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Inverted Papillomas of Nasal Cavity and Paranasal Sinuses

Ultrastructural Investigations on Epithelial-Stromal Interface

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Summary. 10 cases of inverted papillomas of nasal cavity and paranasal sinuses were examined electron microscopically with particular regard to the epithelial-stromal interface. The papilloma cells were clearly demarcated from the stromal tissue by a basement membrane-like material. However, this structure mainly consisted of two or more layers, occasional breaks in the basement membrane were visible. Within the basal epithelial cells resting on the basement membrane accumulations of actin-like microfilaments could sometimes be observed. Furthermore, in the stromal tissue some myofibroblasts were present in the vicinity of the papillomatous cell complexes and capillary vessels also demonstrated several layers of basement membrane. All the changes presented here are considered to be the result of a permanent interaction between aggressive forces of papilloma cells and the defence mechanism of stromal tissue. The results allow the conclusion that inverted papillomas of nasal cavity and paranasal sinuses are true neoplasms with very low malignancy.

Key words: Inverted papilloma – Low malignancy – Multilayered basement membrane – Myofibroblastic cells – Filament accumulations

Inverted papillomas belong to the papillomatosis group of nasal cavity and paranasal sinus tumors, they are defined by their typical inverted growth pattern (Kelly et al. 1980). Their nature is uncertain and continues to be a matter of controversy. In the literature inverted papillomas have been considered to represent hyperplasias secondary to chronic inflammations or as benign or malignant neoplasms (Snyder and Perzin 1972).

There are many comprehensive reports on the clinico-pathological examination of these lesions (Hyams 1971; Trible and Lekague 1971; Snyder and Perzin 1972; Vrabec 1975; Calcaterra et al. 1980). Most authors agree

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that inverted papillomas have only a small propensity to transform into frankly invasive carcinomas although such transformations have been described (Mabery et al. 1965; cp. also Batsakis 1981). However, patients with an inverted papilloma are clearly at risk for the development of an associated carcinoma (Norris 1963; Skolnik et al. 1966; Clairmont et al. 1975). Such an event may happen at a frequency between 5% and 20% (Michaels and Hyams 1975; Yamaguchi et al. 1979).

At light microscopic level invasive growth of inverted papillomas cannot be seen. Occasional bone erosions are said to be caused only by expansive growth. Metastases of a typical inverted papilloma have never been described (cp. also Fechner and Sessions 1977).

The clinical course of the disease is characterized by relentless progression and a high rate of recurrence (Batsakis 1979). This rejects the interpretation of inverted papillomas as a form of simple hyperplasia and is also unusual for benign tumors.

Recently, alterations in epithelial basement membranes and changes of stromal cells in several epithelial neoplasms were reported which seem to provide suitable criteria for evaluation of the malignant potential of such tumors (Alroy and Gould 1980; Schürch et al. 1981). Therefore we examined own cases of inverted papillomas of nasal cavity and paranasal sinuses light and electron microscopically, with particular regard to the epithelial-stromal interface. Our results allow the conclusion that inverted papillomas are true neoplasms with very low malignancy.

Material and Methods

Out of 26 cases 10 inverted papillomas of nasal cavity and paranasal sinuses were examined.

There were 5 male and 5 female patients. The age ranged from 30 to 78 years (mean age: 52.4 years). In all cases the inverted papillomas were localized in the region of the lateral wall of nasal cavity, papillomatous tissue was additionally encountered in the ethmoidal and maxillary sinuses (7 cases), in the sphenoidal sinus (2 cases) and in the frontal sinus (1 case).

At operation the papillomas covered a broad surface of the mucous membrane and showed a verrucous, papillary or frond-like pattern.

The tumor tissue removed surgically was fixed in neutral formalin and embedded in paraffin. The following stains and histochemical reactions were performed: H & E, elastica-Domagk for demonstration of connective tissue fibers, Giemsa stain, methyl green-pyronine, silver impregnation after Gömöri, Ladewig stain, PAS, alcian blue at pH 0.5, 1.0 and 2.5, alcian blue (pH 2.5)-PAS, colloidal iron reaction after Hale and Hale-PAS.

In each case small parts of the tumor specimens were prepared for electron microscopic examination. After fixation in a mixture of 1.25% glutaraldehyde and 1.5% paraformaldehyde (buffered with 0.05 M phosphate buffer at pH 7.3) for 2 h at 4° C a postfixation with OsO_4 for 1 h followed. Embedding in Vestopal or Mikropal. Semithin sections were stained with toluidine blue, ultra thin sections were contrasted with uranyl acetate and lead citrate.

