Electron Microscopic Study of Human Laryngeal Papillomatosis*

DONALD J. SVOBODA, FERNANDO R. KIRCHNER, AND G. O. PROUD

(Dept. of Pathology and Oncology and Dept. of Otolaryngology, University of Kansas Medical Center, Kansas City, Kansas)

SUMMARY

Sixteen biopsies of laryngeal papillomas from patients 4–21 years old, as well as tissue from scarred areas of laryngeal mucosa which previously bore papillomas, were studied with the electron microscope and compared with normal laryngeal mucosa. Tissue blocks that contained cytoplasmic bodies by light microscopy were serially sectioned for electron microscopic examination. The bodies appeared to consist of membrane-bound cell remnants such as mitochondria, lipides, endoplasmic reticulum, and nucleus. No viral particles were detected. Several ultrastructural aspects of laryngeal papillomas, including forms of cytoplasmic bodies not apparent by light microscopic examination, are illustrated. The need for further biologic studies of laryngeal papillomas and for careful electron microscopic investigation of the spectrum of cellular alterations in human neoplasms is indicated.

The etiology of laryngeal papillomas in humans has been a matter of controversy. Ullmann (45), using a bacteria-free filtrate of juvenile papillomas, reported transmission of the tumor to the vaginal mucosa of one dog and to human skin through three successive passages. He postulated, without proving beyond reasonable doubt, that the agent that causes verruca plana and condyloma acuminate also causes human laryngeal papillomas. Subsequent attempts to transmit laryngeal papillomas by cell transfer and/or transmission with cell-free filtrates to many sites (scarified skin, vaginal and oral mucosa) in several species have yielded largely negative results.

Meessen and Schulz (25), in an electron microscopic study of laryngeal papillomas from two patients, 2 and 29 years old, described cytoplasmic structures which they regarded as viral particles and viral inclusions. Friedmann (18), in an electron microscopic study of 29 biopsies from tumors of larynx and pharynx, reported that no viral particles were present. His report did not indicate how many of the tumors studied were laryngeal papillomas, nor were the ages of the patients given. Price, Henken, Nishi, and Broido (35) observed two types of inclusions in laryngeal papillomas but pointed out that their nature and significance were undetermined.

Clinical reviews (37, 46) of juvenile laryngeal papillomatosis show little agreement concerning etiology. Le Jeune and Lynch (22) state that “several definite characteristics of the papilloma virus are recognized and others are presumed.” On the other hand, Rock and Fisher (36) as well as Ferguson and Scott (14) recognized that no convincing proof for specific etiology of laryngeal papillomas exists.

Bernhard (4), referring to the report of Meessen and Schulz, indicated the necessity for further electron microscopic study of laryngeal papillomas. In addition, Luse (24) emphasized the necessity to study, with the electron microscope, human tumors in significant numbers of each type to learn the morphologic variations in any one type of tumor and to assess the importance of viruses in spontaneous human tumors.

The purpose of the present study is, first, to describe the ultrastructural features of laryngeal papillomas as related to other forms of keratinizing epithelium; and, second, with a large number of specimens obtained from several patients during recurrent florid papillomatosis and during remission, to demonstrate the spectrum of cytoplasmic changes with particular emphasis on cytoplasmic bodies in these tumor cells.

* This study was supported in part by U.S.P.H.S. Grants Numbers C-5680 and CA-06791.

Received for publication March 15, 1963.
MATERIALS AND METHODS

Sixteen biopsies of papillomas were obtained from five patients, from 4 to 21 years old, and from this material 277 blocks of tissue were prepared for electron microscopic study. Biopsies of residual mucosa were taken from two of these patients on five occasions when papillomas were not present. Twenty-four blocks of this tissue were prepared for electron microscopy. In this manner the cyclic behavior of these tumors is represented in the experimental material. Two biopsies of normal control mucosa were obtained from children who had never suffered from laryngeal papillomas. Immediately upon removal, the tissue was minced in cold (0°C) 2 per cent osmium tetroxide buffered with veronal acetate to a pH of 7.4, to which sucrose had been added. Blocks of 1 cu. mm. were dehydrated in a graded series of alcohols and in propylene oxide and embedded in epon 812 according to the method of Luft (23). Sections with gold or silver interference colors were prepared with glass knives on a Porter-Blum microtome and stained with lead. They were studied with an RCA-EMU 3B electron microscope with an objective aperture of 35–40 µ. Micrographs were taken at original magnification, ranging from 2400 to 18,000 and enlarged photographically.

