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Histological changes in the hamster cheek pouch epithelium induced by topical application of sodium lauryl sulphate

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Summary. Dentifrices, two containing sodium lauryl sulphate (SLS) and one containing stearylethoxylate as surfactant, were gently rubbed on the mucosal surface of the medial wall of cheek pouches in adult male Syrian golden hamsters. Four daily applications were performed. On the fifth day, the animals were sacrificed and cheek pouch mucosal tissue was routinely processed for light microscopy. After applications of SLS containing dentifrices, the epithelium showed consistently prominent structural changes, especially hyperkeratinization, including ortho and para-keratinization, acanthosis with widening of the intercellular spaces, and varying degrees of basal hyperplasia.

Identical morphological changes were also observed after application of a paste containing only SLS as an agent. In contrast, after application of the stearylethoxylate containing dentifrice, as well as of a paste containing only stearylethoxylate as an agent, the epithelium remained essentially identical to the epithelium of cheek pouches treated with sterile saline, and to the non-treated cheek pouches. From these results, we may conclude that SLS is the agent responsible for the striking changes in the epithelial structure. The specific cytological effects of SLS on the epithelial cells remain to be further studied. Interestingly, application of SLS provides a useful system for the rapid production of acanthosis and hyperkeratinization in the stratified squamous epithelium of the hamster cheek pouch.

Keywords: hamster cheek pouch, mucosa, hyperkeratinization, sodium lauryl sulphate, stearylethoxylate, acanthosis

Little is known about the possible side-effects of dentifrices upon the histological structure of oral mucosa. Most of the studies dealing with side-effects of dentifrices focus on the hard tissues, enamel and dentin. In particular, the adverse effects caused by the abrasive components in dentifrice have been reported (Stookey & Muhler 1968). Most of the soft tissue reactions reported involve hypersensitivity to components of dentifrices (Fisher & Tobin 1953; Millard 1972; Kirton & Wilkinson 1973).

Dentifrices enhance the mechanical cleansing of the dentition by means of abrasive substances and surfactants. The anionic sodium lauryl sulphate (SLS) is one of

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Table 1. Main composition (per cent) of the dentifrices used in this study

	Dentifrice A	Dentifrice B	Dentifrice C
Abrasives	Calcium pyrophosphate	Calcium pyrophosphate	Aluminium hydrate
	42	29	35
Water	36	49	38
Humectants	Glycerol	Glycerol/sorbitol	Glycerol/sorbitol
	20.0	15.0/3.0	1.5/14.0
Surfactants	Sodium lauryl sulphate	Sodium lauryl sulphate	Stearylethoxylate
	1.1	1.4	3.0
Flavouring agents	Menthol/spearmint	Menthol	Menthol/peppermint
	0.6	0.5	0.6
Fluoride	MFP/NaF	None	NaF
	0.40/0.10		0.22

the most widely used surfactants in dentifrices. Some enzyme containing dentifrices use a non-ionic surfactant, stearylethoxylate, because SLS has a denaturating effect on the enzymes added (Tsutzumi *et al.* 1982).

In their study of exfoliative cytology, Flores de Jacoby *et al.* (1975) concluded that dentifrice surfactants are responsible for a reduction in the keratinization index of the human oral epithelium, probably due to rupture of intercellular junctions. Götze (1977) reported increased desquamation of the oral epithelium. Guarnieri (1970) investigated the effects of dentifrice components on the lower labial gingiva of adult guinea-pigs and concluded that sodium *N*-lauroyl sarcosinate, in particular irritated the gingival mucosa. In several studies (for review see Veys *et al.* 1992), it has already been shown that SLS may have some disadvantageous influences in the oral cavity. SLS may cause a moderate etching of the enamel surface (Hamilton *et al.* 1975), reduce the clinical effect of a topically applied monofluorophosphate solution (Melsen & Rölla 1983) and reduce the uptake of alkali soluble fluoride by enamel (Barkvoll *et al.* 1988). SLS may have affinity for proteins and causes denaturation of many proteins (Tsutzumi *et al.* 1982). It may increase the permeability of the oral mucosa to substances with different chemical and physical properties (Siegel & Gordon 1985). Also, SLS is not compatible with the antiseptic chlorhexidine, even when these two substances are introduced separately into the oral cavity (Barkvoll *et al.* 1989).

