Virchows Arch [Pathol Anat] (1981) 393:273–286

Virchows Archiv A

Intermediate-Sized Filament Proteins (Prekeratin, Vimentin, Desmin) in the Normal Parotid Gland and Parotid Gland Tumours

Immunofluorescence Study*

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Summary. Antibodies to different intermediate-sized filament proteins can distinguish cells and tissues of epithelial, mesenchymal, muscle, astrocytic and neural origin. Antibodies to prekeratin, vimentin and desmin have been used to distinguish cells of epithelial, mesenchymal and muscle origin in the normal human parotid gland, and in addition to study some common tumors of this gland. Prekeratin-positive and vimentin-positive cells are found among the tumor cells in the pleomorphic adenomas. In contrast the tumor cells of the mucoepidermoid tumors and squamous cell carcinomas are prekeratin-positive but vimentin-negative.

Key words: Parotid gland tumors – Intermediate-sized filaments of the prekeratin-, vimentin- and desmin-type

Introduction

The diagnostic classification of salivary gland tumors is based upon histological and cytological patterns in fixed and paraffin-embedded material (Thackray and Lucas 1974; Eneroth 1976; Seifert and Donath 1976; Woods et al. 1977). Although these methods have proved to be valuable, problems still remain both in the diagnosis of certain parotid gland tumors, and with regard to the histogenesis of some of these tumors (Eversole 1971).

Intermediate-sized filaments are an ubiquitous component of cells and tissues. They can be distinguished by their diameter (7–11 nm) from the other two

* Supported by the Deutsche Forschungsgemeinschaft (Ca 93/1-1) and by the Hamburger Stiftung zur Förderung der Krebsbekämpfung

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0340-1227/0393/0273/$02.40

filamentous components of the cytoskeleton, i.e., microtubules (diameter 22 nm) and microfilaments (diameter 6 nm). Use of immunological methods to analyze the intermediate-sized filaments present in cells and tissues has shown the existence of several classes of these filaments (see e.g., Franke et al. 1978a; Bennett et al. 1978; Lazarides and Balzer 1978; Sun et al. 1979; Bignami et al. 1980; Yen and Fields 1981). Thus antibodies to different intermediate-sized filament proteins can be used to decide whether cells are of epithelial, mesenchymal, muscle, astrocytic or neural origin (for reviews see Lazarides 1980; Weber and Osborn 1981a, b). The striking agreement of this classification with known systems of histological classification suggests that such antibodies may be of use in classifiying human pathological material. In this study we have used three such antibodies, i.e., an antibody against bovine prekeratin which identifies cells of epithelial origin, a vimentin antibody which identifies cells of mesenchymal origin, and a desmin antibody which distinguishes skeletal, cardiac and certain smooth muscle cells. These have been used to characterize the cell types present in normal human parotid gland, and in a collection of parotid gland tumors.

Materials and methods

Tissue Specimens

Human parotid glands were removed during head and neck surgery. The following glands were made available to us through the courtesy of Prof. Herberhold (Ear, Nose and Throat Clinic, Hamburg):

Normal parotid glands	5
Pleomorphic adenomas	6
Mucoepidermoid carcinomas	1
Squamous cell carcinomas	2

The tumors were all detected in the region of the parotid gland and were classified as primary tumors, except for the two advanced squamous cell carcinomas where the possibility of secondary invasion cannot be completely excluded. The normal glands and the tumors were quick frozen in liquid nitrogen and stored at −70° C.

Immunofluorescence Procedures

The material was sectioned on a Reichert OM U2 cryostat at −20° C, and transferred to glass slides. The sections were allowed to dry for 1 h and then either processed directly or stored at −70° C. The position of the section was marked on the slide with a diamond pencil, and the slides were then treated as follows:

1. Fixation in acetone for 10 min, then air dried.
2. Incubation with the primary antibody (see below) for 45 min at 37° C.
3. Washing in phosphate buffered saline.
4. Incubation with a second antibody (see below) coupled with FITC for 30 min at 37° C.
5. Washing in phosphate buffered saline.
6. Treatment for 5 min in 96% ethanol.
7. The slides were mounted by placing a coverslip, on which a drop of Mowiol 4–88 (Hoechst, Frankfurt) had been placed, over the section.
8. The slides were observed and photographed using a Zeiss Photomicroscope equipped with epifluorescent optics. Controls were performed by omitting the first antibody and instead using phosphate buffered saline.

For further details of the immunofluorescent procedures see Osborn and Weber (1981).

Antibodies

The following affinity purified antibodies were available:

1. Prekeratin. The antibody was raised in guinea pigs against prekeratin purified from cow hoof (Franke et al. 1978b) and was affinity purified on the antigen covalently coupled to Sepharose 4B.

2. Vimentin. The antibody was raised in guinea pigs against vimentin from mouse 3T3 cells (Franke et al. 1979c) and was affinity purified on vimentin isolated from rabbit chondrocytes and bound to Sepharose 4B.