Results

Light Microscopy. The structure of inverted papillomas was characterized by a marked proliferation of epithelial elements with formation of papillary fronds. In most cases this process was restricted to the superficial part of the mucous membrane, in only 3 cases were papillomatous complexes

also found deep in the connective tissue. The predominent cells were mostly polygonal basal cell or reserve cell like elements replacing the columnar ciliated cells of normal respiratory epithelium more or less completely. In 9 out of 10 cases epidermoid differentiation was also visible. Mild dysplasia was encountered in 4 cases.

The demarcation of non-invasive papillary epithelium from connective tissue was always sharp. There seemed to be a small zone of a clear PAS positive and alcianophilic lamella-like material in close vicinity of epithelial complexes. The loose connective tissue contained a variable number of vessels.

Electron Microscopy. The ultrastructural findings were rather similar in all cases of inverted papillomas independent of the presence of normal or slightly dysplastic epithelial cells. Thus a summary description can be presented.

Epithelial cells showed numerous short microvilli-like cellular projections which were often joined to projections of adjacent cells by well-developed desmosomes (Figs. 1 and 2). The epithelial cells possessed activated nuclei with round or somewhat irregular contours and often showed a large nucleolus. The organelles varied slightly in relative proportions. Thus a moderate number of mitochondria, some tubes of smooth and rough endoplasmic reticulum and many free ribosomes or polysomes could be found. Sometimes a well-developed Golgi apparatus and some lysosomal bodies were seen. In many cells typical bundles of tonofilaments could be observed (Figs. 1 and 2). Due to a different density of cytoplasm light and dark epithelial cells were distinguished, the latter seemed to be degenerating cells (Fig. 1). Between the epithelial cells we often observed monocytoid, lymphoid and granulocytic cells. The papillomatous structures were demarcated from the connective tissue stroma by basement membrane-like structures (Fig. 3).

Cells of the papillomatous formations sometimes exhibited an accumulation of thin microfilaments near the plasma membrane which was next to the basement membrane. These microfilaments had a diameter of 4–6 nm and therefore were thought to be actin filaments (Fig. 3b).

Over large distances the epithelial basement membrane already alluded to showed remarkable alterations and it was of regular configuration only in small areas. The most conspicuous feature was a reduplication or multilayering of the basement membrane (Fig. 3). Between the single layers of basement membrane remnants of organelles could be seen here and there. Furthermore, in different areas the basement membrane seemed to be of varying thickness (ranging from 40–120 nm) and occasionally it appeared somewhat indistinct. Only very seldom was a breakage of the basement membrane identified, which was caused in most cases by an obvious finger-like projection of an epithelial basal cell (Fig. 4).

The connective tissue stroma near to the papillomatous structures also revealed some pecularities. The vascular structures which were often localized near by the epithelial front were characterized by activated and swollen endothelial cells and a multilayered basement membrane (Fig. 5). Sometimes

