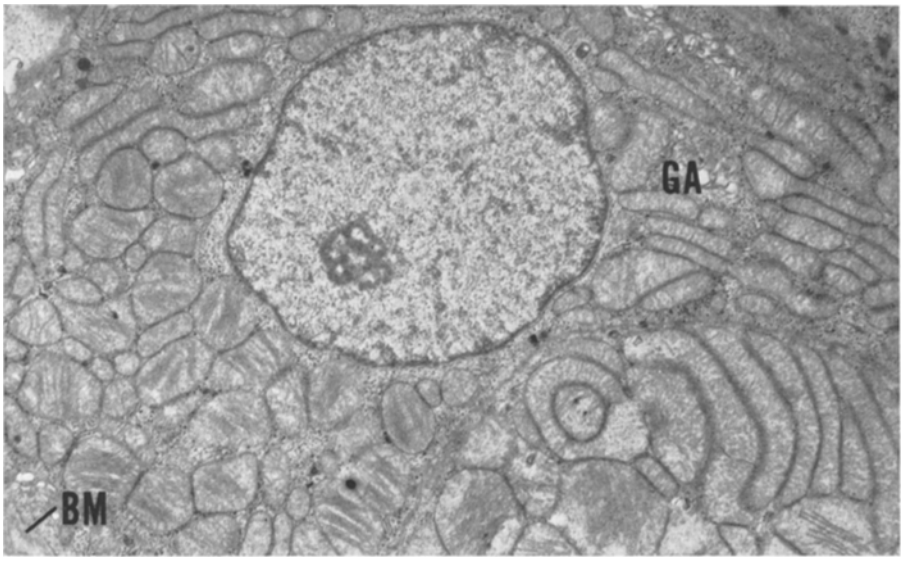

Fig. 7. Oncocytes in a striated duct of the submaxillary gland. The lumen (not shown) is to the right and the basement membrane (*BM*) is to the left. These cells lack the highly complex membrane specializations normally found in the base of striated duct cells. Mitochondria are abundant in both the apical and basal cytoplasm, and show great variability in form. A rouleaux-like aggregate of these organelles is present in the cell at the lower right. GA, Golgi apparatus. ×9,000

in cytoplasmic processes at the base of the cells (TANDLER, 1963). In oncocytes of the striated ducts, the basal processes are absent, and randomly oriented mitochondria are abundant not only in their basal cytoplasm, but in their apical cytoplasm as well (Fig. 7). The mitochondria of striated duct oncocytes display a greater degree of pleomorphism within individual cells than they do in oncocytes in other sites. They range in form from small rods to large, ovoid structures with an increased number of cristae. In many of the larger mitochondria, the cristae are observed to be finger-like. Flattened mitochondria are frequently observed in aggregates similar to those found in some acinar oncocytes. In duct oncocytes, such aggregates are most frequently found in the supranuclear cytoplasm.

Intramitochondrial dense granules were not observed in any of the oncocytes in the salivary glands, but were plentiful in neighboring cells. Unlike mitochondria in oncocytes of Warthin's tumor (TANDLER and SHIPKEY, 1964; TANDLER, 1966) or of oncocytoma (BAUER and MCGAVRAN, 1964) none of the mitochondria in salivary gland oncocytes contained deposits of glycogen or accumulations of dense, amorphous material.

Discussion

On the basis of light microscopical observations, HAMPERL (1931, 1933, 1962a, b, c) concluded that oncocytes represent a form of cellular degeneration or "regression". This contention is supported by the present report, since oncocytic cells, regardless of their location in salivary glands, appear to lose all signs of organ specific differentiation. That the change from a normal to an oncocytic cell may be gradual is indicated by the observation of transitional cells in the parathyroid gland (MUNGER and ROTH, 1963). In the human salivary glands, the earliest observable change is a great increase in the number of mitochondria. In this stage, these organelles are of typical morphology, but consistently lack the intramitochondrial dense granules present in the mitochondria of adjacent normal cells. The absence of these granules from oncocyte mitochondria is not an artifact, but may reflect a change in function.

In addition to the lack of intramitochondrial dense granules, these nascent oncocytes are deficient in cytoplasmic membrane systems. Remnants of specialized structures, such as basal folds, may nevertheless remain. As oncocytic transformation progresses, the cells become completely devoid of any trace of their former specialized cyto-architecture, and the mitochondria undergo the drastic changes in number and morphology which have been described.