3. Desmin. The antibody was raised in rabbits against desmin purified from chicken gizzard (cf. Lazarides and Balzer 1978) and was affinity purified on the antigen bound to Sepharose 4B.

These antibodies, which have been fully characterized on animal (e.g., Franke et al. 1978a, b, 1979a, b; Weber and Osborn 1981a, b) and human tissues (Altmannsberger et al. 1981) were used at a final concentration of 50 µg/ml.

FITC-labelled goat anti-guinea pig IgG, or FITC goat anti-rabbit IgGs were purchased from Miles-Yeda, Israel. To reduce non-specific staining by the FITC-labelled antibodies these were absorbed after a 1:5 dilution on lyophilized human parotid gland material (~10 µg/ml). FITC-labelled second antibodies were then used at a final dilution of approximately 1:20.

Results

Normal Parotid Gland

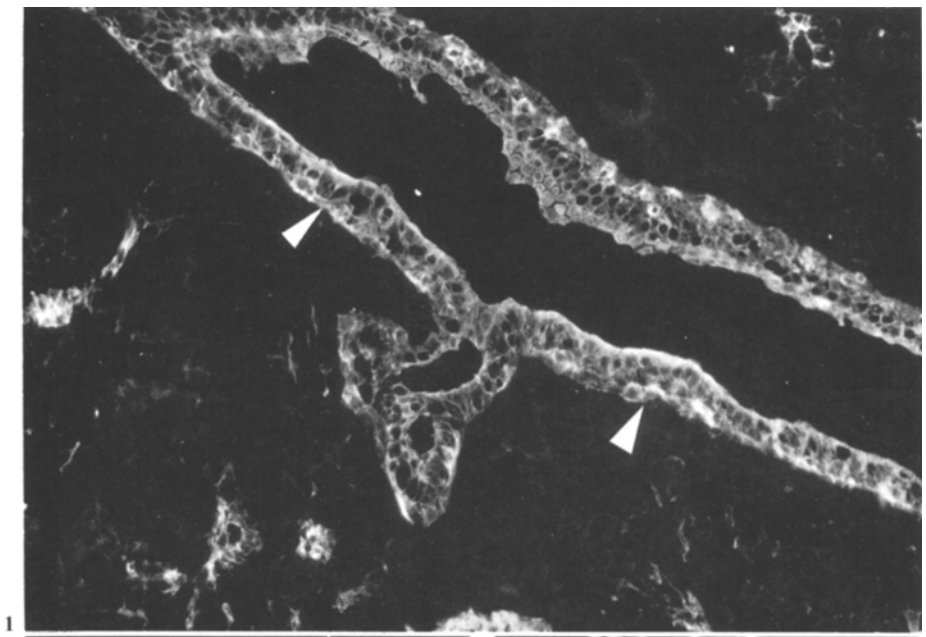
Prekeratin. When antibodies to prekeratin were used in immunofluorescence microscopy, intense staining of the myoepithelial cells and the duct cells was observed. The myoepithelial cells displayed a spindle-shaped cell body with branches surrounding the different acini. Myoepithelial cells were detected at the periphery of intercalated ducts, as well as of the striated ducts and the large excretory ducts. All duct cells were clearly stained for prekeratin. In contrast the acinar cells appeared to be negative (Figs. 1, 2).

Vimentin. Antibodies to vimentin revealed a different staining pattern. Vimentin filaments could be observed in the cytoplasm of the fibroblasts of the connective tissue between the acini and the ducts (Fig. 3). A layer of less strongly positive cells was sometimes observed at the peripheral border of the ducts. In some cases, triangular-shaped cells could be observed between the duct cells of striated ducts. Endothelial, and vascular smooth muscle cells were strongly stained for vimentin.

Desmin. When antibodies to desmin were used in immunofluorescence microscopy, desmin was detected in some cells present in the walls of blood vessels. The surrounding tissue was negative.

Pleomorphic Adenoma

Prekeratin. In the pleomorphic adenomas, the tumor cells were positive for prekeratin. Two different patterns could be distinguished. The first, characterized