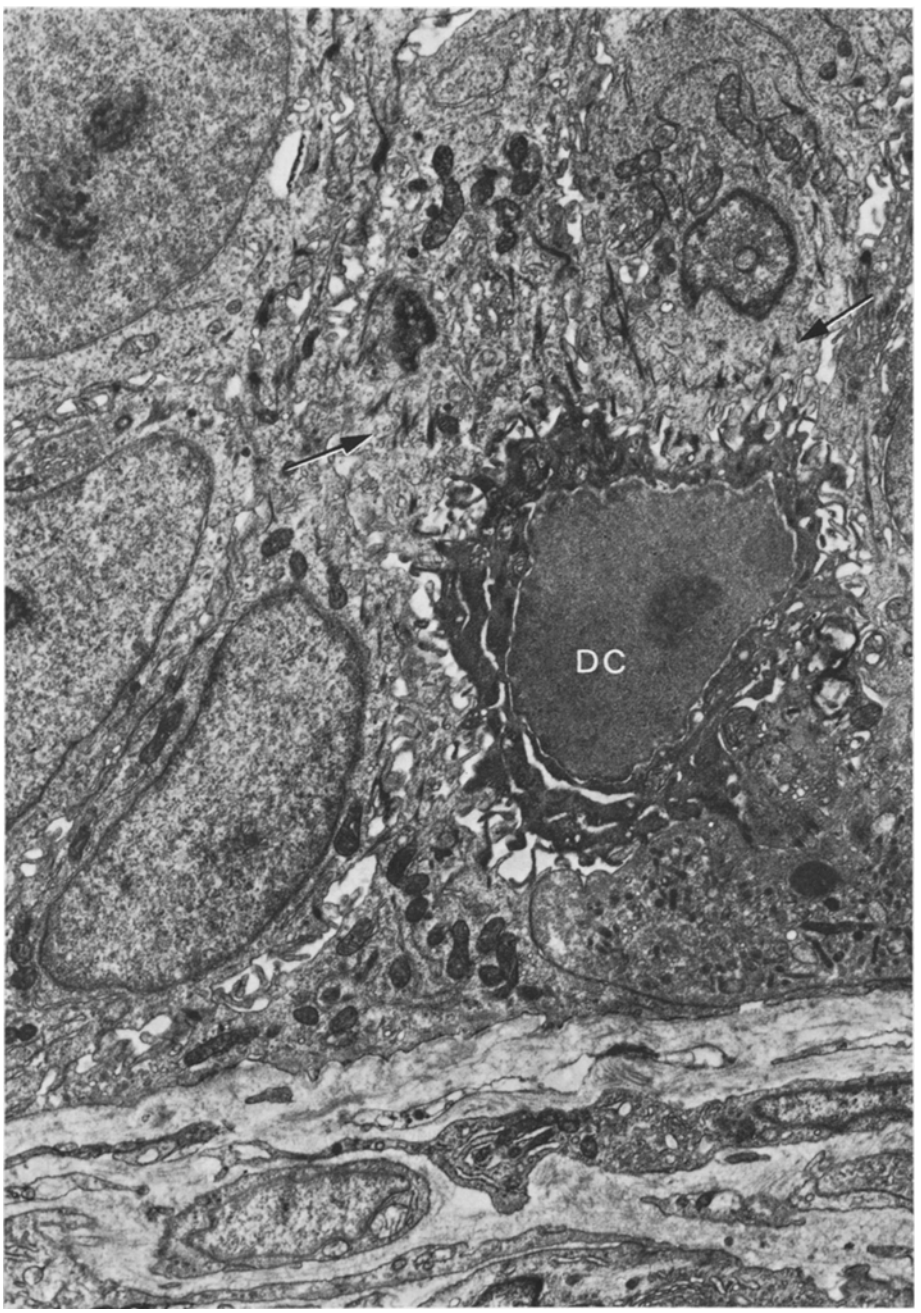

Fig. 1. Papilloma cells with activated nuclei, some tonofilament bundles (→) and a moderate amount of organelles. Besides several "light" cells a "dark" cell (DC) is present. Papilloma cell complex is clearly to be distinguished from the spindle-shaped mesenchymal cells in the stromal connective tissue (lower quarter of the picture) (9,600:1)