The enormous increase in size of the mitochondrial population in oncocytes may be expected to be accompanied by a corresponding elevation in the level of activity of the various mitochondrial enzymes. When oncocytes are compared with their normal counterparts by histochemical means, the results vary. Those oncocytes whose mitochondria are of normal morphology show considerably enhanced enzymatic activity (FISCHER, 1961; TREMBLAY and PEARSE, 1959), while those oncocytes whose mitochondria are altered in size or form display little or no enhancement of oxidative enzymes (BALOGH and ROTH, 1965; CHAUNCEY, SHKLAR and BROOKS, 1962; SHKLAR and CHAUNCEY, 1965). It has been shown (KARNOVSKY, 1963) that under certain environmental conditions the mitochondria

of the tubule cells of the frog nephron undergo morphological changes resembling those in oncocytes, and that their cytochrome oxidase activity virtually disappears. Similarly altered mitochondria have also been observed in many pathological conditions, and have been related to impaired function (TRUMP and ERICSSON, 1965).

Recent studies have firmly established the presence of DNA within mitochondria. It has been suggested that this mitochondrial DNA may carry genetic information involved in the control of mitochondrial structure, function, and number (WILKIE, 1964; RABINOWITZ et al., 1965). It is possible, therefore, that changes in the mitochondrial genome may be responsible for initiation and promulgation of the oncocytic process in salivary gland cells and in cells of other organs. This does not exclude the possibility that some mitochondrial components may be altered by nuclear mutation (EPHRUSSI, 1953).

Since oncocytes retain the ability to divide (HAMPERL, 1962a), supervention of neoplastic transformation would result in a tumor composed of oncocytes, such as the oncocytoma. This sequence is not fixed, and cells that are already neoplastic may secondarily undergo oncocytic change. For example, FOOTE and FRAZELL (1953) reported that foci of oncocytes are present in about 10 per cent of mixed tumors of salivary gland origin.

Summary

Oncocytes, cells displaying marked cytoplasmic acidophilia and granularity, are present in the acini and ducts of normal human salivary glands. These cells apparently originate by a process of sequential transformation of normal epithelial cells. In the early forms, there is a great increase in the number of mitochondria, which are typical in morphology. In later oncocytes, these organelles undergo striking changes in form and size. These mitochondrial changes are accompanied by the gradual disappearance from the oncocyte of other cytoplasmic membrane systems and of plasmalemmar specializations. It is suggested that the structurally modified mitochondria are biochemically deficient.

The author acknowledges the expert technical assistance of Mr. ROY R. KEPPIE and Mrs. MONA BRANDRETH.

Fine Structure of Oncocytes in Human Salivary Glands
Die Feinstruktur der Onkocyten in Speicheldrüsen der Menschen

Zusammenfassung

Onkocyten finden sich sowohl in den Acini wie in den Ausführungsgängen normaler Speicheldrüsen. Sie besitzen ein eosinophil granuliertes Cytoplasma und sind wahrscheinlich Abkömmlinge normaler Drüsenzellen, aus denen sie sich über eine Reihe von Zwischenstufen entwickeln. Die Frühformen zeigen eine starke Zunahme der Mitochondrien. In den späten Stadien erfahren die Mitochondrien charakteristische Veränderungen in bezug auf Form und Größe, begleitet von einem Schwund der cytoplasmatischen Membransysteme und weiterer plasmacellulärer Spezialorganellen. Die geschädigten Mitochondrien dürften auch in ihrer biochemischen Leistung beeinträchtigt sein.

References

BALOGH, K., JR., and S. I. ROTH: Histochemical and electron microscopic studies of eosinophilic granular cells (oncocytes) in tumors of the parotid gland. Lab. Invest. **14**, 310—320 (1965).

BAUER, W. C., and M. H. MCGAVRAN: Ultrastructure and surgical pathology. In: Surgical pathology, 3rd ed. (L. V. ACKERMAN), p. 27—54. St. Louis: C. V. Mosby Co. 1964.

CHAUNCEY, H. H., G. SHKLAR, and R. A. BROOKS: Histochemistry of human salivary gland tumors. Oral Surg. **15**, 950—964 (1962).

EPHRUSSI, B.: Nucleo-cytoplasmic relations in microorganisms. New York: Oxford University Press 1953.

ERLANDSON, R. A.: A new Maraglas, D.E.R. 732, embedment for electron microscopy. J. Cell Biol. **22**, 704—709 (1964).

