The snuff-induced lesion

A clinical and morphological study of a Swedish material

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Snuff-induced oral lesions were studied clinically, histologically and by electron microscopy. The material was collected from 21 male snuff users. The lesions had a characteristic whitish appearance with a wrinkled swollen texture and the most common localization was in the vestibular area of the upper jaw. In two cases gingival retraction was observed. The light-microscopical examination showed a mild epithelial dysplasia in five cases. No carcinoma in situ or invasive carcinoma was recognized. In some of the cases ultrastructural changes in the basement lamina region, such as defects of the basement lamina and the occurrence of basal cell processes in the connective tissue, were observed. Odland bodies were seen in the epithelium. In the connective tissue filamentous material of unknown nature was found. The ultrastructure of the filamentous material suggested that it might be amyloid. In summary, snuff-induced lesions should be diagnosed, patients should be informed and made to quit the habit, and remaining lesions should be followed up regularly. □ *Snuff-induced leukoplakia; clinic; morphology*

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Swedish snuff is made of tobacco leaves, which after drying, grinding and storing are mixed with water, salts and flavors. Fresh snuff contains about 0.88% nicotine. In 1978 approximately 3400 tons of snuff were sold in Sweden and consumed by about 7-800,000 persons. The sale of snuff in USA was estimated at 15,000 tons in 1961 (21). The habitual user applies the snuff in one or two favourite regions, usually the vestibular area in the upper or lower jaw. The resulting tissue changes in the oral mucosa are usually called 'snuff dipper's lesion' or 'snuff-induced leukoplakia'. The term 'leukoplakia' indicates a white patch or plaque in the oral mucosa, which cannot be scraped off and cannot be diagnosed clinically or pathologically as any other disease (26). Since the snuff-induced lesion, with its typical clinical pattern and its specific etiology, obviously constitutes a definite diagnostic entity, the term 'leukoplakia' is avoided in the following.

The possible influence of the snuff-habit on the periodontal tissues has hitherto attracted only slight attention. The reports concerning the relation between snuffing habits and the development of premalignant lesions in the oral mucosa are contradictory probably due to the differences in snuffing habits and variations in the composition of snuff in different cultural areas (20).

Many questions still remain to be answered concerning the biological effects of snuff on the oral mucosa. Hence, it seemed justified to present a material of snuff-induced lesions which had been studied clinically, histologically and by electron microscopy.

Materials and Methods

The material comprised 21 men, who were referred for treatment at the Dental School, Karolinska Institute, Stockholm, for snuff-induced lesions in the oral mucosa. The age of the patients varied between 31 and 79 years, the mean age being 55.

After clinical registration of the lesion a biopsy was performed under local anaesthesia. The specimen was cut in two parts, one of which was fixed in a 10% buffered formalin solution for light microscopic examination. After embedment in paraffin, the

