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Histochemical and biochemical determination of calcium in salivary glands with particular reference to chronic submandibular sialadenitis

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Abstract. Although salivary calcification is relatively common, little is known about the localization and content of the calcium of normal and diseased human salivary glands. We investigated this in chronic submandibular sialadenitis with a variable mixture of relatively normal and extremely atrophic parenchyma and in normal submandibular, parotid and palatal glands. Calcium was localized histochemically in mucous acinar cells of submandibular and palatal glands at moderate to high levels, in serous acinar cells of submandibular and parotid glands at low to moderate or occasionally high levels, in mucous ductal cells at moderate to high levels, and in extremely atrophic parenchyma at low levels or not at all. Calcium was determined biochemically at relatively high levels in the different glands in the order palatal, submandibular, sialadenitis and parotid. However, the differences were small. The results indicate that most salivary calcium is associated with secretory granules; this is the likely source of the calcium involved in salivary calcification

Key words: Calcium – Ectopic calcification – Salivary gland calculi – Salivary glands – Sialadenitis

Introduction

Salivary calcification is relatively common, either in the form of microlithiasis (Epivatianos et al. 1987; Epivatianos and Harrison 1989) or lithiasis. However, there is little information on calcium in normal and diseased human salivary glands. We decided to investigate this using sensitive histochemical and biochemical methods that have been found to be valuable in investigating the calcium of salivary glands of other species (Schäfer 1979; Westhofen et al. 1984).

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Materials and methods

Salivary glands removed from patients during general anaesthesia were used and comprised 8 normal parotid glands, 2 groups of normal palatal glands, 5 normal submandibular glands and 10 submandibular glands with chronic sialadenitis. The glands were obtained upon removal and pieces were immediately quenched in hexane cooled by solid carbon dioxide and stored at −70° C. Normal glands were removed because of tumours or lymphadenopathy. There was no evidence of compression or obstruction in the normal material taken for this investigation, which was of normal histological appearance.

The histochemical method using glyoxal *bis*(2-hydroxyanil) (GBHA) (Kashiwa and Atkinson 1963; Kashiwa and House 1964) modified for cryostat sections (Schäfer 1979) was used to localize ionized and ionizable calcium. The staining-solution consisted of 3.3% GBHA, 3.4% sodium hydroxide and 75% ethanol. It was freshly prepared and cooled in the chamber of the cryostat. A few drops were placed on a microscope-slide already cooled in the chamber. A section was cut at 20 µm and placed on the staining-solution on the slide, which was removed from the cryostat and warmed on the palm of the hand, which allows the GBHA to react with the calcium in the section as it thaws. The staining-solution was removed by filter paper and rinsing with 75% ethanol. The section was allowed to dry, rinsed in absolute ethanol followed by xylene and mounted in DPX. The stained sections were kept in the dark at 0–4° C and had usually not deteriorated after 2 years. Controls were made by immersing stained sections in 90% ethanol saturated with sodium carbonate and potassium cyanide, to which only calcium-GBHA precipitates are resistant (Kashiwa and Atkinson 1963), and by energy-dispersive X-ray microanalysis of stained sections mounted on carbon stubs in a JEOL 100C scanning-transmission electron microscope equipped with a Link 860 analyser.

A biochemical method of dry ashing that achieves maximum extraction of calcium (method A of Menden et al. 1977) was used to prepare samples of the glands for the measurement of calcium by a Perkin-Elmer atomic-absorption spectrophotometer. Differences between the amounts of calcium in the various glands were tested by the Kruskal-Wallis one-way analysis of variance by ranks (Cohen and Holiday 1982).

Results

The results of the GBHA histochemical method for calcium are summarized in Table 1 and illustrated in

Table 1. Glyoxal *bis*(2-hydroxyanil) (GBHA) staining of human salivary glands

	Parotid	Palatal	Submandibular	Sialadenitis
Mucous cells		++, +++	++, +++	++, +++
Serous cells	+, ++, (+++)		+, ++, (+++)	+, ++, (+++)
Extremely atrophic parenchyma				0, +

0, unstained; +, weakly stained; ++, moderately stained; +++, strongly stained; (+++), occasionally strongly stained