The aim of the present study was to investigate the possible effects of dentifrices on the histological structure of the epithelium of the hamster cheek pouch. We used hamster cheek pouch as target site because it has already been widely used in other experimental procedures. It provides a suitable model for testing the irritant effect of substances on the mucous membrane (Cutright *et al.* 1974; Eveson 1981; Harsanyi *et al.* 1991). Moreover, it has been shown that the histological and histochemical features of cheek pouch lesions induced by carcinogens are closely similar to those of human oral mucosal lesions (MacDonald 1981; Smith 1968).

Materials and methods

In a first experiment, 12 adult male Syrian golden hamsters were used. The effects of three dentifrices on the histological structure of the cheek pouch epithelium were investigated. The main compositions of these three dentifrices are given in Table 1. Two dentifrices (A and B) contain the anionic sodium lauryl sulphate (SLS), and one dentifrice (C) contains the non-ionic stearylethoxylate as a surfactant. Each dentifrice was applied on the mucosa of the medial wall of the cheek pouch in four hamsters. The animals were slightly anaesthetized with chloroform. Using a cotton bud, the dentifrices were gently rubbed on the pulled out mucosa of the right cheek pouch for 15 seconds. An area of approximately 1 cm^2, distal to the anterior vein, was carefully rubbed. On the left pouch, the same treatment was carried out with sterile saline instead of dentifrice, in order to investigate the possible effects of the mechanical manipulation. The pouch treated with dentifrice is called the test pouch, the one treated with sterile saline the control pouch. In each animal, four applications were performed at intervals of one day (24 hours). On the fifth day, specimens of the mucosa were carefully excised from the painted sites from the anaesthetized animals. The animals were then killed by cervical dislocation.

The mucosal specimens were stretched and pinned down, with the connective tissue surface downwards, on cork sheets to keep them flat during processing. The tissues were fixed by immersion in 10% formalin for 48 hours. After fixation, the specimens were trimmed to approximately 8 × 8 mm, to ensure that the samples are representative of painted mucosa, then cut into two pieces and routinely processed for paraffin embedding. Tissue sections were cut at 5 μm perpendicular

to the epithelial surface, stained with haematoxylin and eosin, or with a routine trichrome staining, and examined with a Zeiss Photomicroscope III.

The thicknesses of the full epithelium, of the stratum corneum and of the epithelium without stratum corneum were measured by an eyepiece micrometer at a magnification of ×320. In each animal, 40 measurements of each parameter were randomly performed using 15 tissue sections from each of the test pouch and control pouch. Between sample sections there was a space of at least 250 μm.

In a second experiment, in order to examine specifically the possible effects of the surfactants in the dentifrices used, the following two pastes were applied on cheek pouch mucosa: (1) a paste containing 1.25% sodium lauryl sulphate alone and (2) a paste containing 3% stearylethoxylate alone, each with alumina hydrate and glycerol/sorbitol as a vehicle.

Results

The mucosal epithelium from the control pouch, treated with sterile saline, was a thin stratified keratinized squamous epithelium (Figure 1). The histological structure was identical to that of non-treated areas, showing four cell layers: a basal, a spinous, a granular and a superficial layer. The basal cell layer, or stratum germinativum, consisted of a single row of more or less cuboidal or low columnar cells, resting upon the underlying basement membrane. These cells had a relatively large, oval and dense nucleus. The interface between the basal cell layer and the underlying connective tissue was rather flat. The intermediate layer, called stratum spinosum, consisted of one to two layers of polyhedral and somewhat flattened cells (spinous or prickle cells), which were larger than the basal cells. They contained an oval nucleus, in which the nucleolus was sometimes visible. No obvious intercellular spaces were observed between these spinous cells. The granular layer, or stratum granulosum, consisted of one or two layers of flattened cells, with a pycnotic nucleus. The cytoplasm contained fine keratohyalin granules. The superficial layer, or stratum corneum, was made up of several tightly packed layers of orthokeratinized cornified cells.

