Virchows Arch A (1987) 412:73–78

Virchows Archiv A

Chronic sialadenitis

An immunocytochemical study in humans

Richard M. Palmer [1] **and John W. Eveson** [2]

[1] United Medical and Dental School's of Guy's and St. Thomas's Hospitals. London Bridge, London SE1 9RT, UK
[2] Department of Oral Medicine, Surgery and Pathology, Bristol Dental School and Hospital, Lower Maudlin Street, Bristol BS1 2 LY, UK

Summary. The cellular changes in salivary gland parenchyma with chronic inflammation were studied immunocytochemically with a panel of antibodies. Myoepithelial cells were labelled with anti-myosin, duct cells with a polyclonal anti-callus prekeratin, a monoclonal anti-keratin CAM 5.2 and a monoclonal anti-keratin 7 (RPN 1162), and a subpopulation of basal duct cells with a monoclonal anti-keratin 16a. The wide range of changes observed were similar to those described following experimental duct ligation. One of the most striking features was the survival of myoepithelial cells surrounding persisting acini and ductal structures. Most of these ductal structures appeared to be either surviving intercalated ducts or were altered acinar cells. There was no evidence of myoepithelial or ductal hyperplasia. The 16a positive basally located duct cells which are conspicuous in normal glands, pleomorphic adenomas and in the epithelial islands in lymphoepithelial lesions (Palmer et al. 1985; 1986) were virtually absent, except in one specimen with mild inflammatory changes. If this cell type represents a reserve cell, then loss of it may preclude recovery of the remaining parenchyma following resolution of inflammation.

Key words: Sialadenitis – Salivary gland – Myoepithelial – Keratins

Introduction

Chronic sialadenitis is a common complication of duct obstruction usually due to calculi, and is most common in the submandibular gland (Mason and Chisholm 1975). This results in gradual loss of acinar cells, an increase in the number of duct cells, periductal lymphocytic infiltration and fibrosis.

Offprint requests to: R.M. Palmer

There has been comparatively little research into the cellular changes in the parenchyma during and following inflammation in human salivary glands and the submandibular gland has received most attention (Tandler 1977). However, the effects of duct ligation have been studied in a number of animal species using histology, enzyme histochemistry and electronmicroscopy (Harrison and Garrett 1976a, b; Tamarin 1971a, b), and the observed results appear similar to many of the changes described in chronic sialadenitis. It has been suggested by Tamarin (1971a), that the alterations in the parenchyma of glands during recovery from duct ligation are very similar to changes during embryological development. Sialadenitis may, therefore, be an interesting model to study the effects it has on cellular differentiation, and may provide further insight into the changes during salivary gland neoplasia.

The application of immunocytochemical techniques using antibodies to cytoplasmic filaments allows characterization of cell types within normal and pathological tissue and has been useful in the study of salivary tumours (Caselitz and Loning 1981; Caselitz et al. 1981a, b; 1982; Palmer et al. 1985) and disorders such as Sjogren's syndrome (Palmer et al. 1986; Saku and Okabe 1984). The aim of the present study was to apply these techniques to specimens of inflamed human submandibular and parotid glands in order to study changes in cellular differentiation.

Materials and methods

Specimens of inflamed submandibular (5 cases) and parotid glands (4 cases) were obtained at the time of surgery. The glands had given persistent signs and symptoms of chronic sialadenitis and many had salivary calculi.

The tissue was fixed in methacarn (60% methanol, 30% chloroform, 10% acetic acid) and paraffin processed before

immunoenzyme staining as previously described (Palmer et al. 1985). The following primary antibodies were used:

1. Rabbit anti-smooth muscle myosin, previously shown to stain myoepithelial cells (Palmer et al. 1985; Palmer 1986).
2. Rabbit anti-human callus prekeratin, shown to stain all duct cells in normal salivary glands (Palmer et al. 1985).
3. A monoclonal anti-keratin CAM 5.2 (Becton Dickinson, Twickenham, UK) to keratins of 39K, 43K, and 50K daltons, recognising mainly secretory epithelia.
4. A monoclonal anti-keratin (16a) to keratin of 45/46K daltons previously shown to stain a subpopulation of duct cells in normal glands (Palmer et al. 1985; 1986).
5. A monoclonal anti-glandular epithelia RPN 1162 (Amersham, Aylesbury, UK) which reacts with keratin 7.
6. A monoclonal antibody to type IV collagen shown to stain basement membranes (Gusterson et al. 1984; Palmer et al. 1985).