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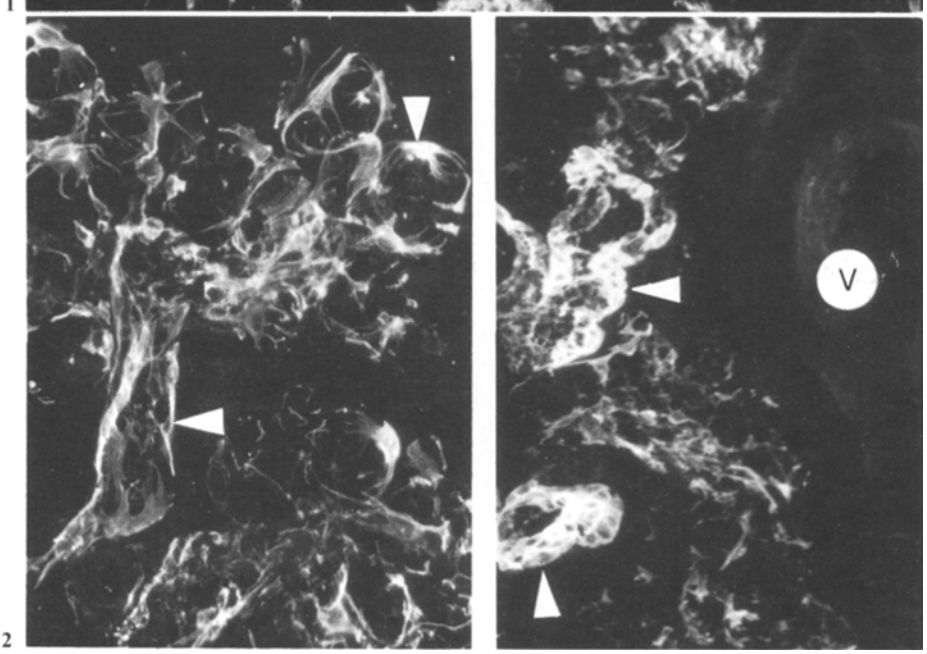

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Fig. 1. Normal parotid gland. Intensely stained cells of a large excretory duct. Immunofluorescence staining for prekeratin. ×210

Fig. 2a, b. Normal parotid gland. **a** Intensely stained myoepithelial cells arround acini and intercalated ducts (*arrowheads*). **b** Intensely stained duct cells (*arrowheads*) on the right negative glood vessel (*v*). Immunofluorescence staining for prekeratin. **a** ×330, **b** ×210

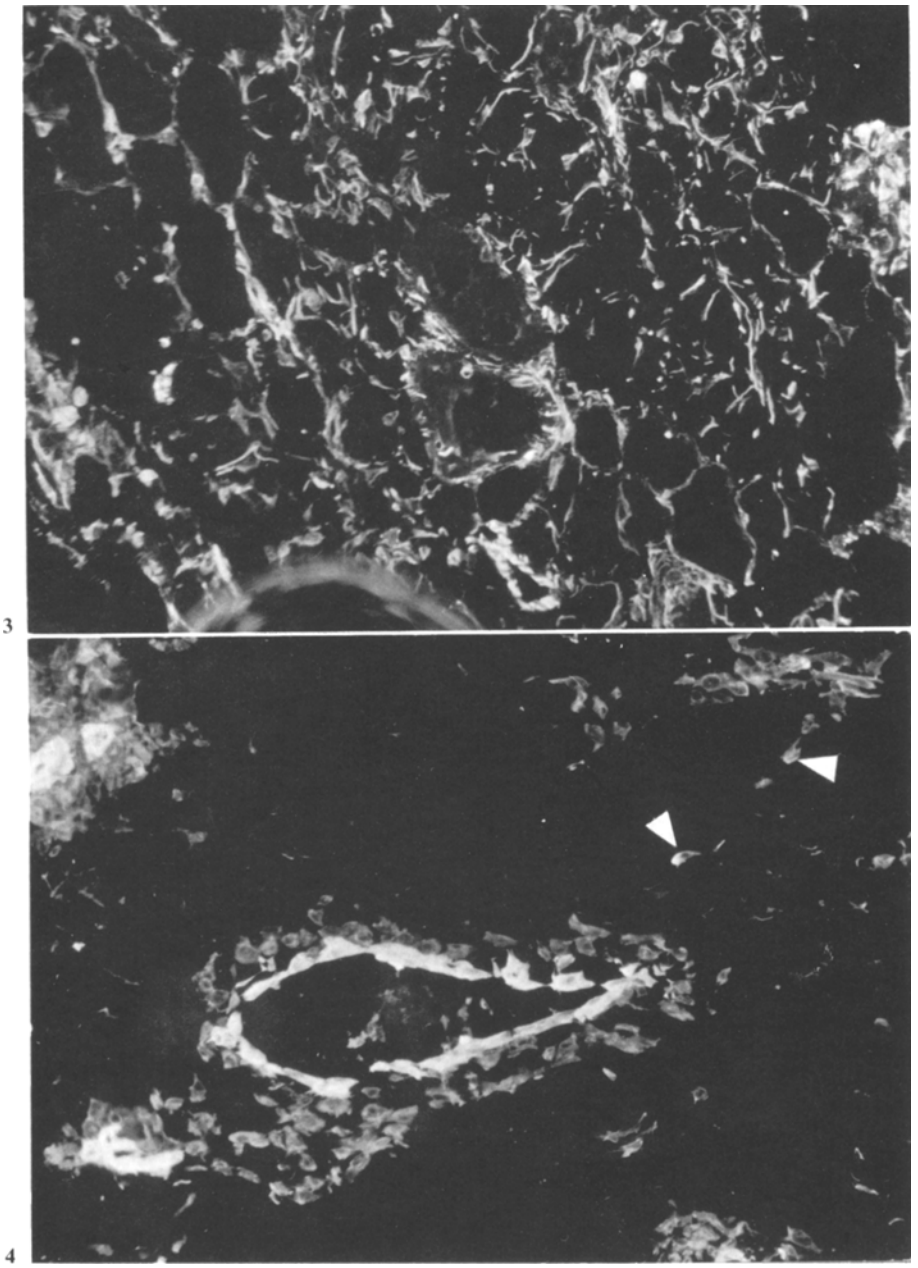


Fig. 3. Normal parotid gland. Meshwork of intensely stained cells between the acini and ducts (predominantly fibroblasts). Staining of the vimentin filaments inside the cells. Immunofluorescence staining for vimentin. ×210