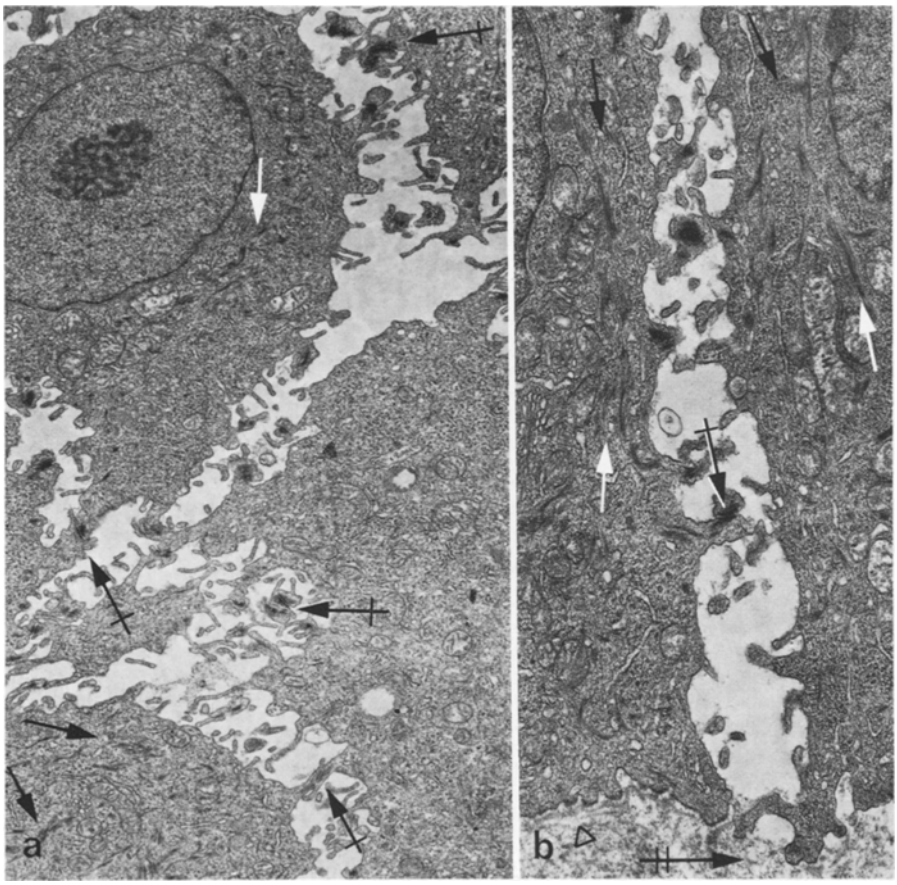

Fig. 2. Details of papilloma cells. (**a**) Papilloma cells show numerous microvilli-like cellular projections. Such surface formations of adjacent cells are often junctioned by desmosomes (↔). Note tonofilament bundles within the cytoplasms (→) (7,820:1). (**b**) At this magnification desmosomes (→) and tonofilament bundles (→) are clearly visible. Furthermore, a subepithelial basement membrane-like structure can be seen (▷), which is partially interrupted (↔) (13,760:1)

degenerating endothelial cells were obvious. In some vessels proliferating and very enlarged pericytic cells could be detected, they exhibited microfilament accumulations within the cytoplasm (Fig. 5b) in the vicinity of degenerating endothelial cells. Pericytic cells were also enveloped by a basement membrane. Between the single layers of the multilayered basement membrane of vessels organelle remnants and inflammatory cells were occasionally present (Fig. 5a). The vascular basement membranes showed similar dimensions as epithelial basement membranes, they were between 30 and 120 nm wide.

The stromal cells were partly arranged parallel to the direction of the epithelial basement membrane. They were equipped with different organelle