After application of dentifrice A and B (Figures 2–4), the epithelium showed hyperorthokeratinization with

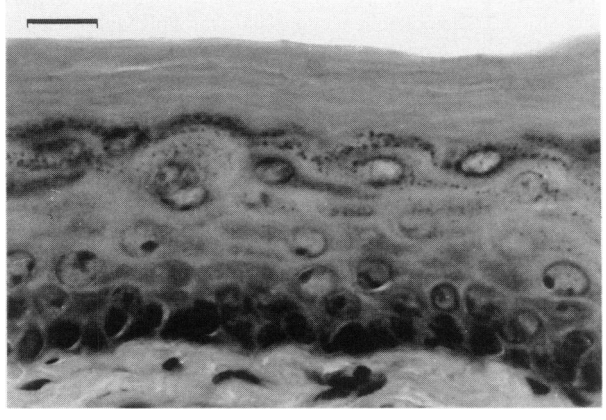

Figure 2. Epithelium of the hamster cheek pouch treated with dentifrice A. The epithelium clearly shows hyperorthokeratinization, thickening of the stratum granulosum, and acanthosis with widening of the intercellular spaces. Haematoxylin–eosin staining. Bar 20 μm.

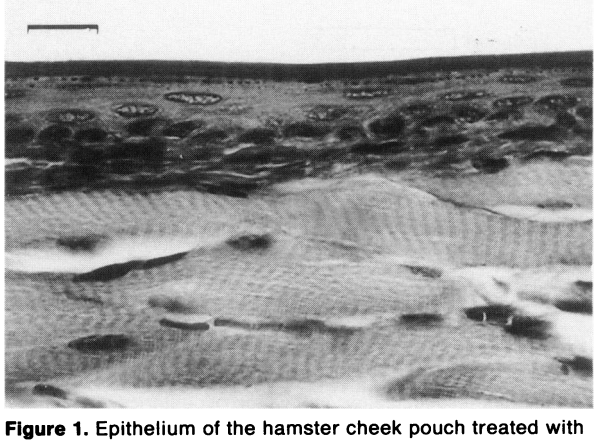

Figure 1. Epithelium of the hamster cheek pouch treated with sterile saline. Trichrome staining. Bar 20 μm.

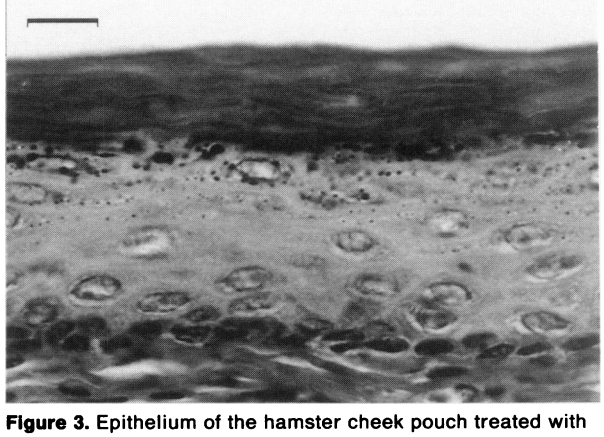

Figure 3. Epithelium of the hamster cheek pouch treated with dentifrice B. As in Figure 2, the epithelium shows hyperorthokeratinization, thickening of the stratum granulosum, and acanthosis with widening of the intercellular spaces. Trichrome staining. Bar 20 μm.