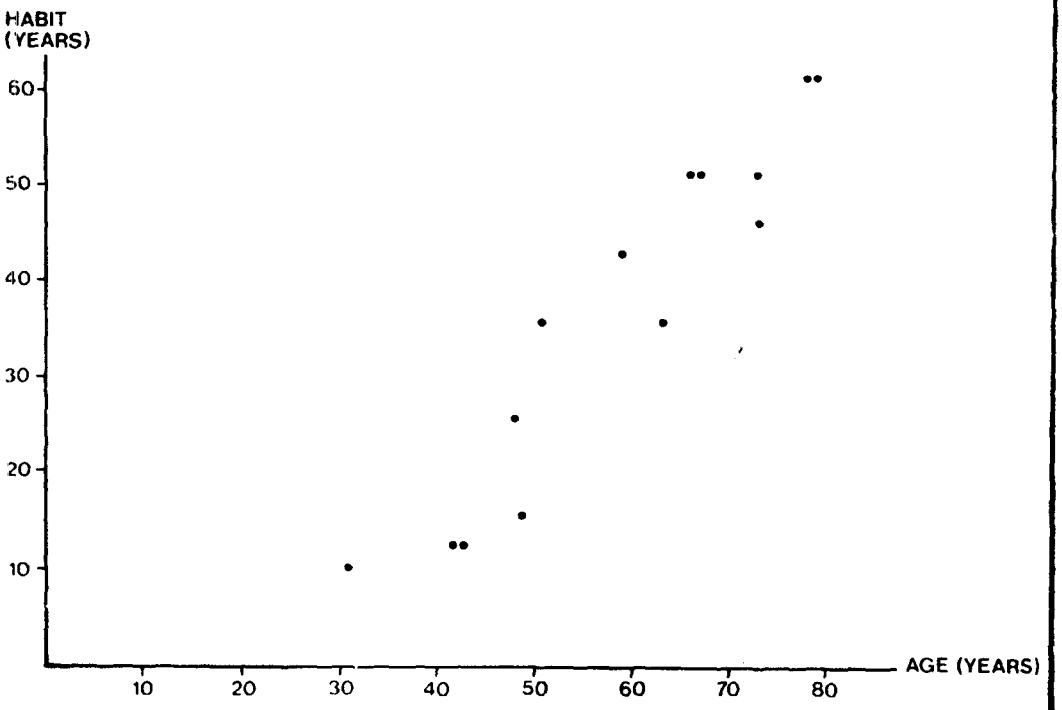

Fig. 1. Snuff-induced oral leukoplakia. Duration of habit and age of 14 of the 21 subjects.

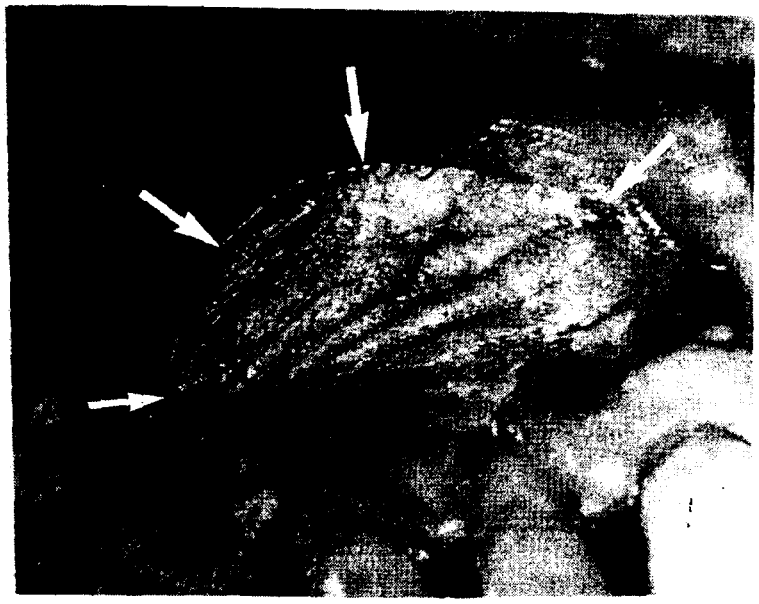

Fig. 2. Snuff-induced lesion in the upper right vestibulum of a 73-year-old man caused by continuous application of snuff during 50 years. The lesion has a characteristic and wrinkled appearance (arrows).

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tissue specimen was serially sectioned in 5-µm-thick sections, which were stained with Mayer's haematoxylin-eosin and Weigert's haematoxylin-van Gieson stain. These specimens were evaluated histologically by two independent observers. The other part of the biopsy was fixed for 2 hours in an ice-cold buffered 1.5% osmium-tetra-oxid solution for electron microscopy. After dehydration and embedding in Epon, the specimen was sectioned using a glass knife into 1-µm-thick sections for light microscopic examination and in about 40 to 60-nm thick sections for the electron microscopy. The latter sections were stained in uranyl acetate and lead acetate and studied in a Siemens Elmiskop I. The ultrastructure of the normal human oral epithelium prepared in an identical manner has been presented previously (8–11).

Results

Clinical observations

Duration. In 14 cases the average duration of the snuff habit was estimated to 36.1 years, varying between 10 and 60 years. The habit started between 16 and 34 years of age, the average age being 22.6 years. A close correlation was noticed between age and duration of the habit (Fig. 1). In the remaining 7 cases no information was available about the duration of the snuff habit.

Localization. In 19 cases the snuff-induced lesions were localized to the vestibular area of the upper jaw (Fig. 2). In the remaining two cases the lesion was located in the corresponding area in the lower jaw. In some cases the lesion was limited to the mucogingival fold and in others it also included the labial mucosa. In a few cases the gingiva propria was engaged as well.

Symptoms. The snuff-induced lesions had a characteristic whitish appearance frequently with a brown discolouration which clearly contrasted with the neighbouring mucosa. In some lesions dark red pin pricks surrounded by an elevated, swollen, whitish zone were observed as well as net-like, whitish tissue changes in combination with reddish areas (Fig. 3). The lesions were also characterized by their wrinkled, swollen texture and by their consistency which was firmer than the surrounding normal oral mucosa. Desquamating epithelium and ulcerations were furthermore observed in some cases.