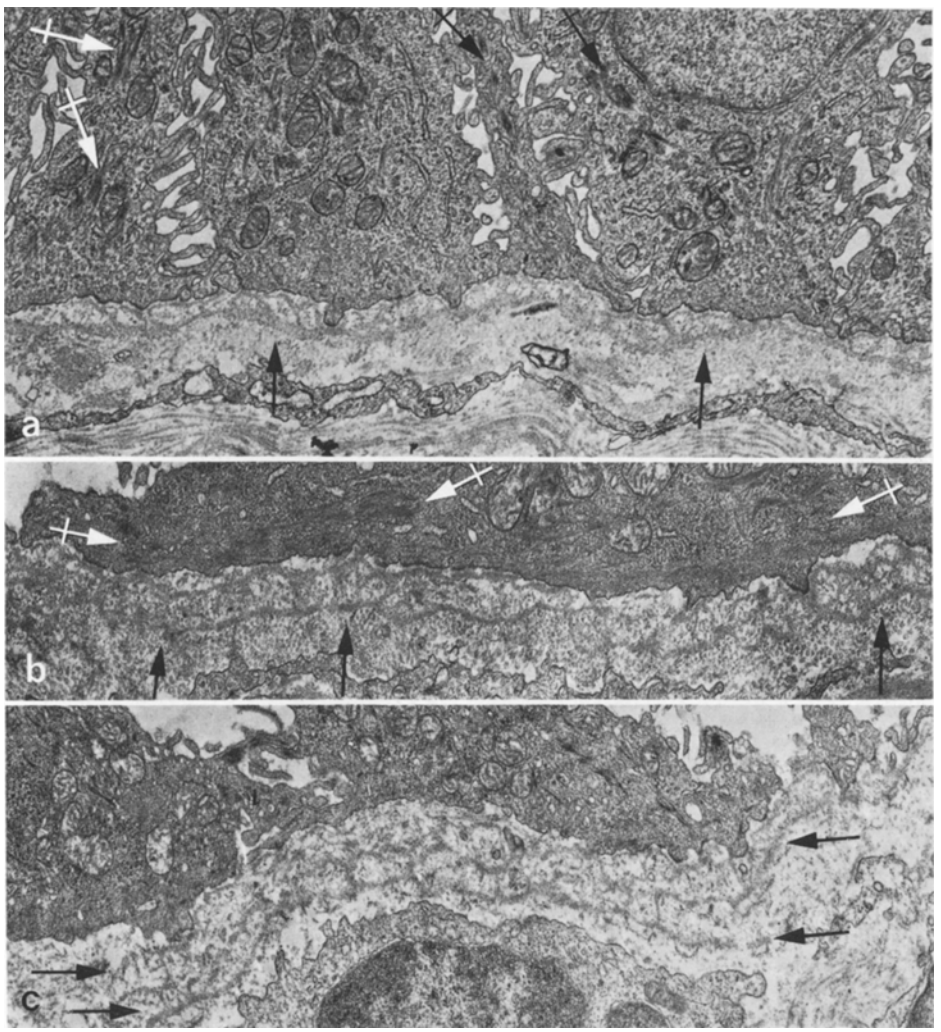

Fig. 3a–c. Epithelial-stromal interface. (**a**) Epithelial papilloma cells show some tonofilament bundles in their cytoplasm (↔) and have microvilli-like cellular projections. Note the duplicated epithelial basement membrane of variable thickness (→) (13,760:1). (**b**) In the basal region of epithelial cells which rest on the basement membrane (→) intracytoplasmic accumulations of thin filaments (↔) in parallel arrangement to the plasma membrane can be observed. Furthermore we can see a duplicated epithelial basement membrane (13,760:1). (**c**) The epithelial basement membrane (→) is multilayered. At the left edge of the picture there is an obvious breaking of the basement membrane (10,880:1)

compositions. We encountered fibroblastic cells with numerous tubes of rough endoplasmic reticulum and histiocytic cells with vesicular structures and lysosomal bodies. The presence of some myofibroblast-like cells in close vicinity of papilloma cells is worthy of note (Fig. 6). Besides rough endoplasmic reticulum, mitochondria and ribosomes this cell type is characterized by bundles of microfilaments within the cytoplasm. Furthermore, uncharac-