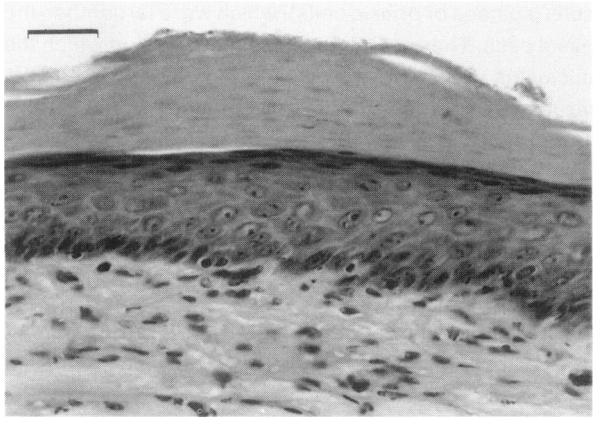

Figure 4. Epithelium of the hamster cheek pouch treated with dentifrice B. The epithelium shows parakeratinization, absence of stratum granulosum, acanthosis with widening of the intercellular spaces, and some basal hyperplasia. Haematoxylin–eosin staining. Bar 40 μm.

local parakeratinization, acanthosis, and varying degrees of basal hyperplasia. In the parakeratinized areas, the keratin layer was sometimes thickened and domeshaped (Figure 4). In the case of orthokeratinization (Figures 2 and 3), there was an obvious thickening of the stratum granulosum, now composed of large cells containing intensely stained coarse keratohyalin granules and a large pale nucleus, which is not pycnotic. On the other hand, in areas with parakeratinization (Figure 3) a definite stratum granulosum was never identifiable. The interface between the basal cell layer and the underlying connective tissue was rather uneven. The basal cell layer, showing varying degrees of hyperplasia (Figure 4), was composed of irregularly shaped and apparently heterogeneous cells. Between these cells intercellular spaces and bridges were found. The stratum spinosum was entirely enlarged (acanthosis) and now composed of three to five layers of prickle cells. These cells seemed to be swollen and contained a relatively large nucleus with a strikingly big nucleolus. The nucleo–cytoplasmic ratio was increased. In the obvious intercellular spaces of the stratum spinosum, intercellular bridges were clearly observed. All morphological changes observed after application of dentifrices A and B were also observed after application of the paste containing the anionic surfactant sodium lauryl sulphate. All the above described morphological reactions of the epithelium were consistently found in all treated areas of all pouches in all animals. In general, the painted mucosa showed no changes in the cellularity of the sub-epithelial connective tissue suggesting inflammation, or dysplastic features.

The light-optical histological structure of the mucosal epithelium seemed not to be affected by the application of dentifrice C, or by the application of the paste containing the non-ionic surfactant stearylethoxylate (Figure 5).

The results of the measurements on the epithelium are shown in Figure 6. The average thicknesses of the total epithelium were, on the test and control sites respectively, 84 ± 25 and $35 \pm 11\,\mu$m with dentifrice A ($P < 0.001$), 82 ± 20 and $41 \pm 10\,\mu$m with dentifrice B ($P < 0.001$), and 33 ± 11 and $33 \pm 9\,\mu$m with dentifrice C ($P > 0.05$). The average thicknesses of the stratum corneum were on the test site and on the control site respectively 33 ± 17 and $9 \pm 2\,\mu$m with dentifrice A ($P < 0.001$), 28 ± 13 and $7 \pm 2\,\mu$m with dentifrice B ($P < 0.001$), and 9 ± 4 and $8 \pm 2\,\mu$m with dentifrice C ($P > 0.05$). The average thicknesses of the epithelium without stratum corneum were for