In addition, thick sections (0.5–1.5 µ) of all blocks were cut, stained with toluidine blue, and examined with the light microscope to assess the depth of the tissue (deep, intermediate, or superficial) from the papilloma, to determine the presence of inclusions, and to note unusual structural features. Features of Laryngeal Papilloma Cells Not Seen in Normal Mucosa or in Residual Mucosa from Papilloma Patients Free from Tumor at the Time of Biopsy

Features of laryngeal papillomas bore many ultrastructural resemblances to nonkeratinizing oral mucosa (51) and to epidermal cells (8, 28, 38–40), but neither the small granular component of human (32) and mouse epidermis (16) nor the intramitochondrial bodies of mouse epidermis (17, 39) were present.

Certain structural features were uniformly present in cells from any level. In typical superficial cells (Figs. 2, 3) tonofilaments were aggregated to form prominent tonofibrils which terminated in desmosomes. Perinuclear fibrils were abundant, and a moderate number of ribonucleoprotein particles as well as homogeneously dense circular bodies 0.3–0.4 µ were present in the cytoplasm. Mitochondria were sparse, and endoplasmic reticulum was poorly developed. Nuclear pores were evident in paracentral sections, and nuclear membranes often had irregular indentations. Intercellular spaces were wide.

Intermediate cells (Fig. 4) were generally smaller than superficial cells, and the intercellular spaces were narrower. Tonofilaments were abundant and, in oblique section, imparted the “crown of thorns” appearance described by Setala et al. (39). Mitochondria were slightly more numerous than in superficial cells but of a similar simple structure. In occasional sections from intermediate layers, polymorphonuclear leukocytes were located between tumor cells or appeared as isolated granular islets invaginated into the cytoplasm of tumor cells (Fig. 5). The granules, measuring approximately 230–300 µ in greatest diameter closely resembled the particles measuring 240–275 µ in the second figure of Meessen and Schulz.

In basal cells, those adjacent to the supporting fibrovascular stalk (Figs. 6, 7) tonofilaments were less prominent than in superficial cells, and RNP particles were moderately abundant. Mitochondria were more numerous than in superficial or intermediate cells, and mitochondrial dense bodies were more prominent than at other levels. Cell membranes showed deep interdigitations, and “half-desmosomes” were apparent at the basement membrane. Endothelial cells also possessed long extensions of cytoplasm which, though extremely attenuated in some portions, appeared to form a continuous lining. The plasma membrane, particularly that portion over the nucleus, formed numerous blunt pseudopodial projections. Mitochondria were similar in size and shape to those in epithelial cells but lacked the intramitochondrial dense bodies. Multivesicular bodies or vesicles within vacuoles were not uncommon near the plasma membrane. The cytoplasm often contained round to oval dense bodies, measuring up to 1.4 µ, containing secondary denser granules situated primarily at the periphery (Figs. 8, 9).
brane-bound aggregates of mitochondria, RNP particles, and a second membrane-bound area composed of a moderately dense amorphous material interrupted by irregular, branched electronlucent areas (Figs. 10, 11). These bodies measured an average of 0.9 μ. Occasionally, the adjacent cells possessed large cytoplasmic vacuoles containing randomly scattered irregular dense particles measuring approximately 0.05 μ. These particles occasionally coalesced to form irregular collections up to 0.55 μ in diameter (Figs. 12, 13).

Smaller cytoplasmic bodies, not detected by light microscopy, were seen rarely. Some were of moderate density, measured 0.1–0.4 μ, were composed of granular or particulate material, and surrounded by one or more membranes (Figs. 14–17). Others of similar size range contained membranous fragments (Fig. 18) or irregularly dispersed dense material, possibly lipide (Fig. 19).

Within macrophages, interposed between tumor cells, were inclusions which consisted of amorphous dense material that appeared partially separated into membrane-bound compartments (Fig. 20).

Examination of chorioallantoic membranes after implantation of papilloma tissue and 4 days' incubation at 37° C. failed to reveal significant membrane thickening or cellular proliferation.