Fig. 4. Pleomorphic adenoma. Groups of intensely stained tumor cells (*center*). Sometimes tubular arrangement, some single cells (*arrowheads*) positively stained. Immunofluorescence staining for prekeratin. ×210

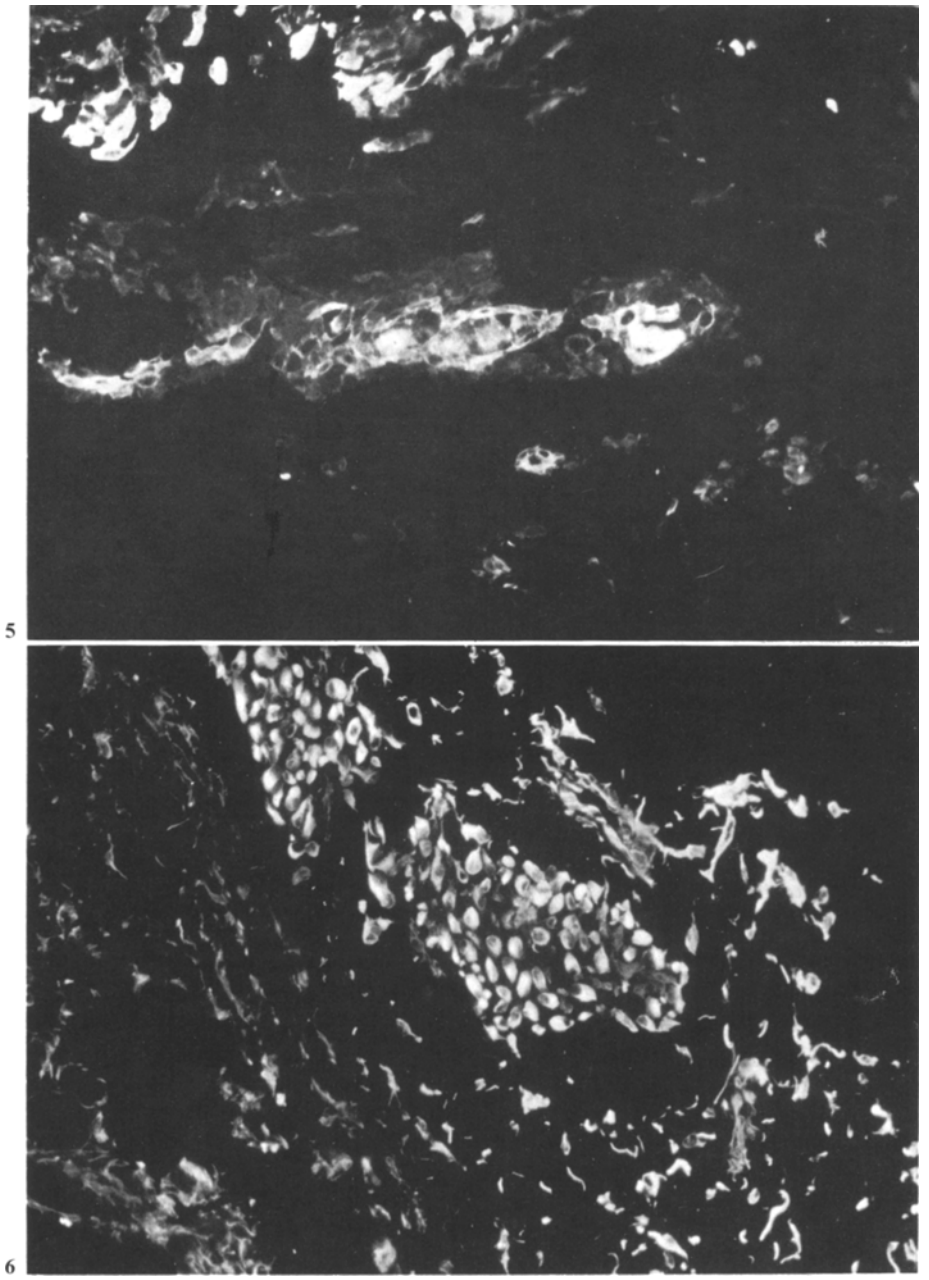


Fig. 5. Pleomorphic adenoma. Solid arrangement of positively stained tumor cells (*center*). Negative background predominately formed by the stromal part of the pleomorphic adenoma. Immunofluorescence staining for prekeratin. ×210

Fig. 6. Pleomorphic adenoma. Many positive tumor cells in loose arrangement. Immunofluorescence staining for vimentin. ×210

by solid cell clusters in which all cells are positive for prekeratin is illustrated in Fig. 5. The second, characterized by the presence of dispersed spindle-shaped cells in the stromal compartments is illustrated in Fig. 4.