The gingival lesions did not display any pin pricks or wrinkles. The affected gingiva was whiter than normal and reddish areas could also be observed. In two cases, gingival retraction was observed (Fig. 4).

Subjective symptoms failed in most cases. In some cases the patient reported that smarting pain occurred. The main part of the patients, however, was not aware of any pathological change in the oral mucosa.

Treatment. All patients were recommended to break the habit of snuffing. On patients who did, the lesion was markedly normalized in structure and colour after one week. After 14 days, only remnants of patches remained and the mucosa had regained most of its soft consistency and normal colour.

Fig. 3. Leukoplakia in a 43 year old man who had used snuff for 12 years in the upper left vestibulum. The lesion involves the alveolar mucosa and the gingiva. Dark-red pin pricks surrounded by an elevated, swollen, whitish zone were observed (arrows).

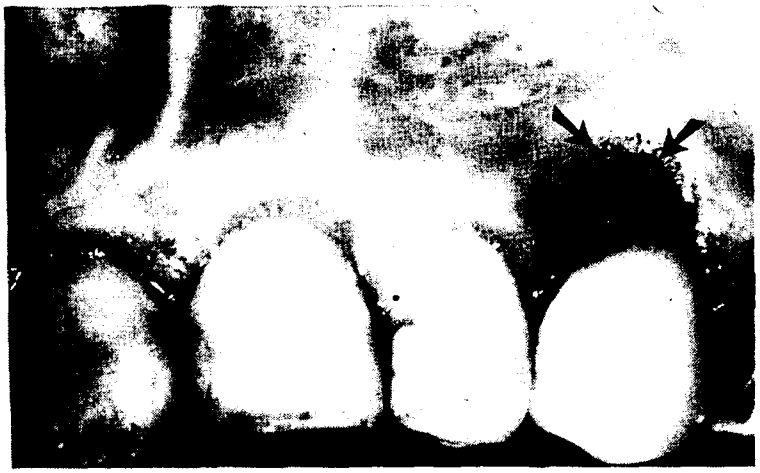

Fig. 4. After 12 years of application of snuff in the upper left vestibulum this 42-year-old man had a moderately pronounced leukoplakia involving the alveolar mucosa and the gingiva in the upper left vestibulum. A gingival atrophy at 23 of 4 mm (arrows) as well as a generalized gingivitis can be observed.

Light-microscopical observations

In all cases epithelial hyperplasia was found. Hyperorthokeratinization was found in 12 cases and hyperparakeratinization in 9 cases. In the surface layer, enlarged, rounded, vacuolated cells containing remnants of cell nuclei were observed. Acanthosis in varying degrees was recognized in all specimens examined. In 11 of the 12 cases with hyperorthokeratinization keratohyaline granules were found.

There were 16 specimens with an inflammatory reaction. In 5 cases a mild epithelial dysplasia was observed in the form of drop-shaped rete processes, reduction of cellular adhesion in the basal and spinous cell layers and slight cellular pleomorphism. In no case was carcinoma in situ or invasive carcinoma diagnosed.

Electron-microscopical observations

Eleven specimens were studied by electron microscopy. The examination included the epithelial cell layers as well as the basement lamina and the subepithelial connective tissue.

The epithelial surface cell layers. The cells in the surface layers were in all cases partly keratinized and contained nuclear remnants. In one specimen the intercellular spaces between the epithelial cells in the stratum corneum were widened but otherwise the intercellular spaces in this layer were narrow (Fig. 5). The number of desmosomes gradually decreased towards the surface.

The epithelial spinous cell layer. An increased concentration of tonofilaments was constantly found in the stratum spinosum (Fig. 6) compared to the normal epithelium. 'Odland bodies' could be identified in 9 specimens where also small keratohyaline granules were observed. The keratohyaline granules were round with peripheral accumulations of ribosomes. In some cases the intercellular spaces were widened and intracellular edema recognized (Fig. 6).

The epithelial basal cell layer. In all specimens an increased amount of tonofilaments was noticed in the basal cell layer (Fig. 7) as compared with the normal condition. The basal epithelium was separated from the connective tissue by a single continuous basal lamina (Fig. 7). In five specimens the lamina densa appeared in double layers. Six specimens showed a discontinuous lamina densa. Cytoplasmic processes from the basal cells without any association to the lamina densa were recorded in four cases.