Appropriate alkaline phosphatase-conjugated secondary antibodies (Sigma, St. Louis, 110, USA) were used in the immunoenzyme technique and fluorescein-conjugated antibodies (Dako, Denmark) for immunofluorescence (Palmer 1986).

Results

The results of immunocytochemical staining in normal salivary glands with anti-smooth muscle myosin, anti-callus prekeratin and monoclonal 16a have been published previously (Palmer 1986; Palmer et al. 1985; 1986).

1. Anti-smooth muscle myosin

Myoepithelial cells were clearly stained surrounding the remaining acini and the simple duct-like structures in both the inflamed parotid and submandibular glands (Figs. 1 and 2). In some specimens the myoepithelial cells were more conspicuous than in normal glands.

2. Anti-callus prekeratin

This antibody produced staining of intercalated, striated and excretory ducts in normal glands. In the inflamed specimens the remaining duct structures stained strongly with this antibody (Fig. 3) and appeared to be more numerous than in normal salivary glands.

3. Monoclonal CAM 5.2

In normal glands this antibody had a very similar staining distribution to the anti-callus prekeratin but there was also some positive staining of the acinar cells which was mainly limited to the cell membrane (Fig. 4). All the duct-like structures in the inflamed glands were positive with this antibody (Fig. 5).

4. Monoclonal 16a

This antibody stained a subpopulation of basally located duct cells in the striated and excretory ducts of normal salivary glands and less consistently intercalated duct cells (Fig. 6). A commercially available monoclonal antibody against keratins 6, 18 (Dako-CK1) showed a very similar staining distribution in methacarn-fixed tissue (unpublished observation).

In the inflamed glands there were no positively stained basally located duct cells in 8 of the 9 specimens. Positively stained cells were observed in only one parotid gland specimen where the basally located cells were not entirely typical as they were spindle shaped rather than cuboidal (Fig. 7). In addition there was staining of the luminal cytoplasm in many of the ducts, a feature which was not present in normal glands. Other specimens were either negative or showed faint or focal staining.

5. Monoclonal RPN 1162

In normal glands there was staining of duct cells which was most conspicuous on the luminal aspect (Fig. 8). The acini were negative. The full thickness of the ductal structures was positively stained in the inflamed glands with no increased tendency towards luminal staining (Fig. 9).

6. Anti-type IV collagen

Type IV collagen staining was positive to a varying degree around the remaining parenchymal tissue and blood vessels (Fig. 10).

Discussion

The specimens described in this paper had been affected by acute and chronic inflammation and obstruction due to salivary calculi. Many of the pathological changes were similar to those described following experimental duct ligation. However, the variation in the cellular differentiation in chronic sialadenitis is probably greater due to the effects of bacterial infection and the length of time the disease has been established.

The role of the myoepithelial cell in normal and neoplastic salivary glands has received considerable attention and therefore the effects of inflammation on this cell type was of particular interest. The myoepithelial cell survives the inflammatory changes extremely well and could be clearly identified immunocytochemically. This has also been