Vimentin. Strong staining of tumor cells of the pleomorphic adenoma was observed with antibodies to vimentin (Fig. 6). These cells appeared similar in morphology to the prekeratin-positive cells.

Desmin. When desmin antibodies were used positive staining of tumor cells was not detected. Some cells present in the walls of blood vessels in the neighbourhood of the tumor were strongly stained.

Mucoepidermoid Tumor

Prekeratin. The tumor cells were stained positively in immunofluorescence with antibodies to prekeratin. The squamous part of the tumor was stained strongly (Figs. 7, 8) – in some cases the picture was similar to that of the squamous cell carcinoma. On the other hand, the mucus-producing cells were only very weakly stained, most of the cytoplasm being filled with mucus. The mucus-containing cells were stained at the peripheral part of the cytoplasm which sometimes appeared as a fluorescent rim. The stromal part of the tumor was clearly unstained.

Vimentin. As in the case of the squamous cell carcinoma, the tumor cells were not stained. However, strong staining of fibroblasts in the stromal part was seen (Fig. 9) and in some cases a fibrous staining was apparent in individual cells.

Desmin. Tumor cells and stroma were negative for desmin. Cells in the walls of blood vessels were strongly stained (Fig. 12).

Squamous Cell Carcinoma

Prekeratin. The tumor cells were positive for prekeratin. The intensity of the staining, however, was different in individual cells: some were stained strongly, others only moderately. The surrounding connective tissue was negative for prekeratin (Figs. 10, 11).

Vimentin. Immunofluorescence microscopy with antibodies to vimentin showed a different pattern from that seen with antibodies to prekeratin. The tumor cells were in general negative for vimentin, whereas cells of the surrounding connective tissue were strongly stained. Interestingly the patterns of vimentin filaments appeared to be somewhat different to that seen in connective tissue of non-tumoral origin. The network of vimentin filaments visualized in the tumoral stromal cells was denser, displaying an irregular appearance of interwo-