DISCUSSION

An evaluation of the literature regarding the etiology of laryngeal papillomas reveals extremely conflicting opinions and no conclusive experimental results. The number of reported successful transmissions of papillomas to animals or humans, with tumor fragments, cell suspensions, or cell-free filtrates, is far fewer than the number of negative results. Bjork and Webber's (5) experiments, using 28 guinea pig inoculations and culture of papilloma tissue from ten patients on chorioallantoic membrane, all yielded negative results. Ono et al. (33), in a study of transplantation of laryngeal papillomas from eleven patients and oral papillomas from four patients inoculated into rabbits, hamsters, and mice, reported negative results. They noted, however, thickening of chorioallantoic membranes in five out of eight instances. These authors quote four personal communications of no definite positive results in animal experimentation. Frugoni (19) and Buratti (11), from their experiments, concluded that laryngeal papillomas were not of viral origin. Text and Hladky (44) attempted to transmit papillomas to animals by direct tissue transplantation, cell suspension, and cell-free filtrates and succeeded in only two instances using a cell-free filtrate. Pinaker and Proud (34) reported no growth of papilloma tissue placed in the anterior chambers of the eyes of five rabbits.

Some explanation is required, however, for the rare apparent transmission of laryngeal papillomas, notably the results of Ullmann and those of Ono et al. Regarding Ullmann's experiments, not only have others failed to repeat his findings under proper conditions but also his series was small and poorly controlled in that he failed to rule out the possibility of the development of native canine verrucae that commonly appear after traumatic manipulation. Similarly, the observation of 1-mm. thickening of five out of eight chorioallantoic membranes by Ono et al. is subject to some reservation, since, from their illustrations, it is difficult to be certain of the type of cells participating in the proliferation. In this connection, Huxley and Murray (21) have pointed out that enormous thickening in the region of vessels of chorioallantoic membranes at points of grafting may occur, apparently nonspecifically, in response to trauma of manipulation or operative stimuli. The thickening, histologically, may consist of vascular mesenchymal tissue or squamous-cell proliferation. The necessity to study critically chorioallantoic membrane proliferation is indicated. Regarding the destruction of monkey kidney cells in tissue culture given injections of suspensions of papillomas (34), Bang (3) has pointed out that several different kinds of changes are produced in tissue culture by different viruses and emphasized that so-called cytopathogenic effect of viruses is nonspecific.

As Williams (50) has emphasized, even the finding of typical virus particles in tissue sections does not provide absolute proof of viral etiology nor identification of the virus but is only inferential evidence for viral etiology that points to further biologic studies. Bearing these reservations in mind, the electron micrographs of Meessen and Schulz cannot be accepted as proof of viral etiology of laryngeal papillomas. In their Figure 1, several of the structures interpreted as particles are empty, near the cell membrane, of variable size and shape and resemble vesicles. Moreover, the host cell has neither desmosomes nor tonofilaments, making its identification as an epithelial tumor cell uncertain. Figure 2 of Meessen and Schulz illustrates granules resembling those of polymorphonuclear leukocytes that occasionally appear as islets in tumor cells (Fig. 5). It is to be noted that occasional phagocytic cells are interspersed among tumor cells and that these phagocytic cells frequently contain inclusion-like material of uncertain nature.

Ullmann originally contended that the virus of
human warts and molluscum contagiosum also causes laryngeal papillomas. From an electron microscopic point of view, this contention is unsupported. The size, ultrastructure, and behavior upon transplantation of human wart virus have been thoroughly studied (6, 9, 10, 26, 27, 41, 42, 47, 48). The results of the present study of laryngeal papillomas bore no similarity to those of investigations dealing with skin papillomas. Nothing was found to suggest resemblance of a proposed laryngeal papilloma virus to the virus of human skin warts, to other dermatotropic viruses such as vaccinia and fowl pox (15, 31), to the Shope virus (49) or to the virus of molluscum contagiosum (2).

Because of the variability in size, site of localization, and mode of maturation of different tumor viruses (12, 20) attention was given to all parts of the papilloma cells—that is, nucleus, nucleolus, cytoplasm and cell membrane. Furthermore, since virus particles, in known viral tumors, may not be present at all levels, cells from each level of papilloma—from those adjacent to the stalk to keratinizing cells at the surface—were studied. Moore et al. (30), in a study of rabbit papilloma virus, identified virus particles only in tissues that were soaked several months in glycerine, because, in freshly fixed papillomas, cytoplasmic granules and particles (keratin and melanin) made identification of virus uncertain. Such a technic was not investigated in the present investigation, because the cytoplasmic clarity of papilloma cells renders masking of viral particles unlikely.