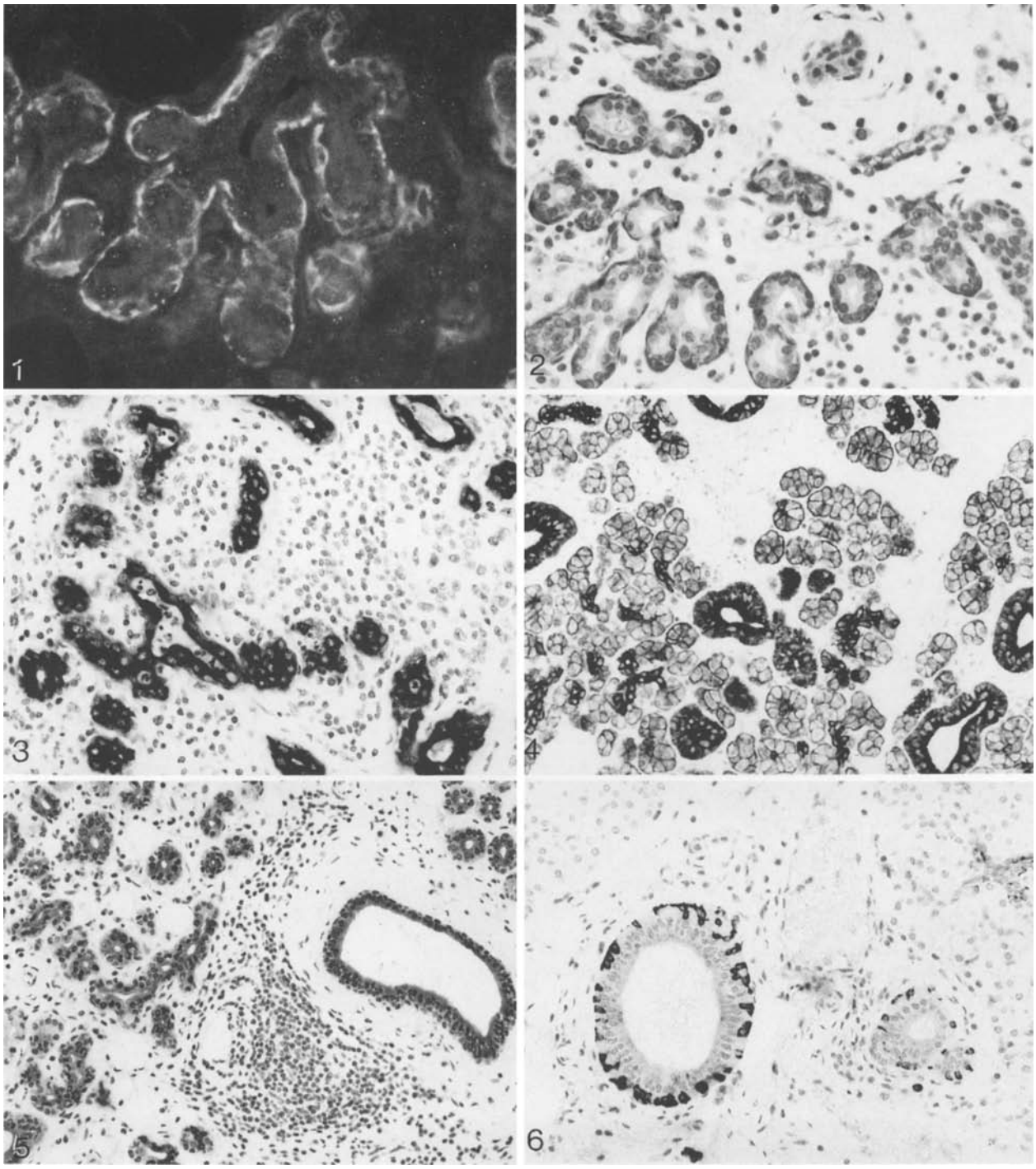

Fig. 1. Immunofluorescence with anti-myosin showing staining of myoepithelial cells in a submandibular gland specimen. ×270

Fig. 2. Immunoenzyme staining with anti-myosin and alkaline phosphatase conjugated secondary antibody showing myoepithelial cells surrounding duct-like structures in an inflamed parotid gland. ×270

Fig. 3. Immunoenzyme staining with anti-callus prekeratin in an inflamed parotid gland specimen. ×180

Fig. 4. CAM 5.2 staining of ducts and the periphery of acinar cells in a normal gland. ×180

Fig. 5. CAM 5.2 staining of multiple duct-like structures in an inflamed gland. ×180

Fig. 6. Monoclonal 16a staining of subpopulations of basal cells in excretory ducts and striated ducts and some luminal staining in intercalated ducts in a normal gland. ×180