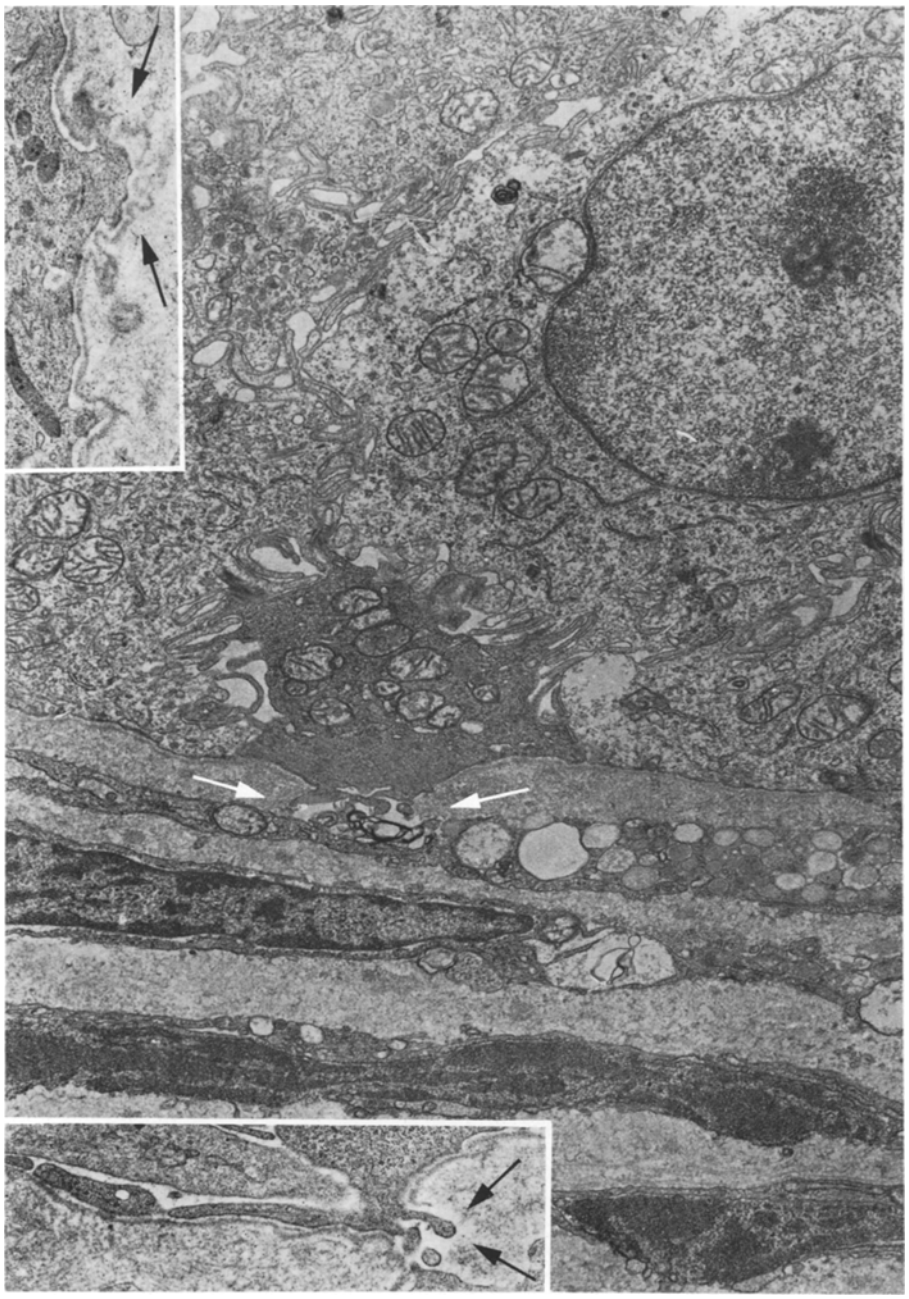

Fig. 4. The papilloma cells consist of light and dark cells. Note the contact between a dark cell and stromal cells (histiocytic?) (→) (13,760:1). *Upper inset:* The distance between lamina densa of the basement membrane and the epithelial cell border is somewhat varying so that the basement membrane appears slightly undulated (→) (13,760:1). *Lower inset:* Interruption of the basement membrane by a cellular finger-like projection (→) (13,760:1)

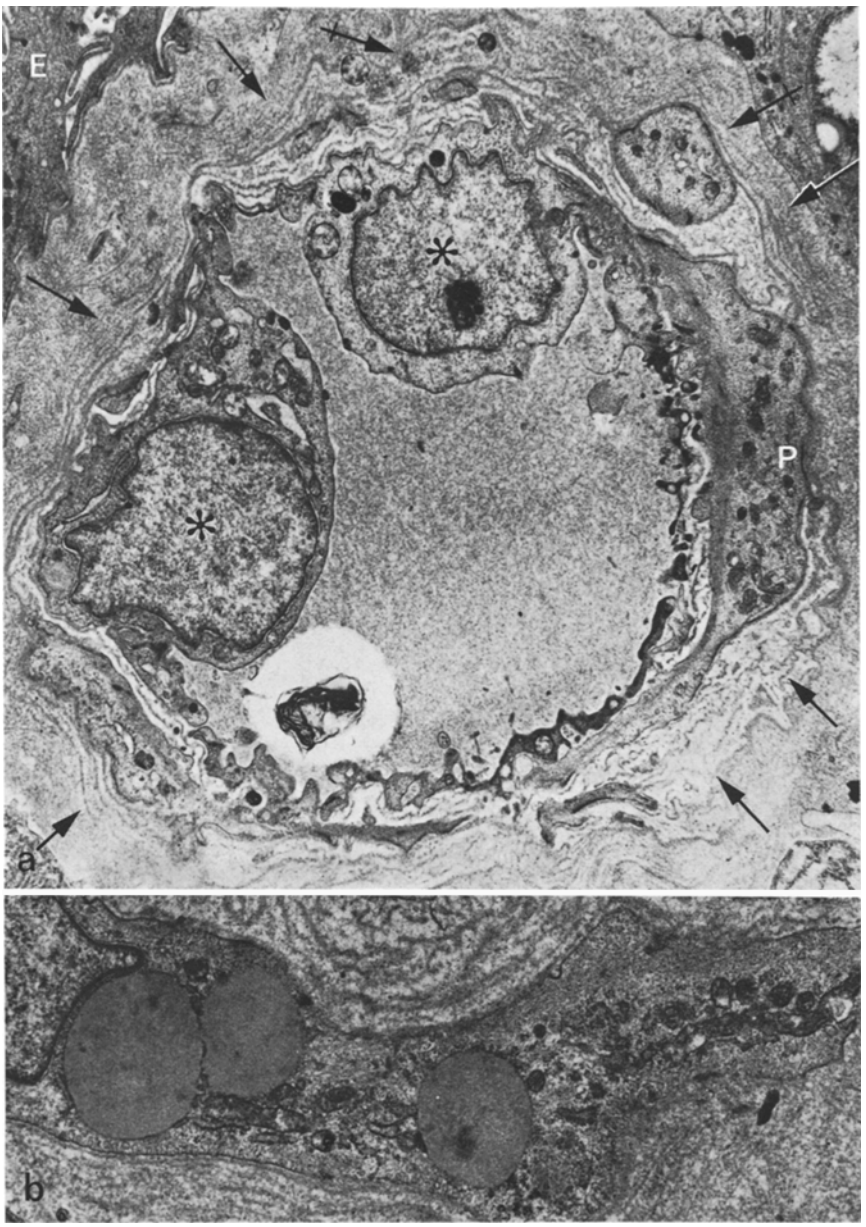

Fig. 5a, b. Activated vessels in the stromal tissue. **(a)** Capillary vessel with activated endothelial cells (*) and a multilayered basement membrane (→). A part of epithelial papilloma cells (*E*) is also visible. A pericytic cell (*P*) is likewise activated and shows microfilament bundles within the cytoplasm (7,820:1). **(b)** Higher magnification of a pericytic cell. There are some lipid droplets and an accumulation of thin filaments within the cytoplasmic region facing the endothelial tube (13,760:1)