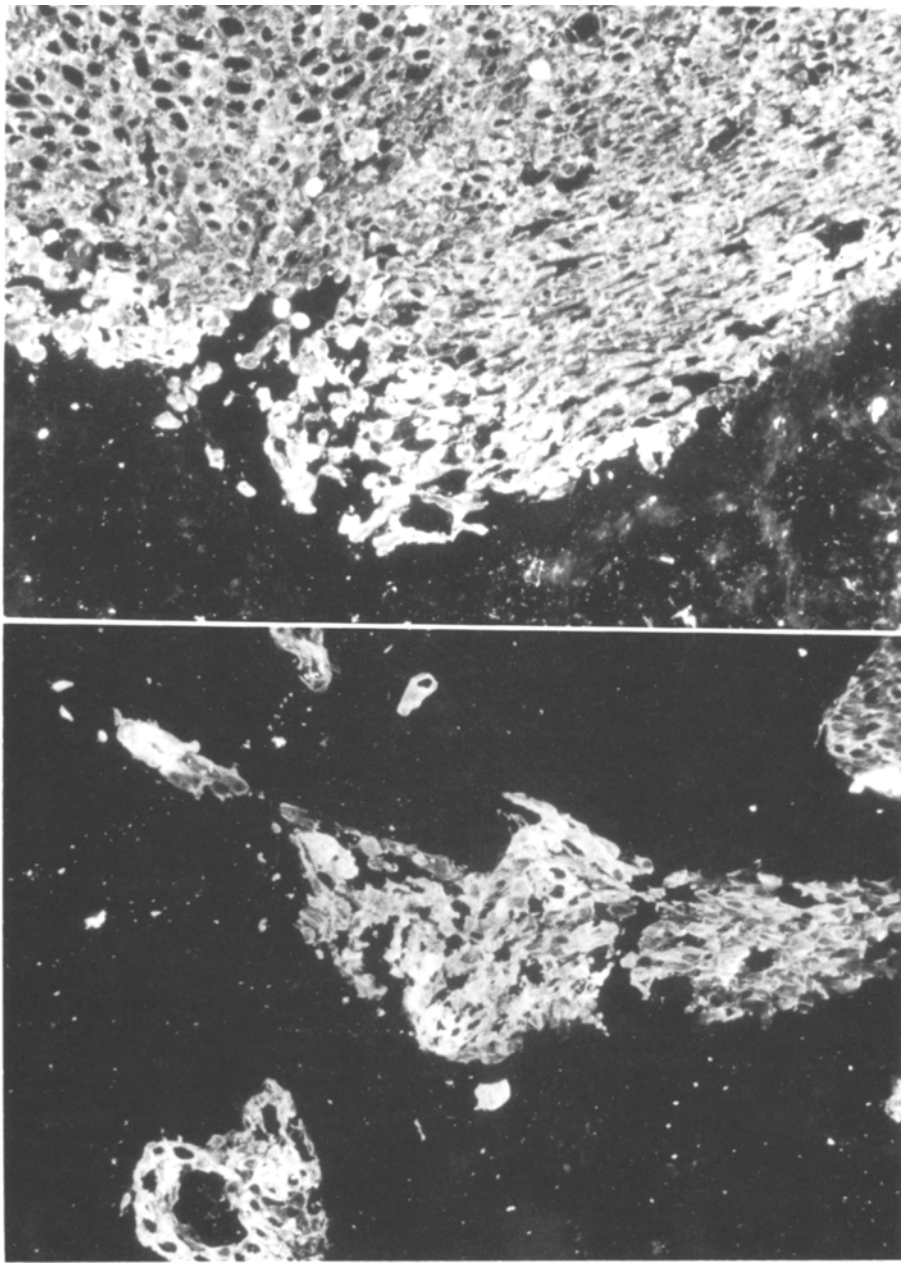
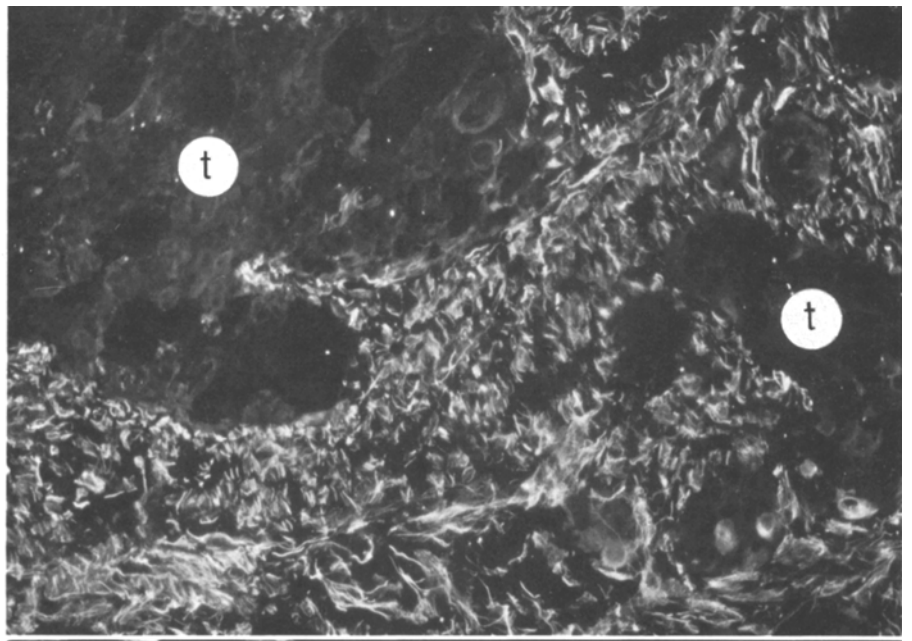


Fig. 7. Mucoepidermoid tumor. Solid arrangement of epithelial tumor cells (*upper part*). Negative staining of the stroma (*lower part*). Immunofluorescence staining for prekeratin. ×210

Fig. 8. Mucoepidermoid tumor. Solid arrangement of positively stained tumor cells in a negative stroma. Immunofluorescence staining for prekeratin. ×210



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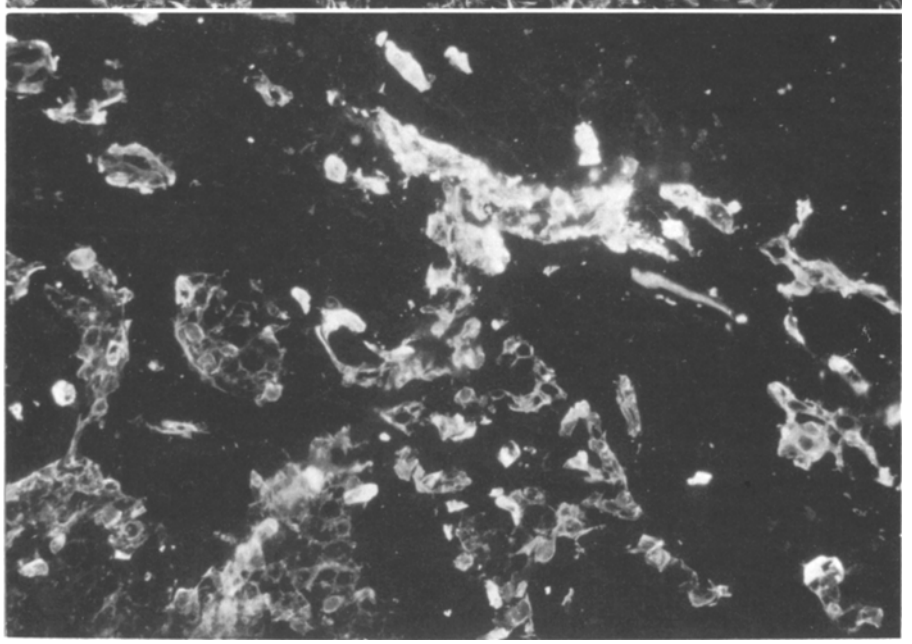