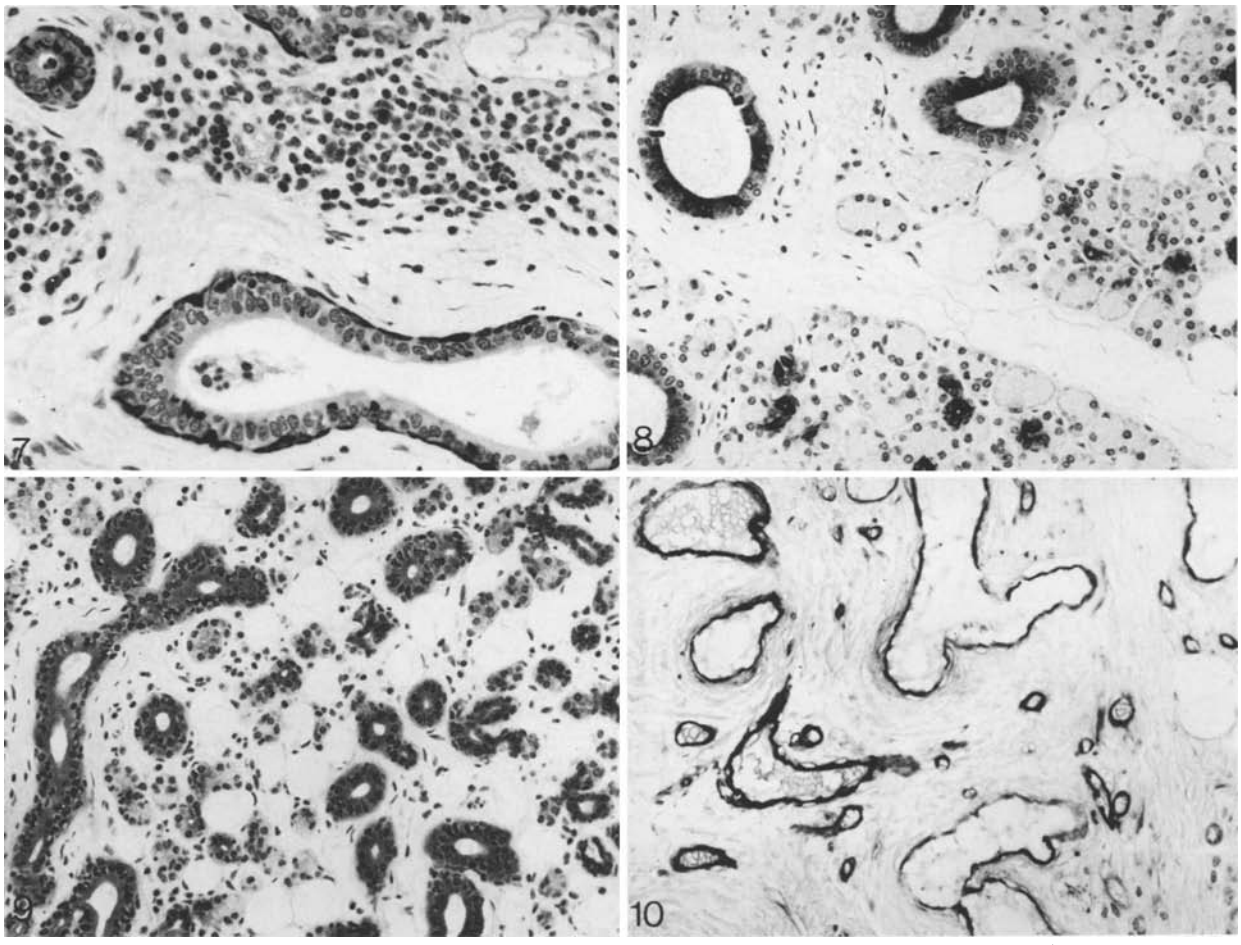

Fig. 7. Flattened basal duct cell staining with monoclonal 16a of excretory ducts and some smaller ducts in a parotid gland specimen. ×180

Fig. 8. Monoclonal RPN 1162 shows staining mainly of the luminal aspects of the excretory, striated and intercalated ducts in a normal gland. There was no staining of acinar cells. ×180