On a 79-year-old man, who had used snuff for 60 years, the electron micrographs showed cellular atypia. Irregular border between the epithelial basal cell layer and the connective tissue with fragmentation as well as absence of the basal lamina was in this case observed (Fig. 8).

Fig. 5. Flattened cell (parakeratosis) with gradual change in st nosomes towards th (× 9000.)

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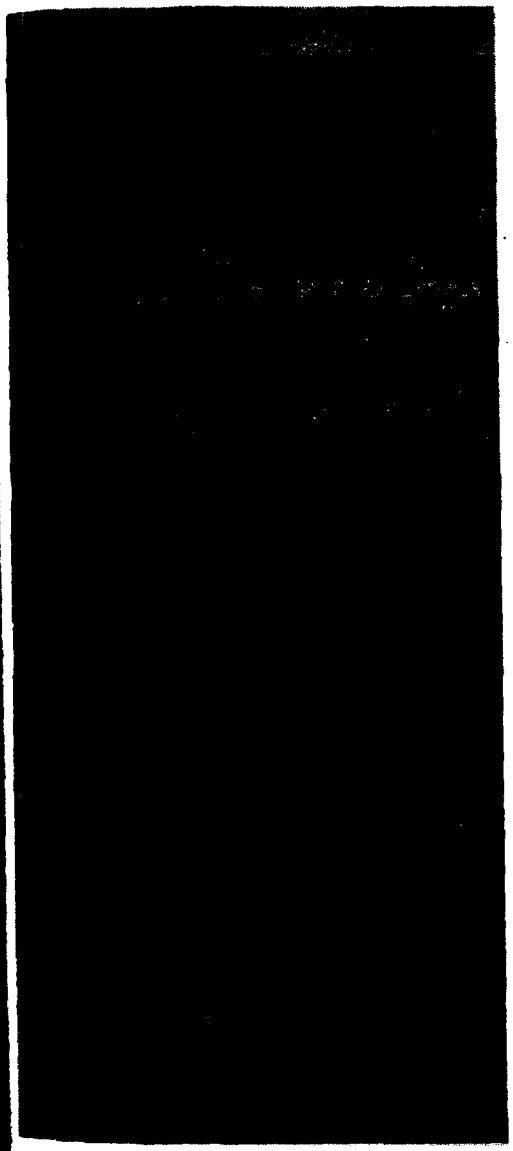

Fig. 5. Flattened cell layers including the surface layer (parakeratosis) with narrow intercellular spaces and a gradual change in structure and number of the desmosomes towards the surface. Electron micrography. (× 9000.)

In the basal cell layer an unusual accumulation of melanin-like dense granules less than 0.1 μm in diameter was recognized in specimen from a man who had used snuff for more than 15 years (Fig. 9).

Connective tissue. In the connective tissue inflammatory cells were observed. Besides collagen fibrils a filamentous material of unknown composition was found in some of the specimens (Fig. 8).

Discussion

Clinical observations

The duration and the debut of the habit (means, 36.1 and 22.6 years, respectively) illustrated that snuff habits established early in life may persist for decades. It was from the result of this study impossible to determine the period of time required for a snuff-induced lesion to develop.

In a study of 25 patients with oral cancer associated with the use of snuff (13) it was shown that the duration of the habit varied between 20 and 50 years with an average of 36 years. In the present study with the same average duration of the snuff habit, however, no carcinoma in situ or invasive carcinoma was found.

The clinical appearance of the lesions was the same as reported by Pindborg & Renstrup (17). The irregularly spread, dark red 'pin pricks' were presumably orifices of salivary glands, enlarged by retention due to constriction of the ductal epithelium. Similar structures in the palatal mucosa of pipe-smokers in leukokeratosis nicotina palati have been reported (5).

The desquamating epithelium and ulceration which was occasionally seen suggested recent intense irritation.

Light microscopy

The histopathological appearance of the examined lesions was uniform and mainly according to findings reported by Pindborg & Renstrup (17) and Axell et al. (2).

Pindborg & Renstrup (17) reported that the main histopathological criteria of snuff-induced lesions were a non-keratinized surface layer with enlarged, rounded and partly vacuolized cells containing nuclear remnants. The results of the present investigation indicated, however, that the epithelium was hyperorthokeratotic in more than 50% of the examined specimens.