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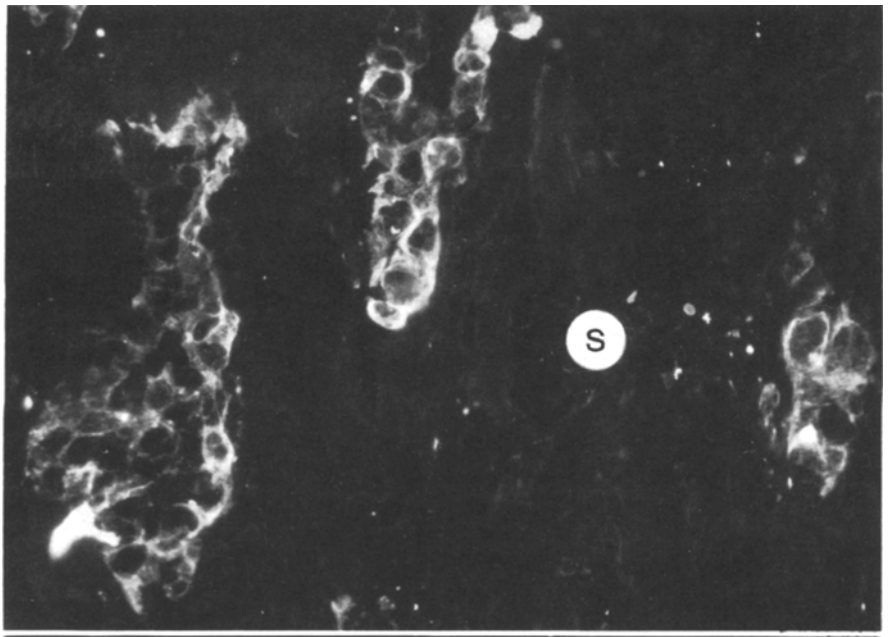
Fig. 9. Mucoepidermoid tumor. Negative staining of the epithelial tumor cells (*t*). Intensely stained stromal cells with densely arranged intracytoplasmic filaments (*center*). Immunofluorescence staining for vimentin. ×210

Fig. 10. Squamous cell carcinoma. Positively stained epithelial tumor cells in solid sheets. Negative stromal part. Immunofluorescence staining for prekeratin. ×210



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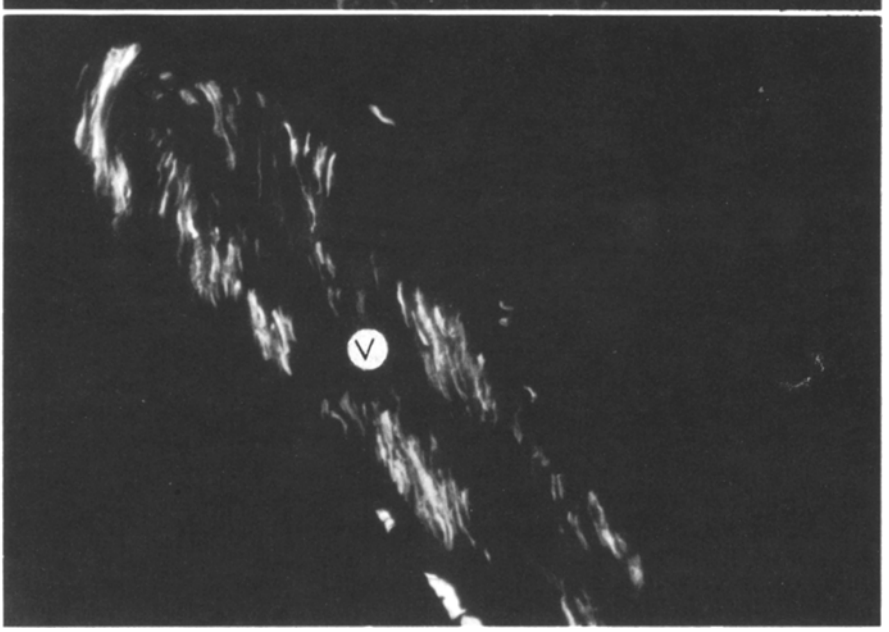

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Fig. 11. Squamous cell carcinoma. Positively stained tumor cells with a varying degree of intensity. Negative stromal part. Immunofluorescence staining for prekeratin. ×330

Fig. 12. Blood vessels. Strong staining of the muscle cells of the vessel wall (*v*). Immunofluorescence staining for desmin. ×210

ven structures inside the cytoplasm. In some rare cases, single positive cells were found in the tumoral tissues. They were generally situated at the border of the invading part of the tumor.

Desmin. The tumor and the surrounding connective tissue was negative for desmin. The blood vessels stained strongly positive, as previously described.