Fig. 9. Monoclonal RPN 1162 shows multiple duct-like structures and some acinar cells showing weak staining in an inflamed gland specimen. ×180

Fig. 10. Remaining parenchyma and blood vessels in an inflamed gland outlined with type IV collagen. ×270

verified ultrastructurally (Unpublished observation – Fig. 11, Tandler 1977). Myoepithelial cells have been shown to be relatively unaltered following experimental duct ligation (Tamarin 1971a) or to assume unusual ultrastructural configurations (Emmelin et al. 1974; Garrett and Emmelin 1979). Tandler (1977) described myoepithelial cells surrounding intercalated type ducts in inflamed submandibular glands. He suggested that there was no evidence of myoepithelial hyperplasia, in contrast to the supposed myoepithelial hyperplasia in benign lymphoepithelial lesion which has since been disputed (Saku and Okabe 1984; Palmer et al. 1986). In the present material the myoepithelial cells often appeared to be more conspicuous but this was probably as a result of the decrease in the volume of parenchyma and the survival of the myoepithelium.

The cellular changes within acinar cells in sialadenitis may be similar to those described in glands which had been experimentally ligated (Tamarin 1967; 1971a, b; Harrison and Garrett 1976a, b). In some areas the acini were of normal appearance and were surrounded by normal myoepithelial cells. Loss of secretory vesicles and the accumulation of more intermediate filaments in the acinar cells, would ultimately result in cells which resemble duct cells. This is seen experimentally within