The possibility that a papilloma virus in the early stage of cell proliferation could become incorporated into the cell genome and be no longer structurally apparent in the florid tumor cannot be excluded in the present study. A situation comparable to that in polyoma-induced hamster tumors, from which virus particles are impossible to visualize or isolate in the later stages, could obtain. Papillomas became available for study only at a relatively late stage when they were symptomatic. It was felt that study of residual mucosa, during periods of remission, might yield additional information, but these tissues were indistinguishable from the controls.

It is of interest that electron microscopic studies of benign and malignant papillomas of the urinary bladder revealed no virus-like particles or inclusions (7, 29).

From current studies of human tumors, not manifestly of viral origin, of liver and nasopharynx,1 wherein cytoplasmic bodies not unlike those in laryngeal papillomas are seen with considerable frequency, it would seem that, until more is known of the ultrastructural alterations in human tumors, a conservative interpretation of "inclusions" and particles resembling viruses would be judicious. It is also important to be certain of the identity of the cell containing particles of unknown nature. Various structures, such as prominent Golgi vesicles, pigment granules, protein droplets, pinocytotic vesicles, fragments of polymorphonuclear cells, and products of phagocytosis and focal intracellular necrosis or cytolysis may have a superficial but confusing resemblance to viruses or viral products, especially at the light microscopic level.

The nature of the cytoplasmic bodies encountered in papilloma cells in this study can only be surmised with caution. Though some (Fig. 11) bear resemblance to bodies seen in mouse lymphatic leukosarcoma their structure is too varied to be definitely assigned. The bodies illustrated in Figures 10 and 11 resemble portions of cells characterized by fibril-free cytoplasm containing scant RNP particles, mitochondria, and a shrunken, irregular, pyknotic nucleus. The cytoplasmic bodies may represent cell remnants which have been phagocytosed by epithelial tumor cells. The vacuoles (Figs. 12, 13) may represent a similar phenomenon differing only in that the nucleus and mitochondria are absent in certain planes of section, leaving only agglomerates of RNP particles and fragments of endoplasmic reticulum membranes. In the absence of crystalline arrays or significant numbers of recognizable viral particles of any morphologically defined type (4, 19) it seems preferable, for the present, to regard the cytoplasmic bodies in these cells as nonspecific alterations, possibly resulting from focal cytolysis or phagocytosis. Final interpretation rests upon further biologic studies of these tumors and more extensive knowledge of the ultrastructural spectrum of human tumors in general.

In conclusion: 1. The cells of human laryngeal papillomas bore many ultrastructural resemblances to epidermal cells and nonkeratinizing oral mucosa.

2. Cytoplasmic bodies, seen by light microscopy were found, with the electron microscope, to consist of material resembling membrane-bound cell remnants segregated within or phagocytosed by tumor cells.

3. Smaller cytoplasmic bodies, not apparent by light microscopy, consisted of granular and dense globular or membranous material of unidentified origin.

4. No typical virus particles or viral inclusions were identified. There was no evidence to suggest resemblance of a proposed laryngeal papilloma

1 Unpublished observations.
virus to the viruses of human skin verrucae or molluscum contagiosum, to the Shope virus, or to other dermatotropic viruses.

REFERENCES

17. ———. Concept Intra Crista: A Dense Body within Mitochondria of Cells in Hyperplastic Mouse Epidermis. Ibid., pp. 784-99.

Fig. 1.—Diagrams illustrate, chronologically, the site and extent of papillomas, areas of scarring, the identification (by letters) and age of patients at successive biopsies.

#1: Patient previously had severe papillomatosis which was in remission at the time of this biopsy. Note extensive scarring due to repeated excision of papillomas.
#2: Papillomatosis in remission, biopsy obtained from scarred area (+).
#3: Tissue obtained from scarred larynx during remission of disease.
#7 & #8: Normal larynx; tissue obtained as control from right vocal cords and arytenoids.
#13 & #17: Tissue obtained from scarred right vocal cord.
FIG. 2.—Broad tonofibrils (t) of two adjacent superficial tumor cells terminate in desmosomes (d). Tonofibrils in oblique section are seen at arrow. ics = intercellular space. ×18,000.