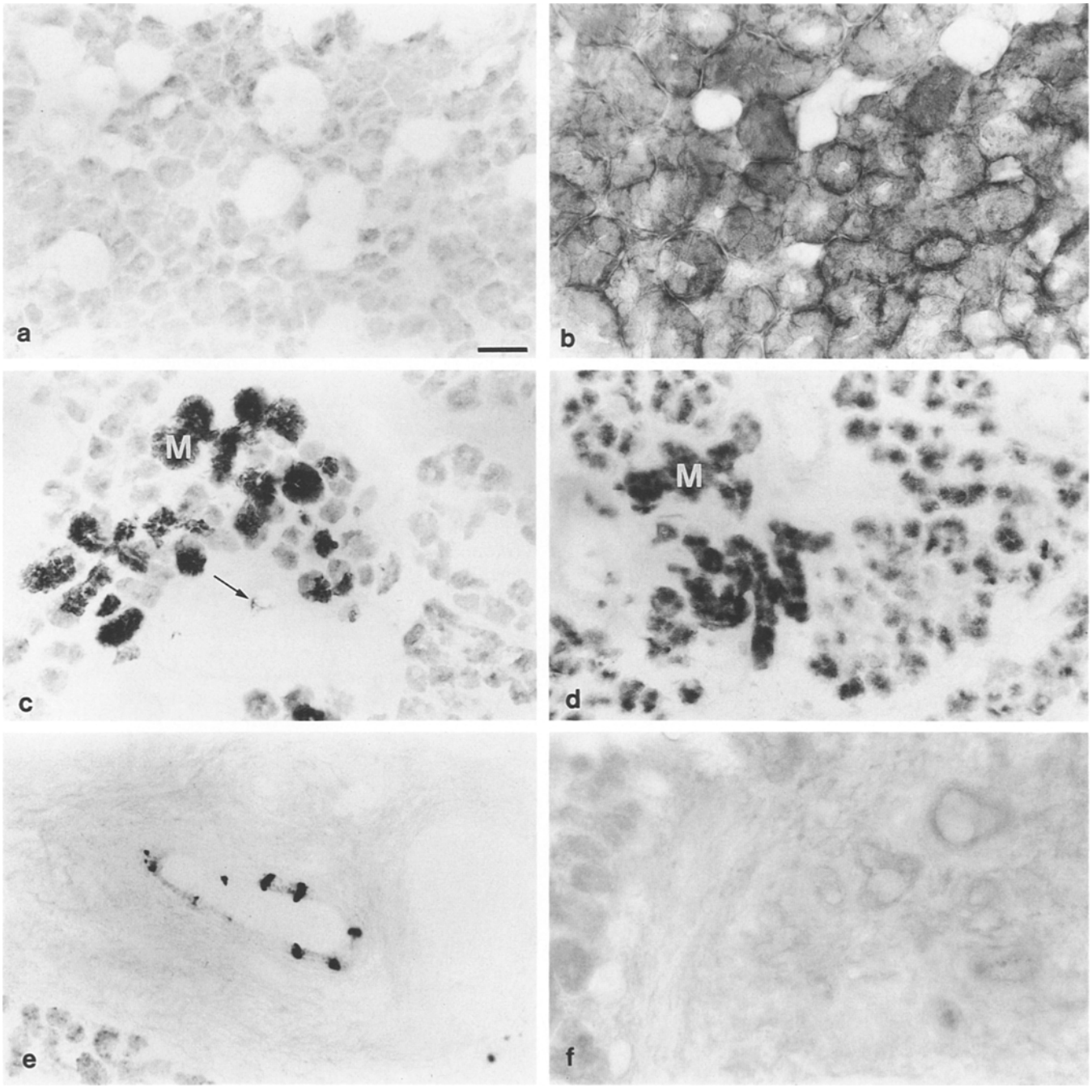

Fig. 1a–f. Cryostat sections of human salivary glands stained by glyoxal *bis*(2-hydroxyanil) (GBHA). Bar 50 µm; × 160. **a** Parotid gland showing weakly stained serous acini. **b** Palatal gland showing moderately to strongly stained mucous acini. **c** Submandibular gland showing strongly stained mucous acinar cells (*M*) and weakly stained serous acinar cells. Stained luminal contents *(arrow)* are seen in an otherwise unstained duct. **d** Submandibular gland showing strongly stained mucous acinar cells (*M*) and moderately to strongly stained serous acinar cells. **e** Submandibular gland showing strongly stained mucous cells in an excretory duct. Weakly stained serous acini are seen in the lower left corner. **f** Chronic submandibular sialadenitis. Weakly stained serous acini are present at the left margin. Elsewhere there is exremely atrophic parenchyma that varies from being unstained to weakly stained