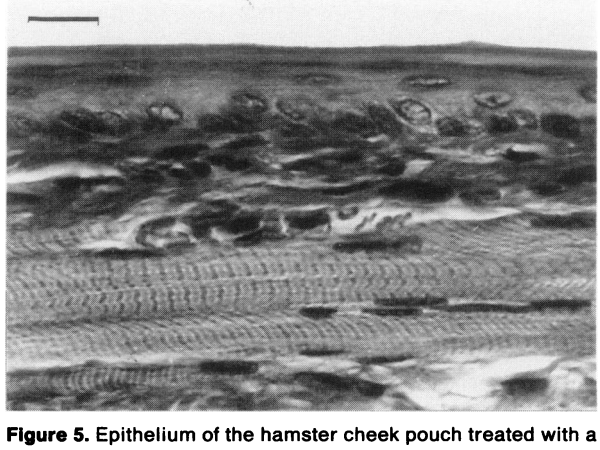

Figure 5. Epithelium of the hamster cheek pouch treated with a paste only containing stearylethoxylate. Trichrome staining. Bar 20 μm.

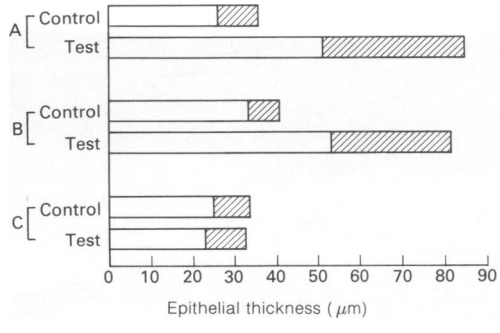

Figure 6. Results of the measurements on the epithelium of the hamster cheek pouch after application of dentifrices A, B and C compared to the control sites. □, Stratum granulosum, stratum spinosum and stratum basale. ▨. Stratum corneum.

test site and control site respectively 51 ± 15 and $26 \pm 10\,\mu m$ with dentifrice A ($P < 0.001$), and 53 ± 14 and $33 \pm 9\,\mu m$ with dentifrice B ($P < 0.001$), and 23 ± 9 and $25 \pm 9\,\mu m$ with dentifrice C ($P > 0.05$). These results reveal that, compared with the controls, and after applications of dentifrices A and B, there is a significant thickening of the total epithelium, of the stratum corneum, and of the epithelium without stratum corneum, whereas, in contrast, there was no significant thickening measured after application of dentifrice C.

Discussion

The general histological structure of the epithelium from the control pouch and the non-treated pouch is in agreement with the structure previously described in normal hamsters (Albright & Listgarten 1962; White & Gohari 1981). Our histological study reveals definite and consistent structural changes in the epithelium of the hamster cheek pouch after application of dentifrices A and B, resulting in a statistically significant ($P < 0.001$) increase in epithelial thickness. In general, the mucosal epithelium showed hyperkeratinization, especially orthokeratinization but locally parakeratinization, acanthosis with widening of the intercellular spaces, and varying degrees of basal hyperplasia. As no structural changes in the epithelium were found after treatment with sterile saline, we may accept that these histological changes were not induced by the mechanical manipulation during application. Moreover, as the observed histological changes were the same in all animals treated with either dentifrice A or B, it may be accepted that we are not dealing with an allergic reaction to components of dentifrice, but with a phenomenon of chemical irritation.

The results presented here relate only to the medial wall of the cheek pouch. This area is normally selected for chemical irritation and cell kinetic studies because of its easy access and the presence of a prominent anterior vein which provides a reproducible marker. It is known that the turnover and response to various chemical stimuli may differ in the medial and lateral walls of hamster cheek pouches (Scragg & Johnson 1983).

The changes in the histological structure of hamster cheek pouch mucosa, which are found after application of dentifrices, appear to be similar but not identical to those produced by other stimuli, including chemical components (Thilagaratnam & Main 1972; Craig & Franklin 1977), ultraviolet light (Rothman 1954), and frictional stimulation (Mackenzie & Miles 1973). Acanthosis and hyperkeratinization, including ortho and para-keratinization, were also observed 3–6 weeks after carcinogen treatment during experimentally induced squamous-cell carcinogenesis in the mucosa of hamster cheek pouch (Tatemoto *et al.* 1986). Although mitotic activity has not been investigated in our study, from the study of Mackenzie and Miles (1973) we may accept that the epithelial thickening is probably also associated with an increased epithelial mitotic rate, besides a reduced desquamation.