FIG. 3.—The cytoplasm of a superficial tumor cell contains homogeneously dense circular bodies (db) measuring approximately 0.6 μ. Cisterns of endoplasmic reticulum (er) and moderate numbers of mitochondria (m) are present. The nucleus (n) shows a deep invagination and pores (arrow) are apparent in the nuclear membrane. ×9,600.

FIG. 4.—A tumor cell of intermediate depth. Tonofibrils (t) are apparent in oblique section. ics = intercellular space. d = desmosome. ×10,300.

FIG. 5.—A polymorphonuclear leukocyte which was situated between tumor cells. Note the absence of tonofilaments in the cytoplasm. In addition to vacuoles (v), cytoplasmic granules (g) of 250 to 290 μ are apparent. n = nucleus. ×45,400.
FIG. 6.—Basal cell adjacent to fibrovascular stalk. Mitochondria (m) are more numerous than in superficial cells; tonofilaments (f) are sparse. Deep interdigitations (id) and microvillous projections (me) of the plasma membrane of adjacent cells are apparent. "Half-desmosomes" (kd) are present at the junction of tumor cells with basement membrane (bm). n = nucleus. X11,500.

FIG. 7.—Basal cells in this section contain mitochondria with matrix granules (mg). An endothelial cell with Golgi apparatus is present at lower right. Note the greater density and thickness of the inner nuclear membrane compared to that of the outer nuclear membrane of the endothelial cell nucleus (ecn). l = lumen. X9,600.
FIG. 10.—A body within the cytoplasm of a papilloma cell. Tonofilaments (f) are also present and aid in identifying the parent cell as epithelial. The body itself appears to contain cell remnants consisting of RNP particles, mitochondria, and a pyknotic nucleus. X18,000.

FIG. 11.—A cytoplasmic body similar to that in the previous figure. Note the circular, interrupted profiles indicated by an arrow. X15,000.
Fig. 8.—Endothelial cell with pseudopodial projections (µ) and a multivesicular body (arrow and inset). X11,500.

Fig. 9.—Tumor cells from the basal level are present at the left (t). Collagen fibers (c) are present in the fibrous stalk and the attenuated process of an endothelial cell (e) contains round or oval dense circular bodies. X9,600.
FIG. 10.—A body within the cytoplasm of a papilloma cell. Tonofilaments (f) are also present and aid in identifying the parent cell as epithelial. The body itself appears to contain cell remnants consisting of RNP particles, mitochondria, and a pyknotic nucleus. ×18,000.

FIG. 11.—A cytoplasmic body similar to that in the previous figure. Note the circular, interrupted profiles indicated by an arrow. ×15,000.
FIG. 12.—A tumor cell (t:) with a large paranuclear cytoplasmic vacuole containing randomly scattered irregular, dense particles that measure from 0.05 to 0.55 μ. n = nucleus. X28,600.

FIG. 13.—Two adjacent tumor cells (t:) contain cytoplasmic bodies. The one at upper left is vacuolar, contains dense particles and indistinct membranous strands (ms). That at lower right is similar to bodies illustrated in Figs. 10 and 11. X12,600.
Fig. 14.—A tumor cell containing a circular body which measures approximately 0.3 μ and is of moderate, homogeneous density (arrow). X5,200.

Fig. 15.—The successive serial section to that illustrated in Fig. 14, at higher magnification, reveals, in this plane, the peripheral membrane (me) which appears folded at z. The granular interior appears condensed at c. X19,800.

Fig. 16.—A granular body is partially surrounded by concentric membranes which, at one pole, resemble a myelin figure (arrow). X39,800.

Fig. 17.—Membranous and particulate collections, of varying size, were occasionally present in cells from deep portions of the tumors. X34,400.
FIGS. 18, 19.—Membranous and particulate collections, of varying size, were occasionally present in cells from deep portions of the tumors. Fig. 18, ×45,800. Fig. 19, ×19,248.

Fig. 20.—Between two tumor cells (t) a macrophage (ma) containing collections of dense material partially separated into membrane-bound compartments (arrows). ×18,000.
Electron Microscopic Study of Human Laryngeal Papillomatosis

Donald J. Svoboda, Fernando R. Kirchner and G. O. Proud


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/23/7_Part_1/1084

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/23/7_Part_1/1084.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.