Table 2. Calcium contents in mmol/g of human salivary glands

	Dry weight			Wet weight
	m	*s*	*n*	m
Parotid	0.035	0.020	8	0.008
Palatal	0.068	0.015	2	
Submandibular	0.048	0.027	5	0.010
Sialadenitis	0.039	0.018	10	

m, mean value; *s*, standard deviation; *n*, number of glands

Fig. 1a–f. The serous acinar cells of the parotid (Fig. 1a) and submandibular (Fig. 1c, d) glands were stained weakly to moderately or occasionally strongly. The mucous acinar cells of the palatal (Fig. 1b) and submandibular (Fig. 1c, d) glands were stained moderately to strongly. Mucous cells were occasionally seen in excretory ducts of submandibular and parotid glands and were stained moderately to strongly (Fig. 1e). The remainder of the lining of excretory and striated ducts was unstained apart from occasional weak staining of ductal cells. Stained luminal contents were seen (Fig. 1c). Much of the parenchyma of the chronic submandibular sialadenitis was histologically similar to normal or only moderately atrophic, and was stained similarly to normal (Fig. 1f). A variable part of the parenchyma was extremely atrophic and showed weak or no staining (Fig. 1f). The stain was resistant to sodium carbonate and potassium cyanide, and calcium was detected in stained parts by microanalysis, which confirm that the stain was a precipitate of calcium GBHA (Kashiwa and Atkinson 1963; Kashiwa and House 1964; Schäfer 1979).

The biochemically determined calcium contents (Table 2) were calculated in terms of dry weight for comparison of the various glands, and those of the parotid and submandibular glands also in terms of wet weight for comparison with other investigations. The differences between the amounts of calcium in the various glands were not statistically significant.

Discussion

The levels of calcium determined biochemically in the glands (Table 2) are higher than in the serum (total calcium 0.0025 mmol/ml), which indicates that they accumulate calcium. The levels determined in the submandibular and parotid glands are higher than those reported previously (Kraintz 1966; Mangos 1979; Mangos and Donnelly 1981), which is apparently due to the maximum extraction of calcium achieved by the method of dry ashing used in the present investigation (Menden et al. 1977).

An experimental investigation on feline salivary glands found that most of the glandular calcium was in secretory granules (Triantafyllou 1991) and the present histochemical detection of calcium in the acini, which contain most of the secretory granules, indicates that this is so in the human salivary glands. This is supported by X-ray microanalytical investigations that detected high levels of calcium in the acinar cells of human parotid and submandibular glands (Roomans et al. 1989) and in the secretory granules of the acinar cells of human labial glands (Izutsu et al. 1991). Calcium appears to be bound to glycoprotein as a cation that shields the polyionic charges of the glycoprotein and allows it to be condensed in the secretory granules (Verdugo et al. 1987). This would explain the accumulation and also the distribution of calcium in the glands. Thus there was generally most calcium in mucous cells, which are packed with glycoprotein, proportionately more of which is acidic (Harrison 1974; Harrison et al. 1987), and least in extremely atrophic parenchyma, which lacks glycoprotein; the content of calcium was greatest in the palatal gland, in which the acini are mainly mucous (Tandler and Riva 1986), less in the submandibular gland, in which mucous acinar cells occupy only about 8% of the acinar volume (Scott 1979), and least in the parotid gland, in which the acini are mainly serous (Tandler and Riva 1986). The greater concentration of calcium detected in some serous acinar cells is possibly due to binding of calcium to other components of the secretory granules, such as proline-rich proteins, which are present in the parotid and submandibular serous cells (Kousvelari et al. 1980). The secretory granules appear to be the source of the calcium in the saliva.

Microlithiasis is found in normal salivary glands (Epivatianos and Harrison 1989) and experimental investigations have shown it to be encouraged by secretory inactivity (Triantafyllou 1991; Harrison and Epivatianos 1992; Triantafyllou et al. 1992). In this state there is crinophagy and luminal stagnation of saliva and degeneration of secretory material rich in calcium; this possibly leads to overwhelming release of ionized calcium that precipitates on to debris, forming microliths in autophagosomes and lumina. The present investigation indicates that such a process may account for the microliths found in human salivary glands. Although there is likely to be stagnation in the extremely atrophic parenchyma of sialadenitis there does not appear to be an increased occurrence of microliths (Harrison and Epivatianos 1990), possibly because of the reduced amount of calcium in such parenchyma. Thus microliths appear more likely to form in normal salivary glands and to lead to localized obstructive atrophy and possibly sialadenitis rather than to be secondary to sialadenitis. However, stones appear to form secondarily to sialadenitis (Harrison and epivatianos 1990), and the secretory granules of the relatively normal parenchyma and the increased numbers of mucous ductal cells are the possible sources of calcium.

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