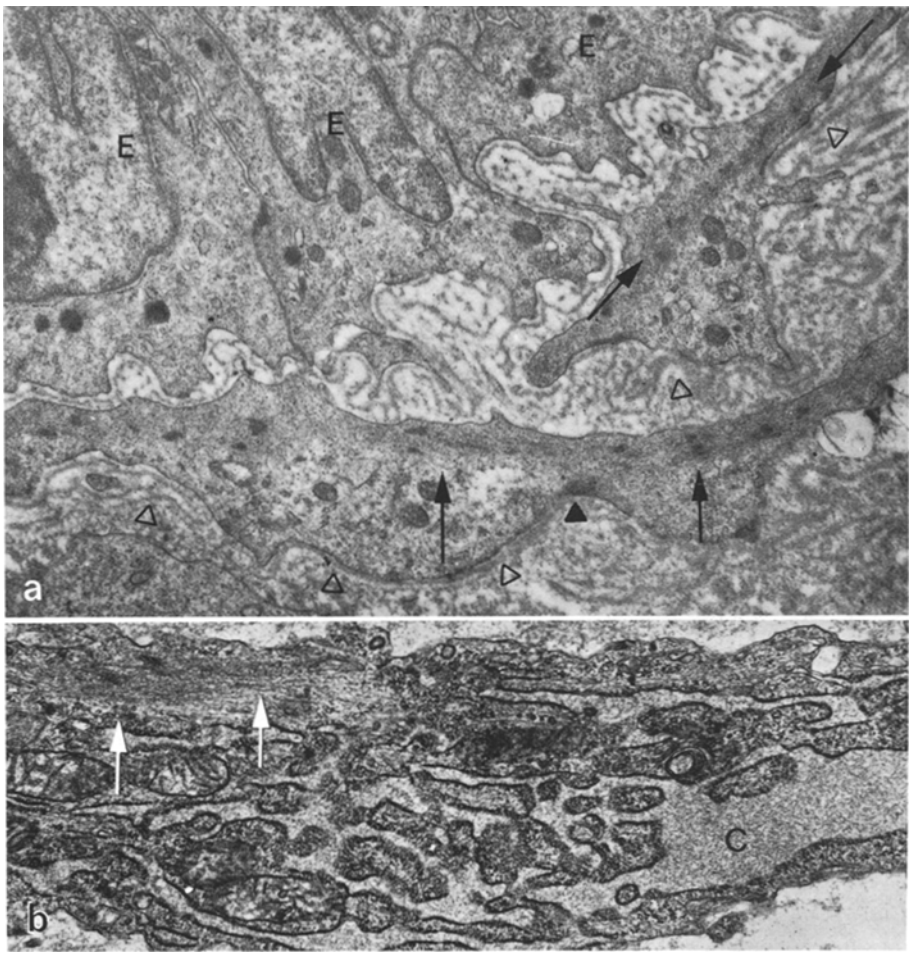

Fig. 6. Myofibroblast-like cells in the stroma. (**a**) Two stromal cells characterized by thin filament bundles with focal densities (→) are localized in vicinity of papilloma cells (*E*). Occasionally attachment sites of filaments are visible (▲). The basement membrane-like structures are partially found in close vicinity of myofibroblast-like cells (△) (13,800:1). (**b**) Filament accumulations with focal densities (→) and abundant rough endoplasmic reticulum with cisterne-like formations (C) (16,900:1)

teristic mesenchymal cells without a typical organelle composition were noted. Numerous inflammatory cells were visible, an interactions between macrophages and lymphocytic cells were occassionally registered. Besides these cells, we observed plasma cells and different types of granulocytes. Uncommonly direct contacts between stromal cells and papilloma cells seemed to be well developed (Fig. 4).

The intercellular substance consisted of thin mature collagen fibers with a periodicity of about 64 nm, thin fibrils without periodicity and an amorphous or finely granular ground substance.