Discussion

The results with antibodies to three different intermediate-sized filament proteins of frozen sections of the normal human paratid gland, and on tumors of this gland confirm and extend results with frozen sections of animal (e.g., Franke et al. 1978a, b, 1979a, b; Sun et al. 1979; Schmid et al. 1979; for review see Lazarides 1980; Weber and Osborn 1981b) and human tissues (Schlegel et al. 1980a, b; Löning et al. 1980; Altmannsberger et al. 1981). They lend support to the idea that also in the human system well-characterized antibodies to different intermediate-sized filament proteins can distinguish the following cell or tissue types: epithelia with antibodies to prekeratin, mesenchymal cells with antibodies to vimentin, muscle cells with antibodies to desmin, astrocytes with antibodies to glial fibrillar acidic protein, and neurons with antibodies to neurofilament components. "Prekeratin" is the term used to describe a group of intermediate-sized filaments in epithelial cells. Antisera against these proteins are raised by using a purification of bovine hoof prekeratin (Franke et al. 1980) and in this study a group of "prekeratin" proteins were detected by this antibody. A subdivision of these proteins was not undertaken.

In the normal human parotid gland, prekeratin filaments were observed in ductal cells and in myoepithelial cells. The presence of proteins of the keratin type in myoepithelial cells is a strong argument for their epithelial nature (Franke et al. 1980; Schlegel et al. 1980b).

With vimentin sera, positive staining of the mesenchymal cells in the tissue was observed, as well as staining of endothelial and vascular smooth muscle cells in blood vessels (cf. Gabbiani et al. 1981). The fibrous networks present in the cytoplasm of vimentin-positive cells seemed to be somewhat less tightly packed than the prekeratin networks present in the myoepithelial cells. Some vimentin-positive cells, in close proximity to striated ducts and to the larger excretory ducts, displayed a triangular shape reminiscent of myoepithelial cells. However, further studies are necessary to determine whether these cells are in fact myoepithelial cells or whether they belong to another cell type present at the periphery of the duct. We cannot currently exclude the possibility that a subset of myoepithelial cells may contain small amounts of vimentin filaments (cf. Franke et al. 1980). Desmin was only found associated with some vascular smooth muscle cells in the walls of blood vessels.

The number of desmin-positive cells was larger than would have been expected from a previous study of the type of intermediate-sized filaments present in certain human blood vessels (Gabbiani et al. 1981). These results also raise

the possibility that some vascular smooth muscle cells contain both vimentin and desmin filaments.

Pleomorphic adenomas are the most common type of tumors in the parotid gland (Thackray and Lucas 1974; Seifert and Donath 1976; Gläser 1979). Although they are a well defined entity on histological grounds (Eneroth 1976; Seifert and Donath 1976), the question of their histogenesis is still open to discussion (Eversole 1971; Gläser 1979; Batsakis 1980). In our collection of six pleomorphic adenomas we were able to demonstrate the presence of tumor cells which stained positively for prekeratin and of cells with similar morphology which were vimentin-positive. Such cells were seen in solid parts of the tumor as well as in parts where the morphology was more reminiscent of mesenchymal tissue. The presence of prekeratin-positive cells in the pleomorphic adenoma is a strong argument for the epithelial origin of this tumor. The presence of vimentin-positive cells in addition, introduces new concepts with regard to the complex nature of this tumor. Three interpretations of these results can be considered. Firstly, pleomorphic adenomas might originate from cells which are characterized in vivo by prekeratin and vimentin filaments. Secondly the tumor cells originate from cells which are characterized by the prekeratin type filaments, but which after neoplastic transformation acquire the ability to produce vimentin filaments. A precedent for this type of conversion might be the fact that on growth of cells in culture many cells of epithelial origin acquire the vimentin system in addition to the prekeratin system (Franke et al. 1979; Osborn et al. 1980). Finally, the pleomorphic adenoma might be a mixed tumor composed of both epithelial and mesenchymal components. Further studies – for example by simultaneous staining of prekeratin and vimentin in the same section, or by studying the tumor after transplantation to the nude mouse or into tissue culture – may help to decide between these possibilities.

The observations on squamous cell carcinomas and on mucoepidermoid carcinomas are easier to interpret. Both tumors were strongly positive for prekeratin in all the cases examined, in agreement with other observations (Löning et al. 1980; Schlegel et al. 1980a, b). Thus the epithelial character of these tumors is clearly demonstrated. The different intensity of staining in the tumor cells may reflect different amounts of keratin-like polypeptides in the different cells.

This study represents one attempt to assess whether antibodies to different intermediate filament proteins are of use in pathology of normal and abnormal human tissues (see also Schlegel et al. 1980a, b; Löning et al. 1980; Altmannsberger 1981). The results show that not only can prekeratin and vimentin antibodies distinguish different cell types in the normal parotid gland, but that these antibodies can also yield information on the arrangement of cells in the common tumors associated with this gland. Thus it seems reasonable to hope that in the future in certain cases these and similar antibodies may be of help in diagnosis, and also in deciding the cell type of origin for particular malignant lesions, not only in the parotid gland, but also in other human malignancies.

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Accepted July 13, 1981