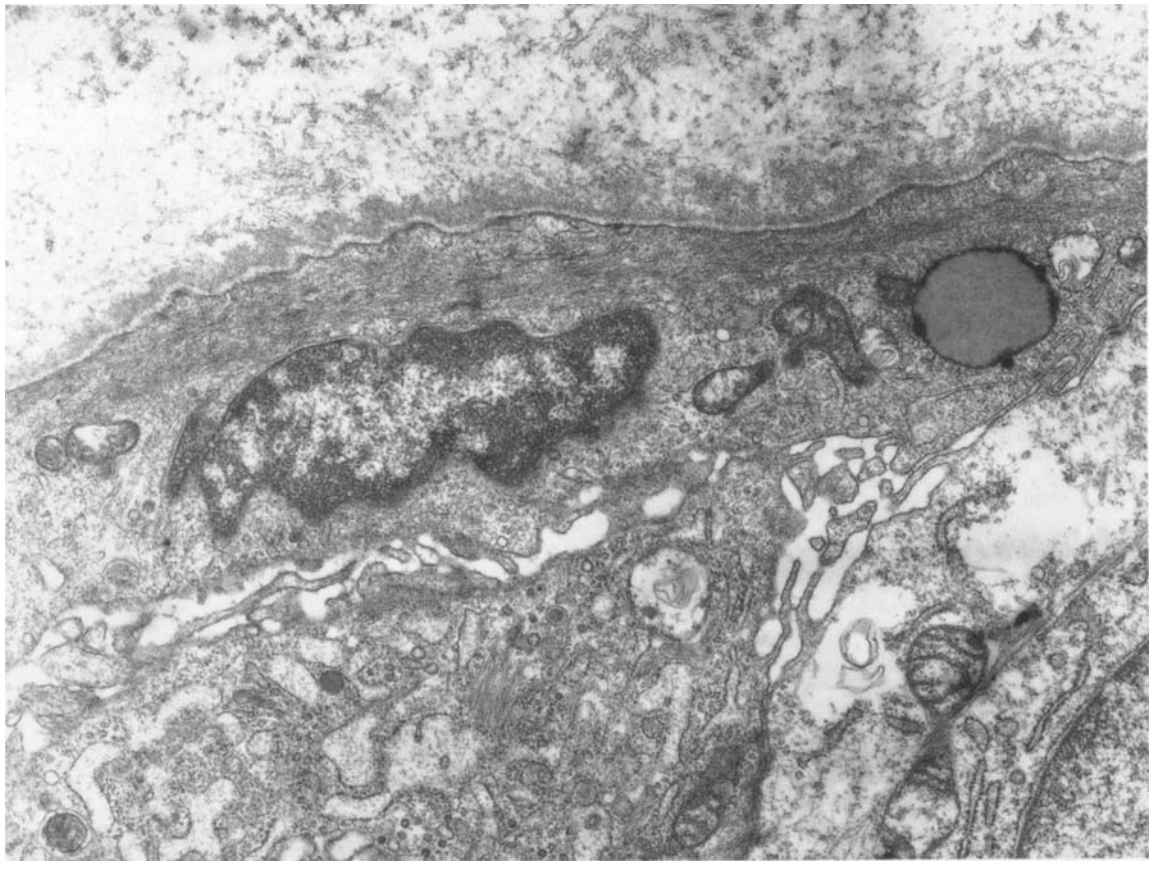

Fig. 11. Surviving myoepithelial cell in an inflamed parotid gland showing typical ultrastructural features including dense bundles of microfilaments, micropinocytotic vesicles and well developed basal lamina. ×16000

31 days of ductal ligation (Tamarin 1967) and a similar sequence of events in sialadenitis would lead to a relative increase in the number of duct-like cells which would be surrounded by myoepithelial cells. The immunocytochemical staining with the anti-callus prekeratin and monoclonals CAM 5.2 and RPN 1162 confirmed the apparent increase in the number of ductal structures. In particular, it could be seen in some specimens that altered acinar cells stained with anti-callus prekeratin and CAM 5.2 whereas they were negative or showed staining only at the periphery of the cells in normal glands. This is most probably due to loss of the specialized characteristics of the acinar cells and change to a more simple duct-like cell type. Keratin 7 expression (which is typical of simple epithelia) by the duct-like cells supports this observation. In the normal process of differentiation epithelial cells usually continue to express the keratins of their cell origin, even in neoplasia (Loning et al. 1980; 1982). The observation that the acinar cells express different keratins following inflammatory changes may be due to the fact that the cytoplasm, which is now devoid of secretory vesicles has an increase in the keratin content which is then detectable with the antibody or to a change in the microenvironment leading to expression of different keratins. This is a much more likely explanation of the apparent increase in the number of ductal structures rather than hypotheses based upon ductal hyperplasia which would also require concomitant myoepithelial hyperplasia to explain the present results. Tandler (1977) suggested that the duct cells in sialadenitis were derived from three possible sources: intercalated ducts, striated ducts or an ambiguous origin. As the duct-like structures of ambiguous origin in his study were surrounded by myoepithelial cells he proposed that the majority were derived from intercalated ducts. Tandler's results could equally be interpreted as showing an origin of these ducts from altered acinar cells.

In ligation experiments where the ligatures were removed and the glands allowed to recover, many of the changes were reversible (Tamarin 1971). During the recovery phase the regenerative chan-

ges resembled differentiation that occurs during embryonic development of salivary tissue. In chronic sialadenitis, similar changes would be prevented by persistent inflammation.

One of the most interesting findings was the sparsity of 16a positive basal duct cells in the inflamed glands. Only one specimen had appreciable numbers of these cells in the expected location. This specimen was only moderately inflamed and probably showed earlier inflammatory changes than the other specimens. Further inflammation may result in alteration of expression of this keratin or loss of this cell type. In this gland, 16a positive cells had lost their normal cuboidal appearance and were flattened or spindle-like. Therefore the morphology of these cells was very similar to myoepithelial cells. However, we have never observed 16a positive myoepithelial cells in any specimens of normal salivary tissue although it is possible that myoepithelial cells may express this keratin following inflammatory changes. It has previously been suggested that the basally located duct cells could represent a 'reserve cell population' and that they may be important in neoplasia and ductal hyperplasia in lymphoepithelial lesions (Palmer et al. 1985; 1986). If this hypothesis is correct then one would expect to see a persistence or increase in these cells if there were true ductal hyperplasia in sialadenitis. The absence of this cell population in most inflamed glands adds support to the earlier suggestion that the apparent increase in duct cells is due to loss of acinar cell characteristics and the assumption of a simple, duct cell phenotype.