Discussion

The light microscopic examination of our material yields common features shared by all inverted papillomas: papillomatous configuration of the lesions and proliferation of cells which correspond preponderantly to reserve cell or basal cell like epithelial elements. There was always some evidence of respiratory differentiation of the epithelium. In most lesions metaplastic stratified squamous epithelium could be seen. The majority of inverted papillomas showed no cellular atypia, but in 4 cases mild epithelial dysplasia was noted. In papillomas a morphological range from typical to dysplastic epithelium has also been reported in the literature (Rudert 1971; Tolsdorff et al. 1972).

Generally, in cases with papillomatous complexes deep in the mucous membrane and with epithelial atypia the differentiation between a pseudoinvasive papilloma and a true invasive carcinoma may be difficult and is dependent on cytological details (Snyder and Perzin 1972).

The epithelial structures of typical inverted papillomas are clearly demarcated from the connective tissue because they are confined by a basement membrane (Kelly et al. 1980). Histochemically we have demonstrated a zone between papilloma and loose stromal tissue which was characterized by an increased amount of fibrillar material and glycosaminoglycans. These findings of histochemical analysis may be interpreted as evidence of a basement membrane-like material around epithelial complexes.

Electron Microscopy. We found in most specimens a reduplicated or multilayered epithelial basement membrane. Such phenomena are considered to be indicators of a tissue alteration (Morgenroth and Themann 1966) or in malignant tumors, of a relatively low degree of malignancy (Gould and Battifora 1976). Here and there breaks in the basement membrane were visible. Generally fragmented or discontinuous basement membranes in epithelial growths correlate with a more or less aggressive behavior; reduction of basement membrane-like material is considered to be a characteristic of invasive carcinomas (McKinney and Singh 1977; Frie 1978).

Sometimes in basal cells resting on the basement membrane actin-like microfilaments accumulated intracytoplasmatically in the vicinity of the plasma membrane next to the basement membrane. Such microfilament organization is known to occur after tissue injury (Fritzsche et al. 1981). In this connection the occurrence of (degenerating) dark papilloma cells and of many intermingled inflammatory cells is emphasized.

Similar ultrastructural alterations to those described here were recently reported in malignant urothelial neoplasms especially in non-invasive transitional carcinomas of the urinary bladder (Alroy and Gould 1980). By analogy, malignant potential of inverted papillomas of nasal cavity and paranasal sinuses may be suggested. In spite of a superficial morphological resemblance between nasal and urothelial papillomas, which is also reflected by the synonyma "transitional cell papilloma" for papillomas of nose, a clear distinction between both the lesions is necessary, because nasal inverted papillomas are not of transitional cell origin but are basal cell or reserve cell tumors of the respiratory epithelium (Michaels and Hyams 1975). Ultra-

structurally we observed tonofilaments and thus an epidermoid differentiation in many papilloma cells.

In the connective tissue close by epithelial complexes, inflammatory cells and mesenchymal cells could be encountered, in particular myofibroblast-like cells were noted. Although myofibroblasts are considered to be an almost ubiquitous modulation of fibroblasts they are probably of some prognostic significance in soft tissue tumors (Stiller and Katenkamp 1975; Vasudev and Harris 1978; Seemayer et al. 1981). These cells can also be found in the stroma of invasive epithelial neoplasms of relatively low malignancy (or in metastases) and may be explained as host stromal response after epithelial stromal invasion (Schürch et al. 1981).

Myofibroblasts are said to be lacking in non-invasive carcinomas (Schürch et al. 1981). In the non-invasive inverted papillomas myofibroblast-like cells appear to be induced in the mesenchymal border zone by extending the stromal connective tissue due to expansive growth. This idea is supported by observations on the development of myofibroblasts after skin stretching in mice (Squier 1981).

Multilayered basement membranes around vascular structures can be seen in inflamed tissues (Cooper and Goodman 1974; Bhawan 1980), after irradiation (Fritzsche et al. 1981) and in several human and experimental tumors (Koss 1977; Lagacé et al. 1979; Nagao et al. 1980). The significance of this vascular alteration is completely obscure and remains a matter of speculation. We suggest by analogy to similar findings in benign lesions with proliferating vessels, that multilayered basement membrane could indicate a vascular cell proliferation (cp. Kindblom and Fassina 1981).

In summary, we consider the inverted papillomas of nasal cavity and paranasal sinuses to be true neoplasms with very low malignancy on the basis of certain features of the epithelial stromal interface. Changes of epithelial cells, of epithelial and vascular basement membranes as well as of stroma cell differentiation may be the result of the permanent interaction between aggressive forces of papilloma cells and the defence mechanisms of stromal tissue.

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