The parakeratinization was always associated with a depletion or absence of the stratum granulosum. Absence of a stratum granulosum is known to be characteristic for parakeratinized epithelium (Wentz *et al.* 1952; Trott 1957). On the contrary, in case of hyperorthokeratinization, we found a hypertrophic stratum granulosum, consisting of larger cells containing a large pale nucleus and strikingly coarse keratohyalin granules.

The acanthosis is especially the result of proliferation of the stratum spinosum, showing an increase in the number of cell layers as well as in the cell sizes. There was also an increase in the nucleo–cytoplasmic ratio. Between the cells of the stratum spinosum, we clearly observed intercellular spaces with intercellular bridges. This may be caused by intercellular oedema, although artefact developed during tissue preparation by shrinking of in-vivo swollen oedematous cells (intracellular oedema) may not be excluded. After application of dentifrice C, the epithelium did not show histologically detectable structural changes.

An important difference in components between dentifrice C and the other two applied dentifrices is undoubtedly the surfactant used: the non-ionic stearylethoxylate versus the anionic SLS. As we found in a second experiment the same morphological effects after application of the paste with SLS as after application of the two SLS containing dentifrices, and because we found no structural effects after application of the paste with stearylethoxylate (as was also the case after application of the dentifrice containing stearylethoxylate), we may conclude that SLS is the agent responsible for the striking histological changes in the hamster cheek pouch epithelium. Moreover, these changes occur strikingly rapidly since the normal cell cycle time for hamster cheek pouch epithelium is generally put at between 142 and 164 hours. However, in hyperplastic epithelium this may shorten to 42–91 hours, and in squamous cell carcinoma 15–22 hours have been recorded (for review Scragg & Johnson 1982).

The specific cytological effects of SLS on the epithelial cells (e.g. on the expression of cytokeratins and of cell surface markers, on the enzyme activity, on the cell kinetics) remain to be further investigated. We are presently studying, by in-situ hybridization, the distribu-

tion patterns of cytokeratin-mRNAs in normal cheek pouch epithelium and in epithelium affected by SLS.

Unlike other chemical irritants and carcinogens (Mohammad & Mincer 1976; Harvey *et al.* 1984), the epithelial structural changes induced by SLS in our experiments were not accompanied by inflammatory cell accumulations in the subepithelial connective tissue. The absence of inflammation was also reported by Mackenzie and Miles (1973), who obtained similar epithelial response after chronic frictional stimulation on hamster cheek pouch epithelium for periods of up to 14 days. The non-occurrence of a subepithelial inflammatory reaction after the applications of SLS should be an indication that the epithelial reaction might result in a decreased permeability of the cheek pouch mucosa, producing a barrier that inhibits SLS penetration. Such alterations have been described by Harvey *et al.* (1984) in a study on effects of chlorhexidine on the structure and permeability of hamster cheek pouch mucosa. Alternatively, in our study the experimental period of 5 days might be too short or the used concentration of SLS too low to produce a clear inflammatory cell infiltration.

Finally, it should be emphasized that the findings reported here are noteworthy in the more general context of the hamster cheek pouch representing a useful model for studying different changes and lesions in a stratified epithelium (Eveson 1981; Craig & Franklin 1977; Tatemoto *et al.* 1986). Indeed SLS can be used as an easy-to-apply, non-carcinogenic substance for producing very quickly (within a few days) a benign epithelial hyperplasia with hyperkeratinization, but apparently without inflammatory changes and without dysplastic features. Comparison between epithelial hyperplasia induced by carcinogens (Tatemoto *et al.* 1986) and non-carcinogens may be helpful in diagnostic histopathology.

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