Further investigations using a wider panel of anti-keratin antibodies on frozen sections for the preservation of optimal antigenicity, and correlation with ultrastructural changes together with quantitative data, would hopefully clarify some of the suggestions made in this paper.

References

Caselitz J, Loning T (1981) Specific demonstration of actin and keratin filaments in pleomorphic adenomas by means of immunoelectronmicroscopy. Virchows Arch A (Pathol Anat) 393:153–158

Caselitz J, Loning T, Staquet MJ, Seifert G, Thivolet J (1981a) Immunocytochemical demonstration of filamentous structures in the parotid gland. Occurrence of keratin and actin in normal and tumoral parotid gland with special respect to the myoepithelial cells. J Cancer Res Clin Oncol 100:59–68

Caselitz J, Osborn M, Seifert G, Weber K (1981b) Intermediate-sized filament proteins (prekeratin, vimentin, desmin) in the normal parotid gland and parotid gland tumours. Immunofluorescence study. Virchows Arch A (Pathol Anat) 393:273–286

Caselitz J, Osborn M, Wustrow J, Seifert G, Weber K (1982) The expression of different intermediate sized filaments in human salivary glands and their tumors. Pathol Res Pract 175:266–278

Emmelin N, Garrett JR, Ohlin P (1974) Secretory activity and the myoepithelial cells of salivary glands after duct ligation in cats. Arch Oral Biol 19:275–283

Garrett JR, Emmelin N (1979) Activities of salivary myoepithelial cells. A review. Med Biol 57:1–28

Gusterson BA, Warburton MJ, Mitchell D, Kraft N, Hancock WW (1984) Invading squamous cell carcinoma can retain a basal lamina: an immunohistochemical study using a monoclonal antibody. Lab Invest 61:82–87

Harrison JD, Garrett JR (1976a) The effects of ductal ligation on the parenchyma of salivary glands of cat studied by enzyme histochemical methods. Histochem J 8:35–44

Harrison JD, Garrett JR (1976b) Histological effects of ductal ligation of salivary glands of the cat. J Pathol 118:245–254

Loning T, Staquet MJ, Thivolet J, Seifert G (1980) Keratin polypeptides distribution in normal and diseased human epidermis and mucosa. Virchows Arch A (Pathol Anat) 388:273–288

Loning T, Viac J, Caselitz J, Thivolet J, Otto HF, Seifert G (1982) Comparative investigation of keratin filaments in normal tissues and tumours of skin, oral mucosa, salivary glands and thymus. Pathol Res Pract 175:256–265

Mason DK, Chisholm DM (1975) In: Salivary Glands in Health and Disease. WB Saunders, London, pp 92–105

Palmer RM (1986) The identification of myoepithelial cells in human salivary glands. A review and comparison of light microscopical methods. J Oral Pathol 15:221–229

Palmer RM, Eveson JW, Gusterson BA (1986) 'Epimyoepithelial' islands in lymphoepithelial lesions. An immunocytochemical study. Virchows Arch A (Pathol Anat) 408:603–609

Palmer RM, Lucas RB, Knight J, Gusterson BA (1985) Immunocytochemical identification of cell types in pleomorphic adenoma, with particular reference to myoepithelial cells. J Pathol 146:213–220

Saku T, Okabe H (1984) Immunohistochemical and ultrastructural demonstration of keratin in epi-myoepithelial islands of autoimmune sialadenitis in man. Arch Oral Biol 29:687–689

Tamarin A (1967) In: Schneyer LH, Schneyer CA (eds) Secretory Mechanisms of Salivary Glands. Academic Press, New York, p 220

Tamarin A (1971a) Maxillary gland recovery from obstruction. 1. Overall changes and electron microscopic alterations of granular duct cells. J Ultrastr Res 34:276–287

Tamarin A (1971b) Submaxillary gland recovery from obstruction. Electron microscopic alterations of acinar cells. J Ultrastr Res 34:288–302

Tandler B (1977) Ultrastructure of chronically inflamed human submandibular glands. Arch Pathol Lab Med 101:425–431

Accepted July 8, 1987