FISCHER, R.: Über den histochemischen Nachweis oxydativer Enzyme in Onkocyten verschiedener Organe. Virchows Arch. path. Anat. **334**, 445—452 (1961).

FOOTE, F. W., JR., and E. L. FRAZELL: Tumors of the major salivary glands. Cancer **6**, 1065—1133 (1953).

HAMPERL, H.: Beiträge zur normalen und pathologischen Histologie menschlicher Speicheldrüsen. Z. mikr.-anat. Forsch. **27**, 1—55 (1931).

— Über besondere Zellen in alternden Mundspeicheldrüsen (Onkocyten) und ihre Beziehungen zu den Adenolymphomen und Adenomen. Virchows Arch. path. Anat. **291**, 704—705 (1933).

— Benign and malignant oncocytoma. Cancer **15**, 1019—1027 (1962a).

— Onkozyten und Onkozytome. Virchows Arch. path. Anat. **335**, 452—483 (1962b).

— Onkozytome der Speicheldrüsen. Z. Krebsforsch. **64**, 427—440 (1962c).

HÜBNER, G., H. J. KLEIN u. N. SCHÜMMELFEDER: Zur Ultrastruktur der Onkocytome. Klin. Wschr. **43**, 798—800 (1965).

KARNOVSKY, M. J.: The fine structure of mitochondria in the frog nephron correlated with cytochrome oxidase activity. Exp. mol. Path. **2**, 347—366 (1963).

MCGAVRAN, M. H.: The ultrastructure of papillary cystadenoma lymphomatosum of the parotid gland. Virchows Arch. path. Anat. **338**, 195—202 (1965).

MILLONIG, G.: Advantages of a phosphate buffer for OsO_4 solutions in fixation. J. appl. Physics **32**, 1637 (1961a).

— A modified procedure for lead staining of thin sections. J. biophys. biochem. Cytol. **11**, 736—739 (1961b).

MUNGER, B. L., and S. I. ROTH: 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).

PALADE, G. E.: A study of fixation for electron microscopy. J. exp. Med. **95**, 285—298 (1952).

RABINOWITZ, M., J. SINCLAIR, L. DESALLE, R. HASELKORN, and H. H. SWIFT: Isolation of deoxyribonucleic acid from mitochondria of chick embryo heart and liver. Proc. nat. Acad. Sci. (Wash.) **53**, 1126—1133 (1965).

SABATINI, D. D., K. BENSCH, and R. J. BARRNETT: Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol. **17**, 19—58 (1963).

SANDBORN, E., P. F. KOEN, J. D. MCNABB, and G. MOORE: Cytoplasmic microtubules in mammalian cells. J. Ultrastruct. Res. **11**, 123—138 (1964).

SHKLAR, G., and H. H. CHAUNCEY: Papillary cystadenoma lymphomatosum, a developmental malformation: histochemical evidence. J. oral. Surg. **23**, 222—230 (1965).

TANDLER, B.: Ultrastructure of the human submaxillary gland. I. Architecture and histological relationships of the secretory cells. Amer. J. Anat. **111**, 287—307 (1962).

— Ultrastructure of the human submaxillary gland. II. The base of the striated duct cells. J. Ultrastruct. Res. **9**, 65—75 (1963).

— Electron microscopical observations on early sialoliths in a human submaxillary gland. Arch. oral. Biol. **10**, 509—522 (1965).

— Warthin's tumor: Electron microscopic studies. Arch. Otolaryng. **84**, 68—76 (1966).

—, and F. H. SHIPKEY: Ultrastructure of Warthin's tumor. I. Mitochondria. J. Ultrastruct. Res. **11**, 292—305 (1964).

TREMBLAY, G., and A. G. E. PEARSE: A cytochemical study of oxidative enzymes in the parathyroid oxyphil cell and their functional significance. Brit. J. exp. Path. **40**, 66—70 (1959).

TRUMP, B. F., and J. L. E. ERICSSON: Some ultrastructural and biochemical consequences of cell injury. In: The inflammatory process (B. W. ZWEIFACH, L. GRANT, and R. T. MCCLUSKEY, ed.), p. 35—120. New York: Academic Press 1965.

WILKIE, D.: The cytoplasm in heredity. London: Methuen & Co. 1964.

ZIPPEL, L.: Zur Kenntnis der Oncocyten. Virchows Arch. path. Anat. **308**, 360—382 (1942).

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