In an investigation of 15,000 snuff users

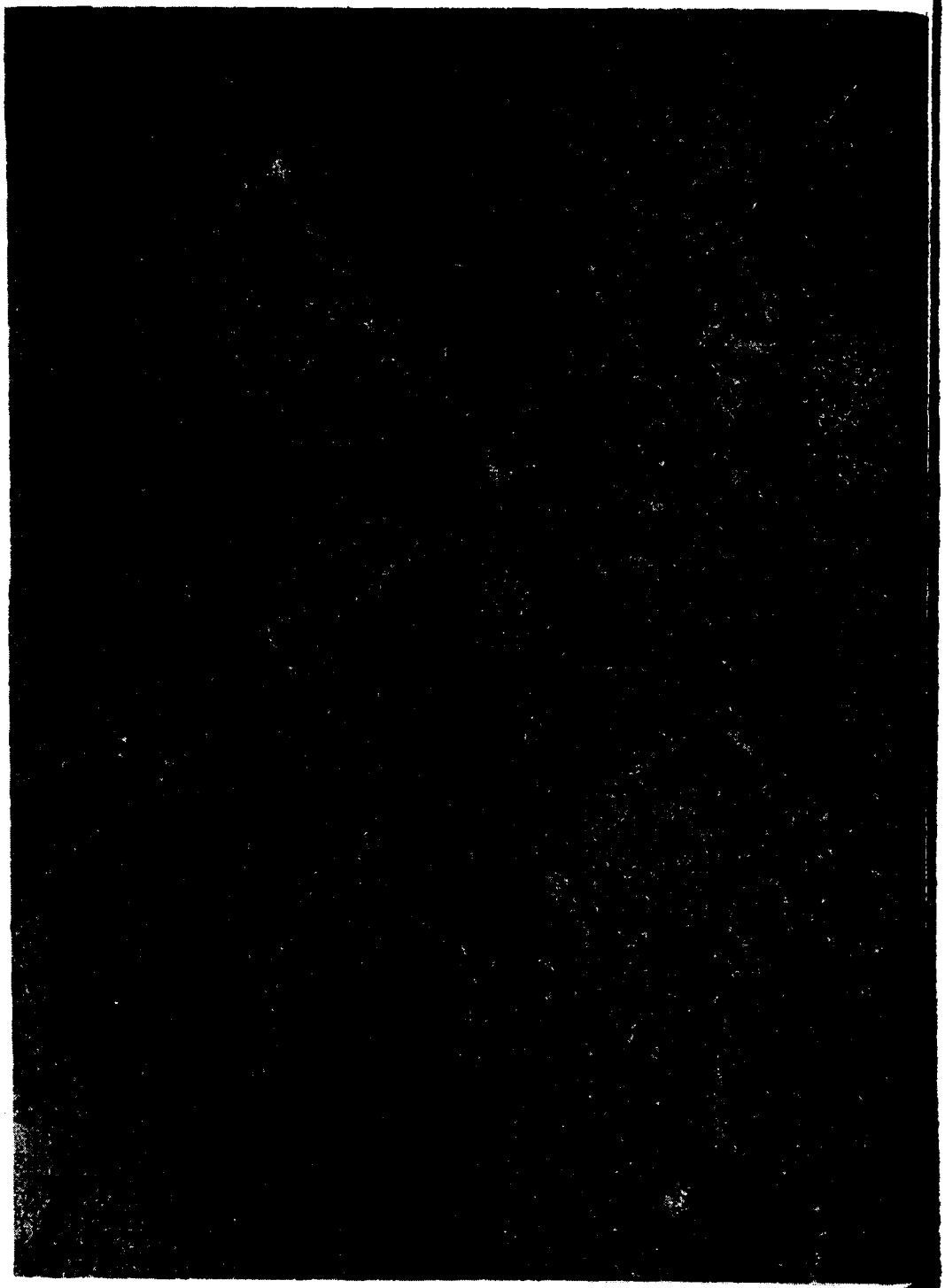

Fig. 6. Stratum spinosum in vestibular leukoplakia after 60 years' use of snuff. The intercellular spaces are wide and intracellular edema is recognized. Increased tonofilament concentration compared to the normal vestibul Electron micrograph. (× 6000.)

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Fig. 7. The epithelial basal cell layer and the subepithelial connective tissue in a vestibular leukoplakia caused by the use of snuff during 42 years. In the basal cell layer an increased amount of tonofilaments as well as widened intercellular spaces are noted. The epithelial cells are separated from the connective tissue by a single continuous basal lamina (arrows). At a distance of less than one micron from the basal cells there is a wide capillary (C) in the connective tissue. Electron micrograph. (× 6000.)

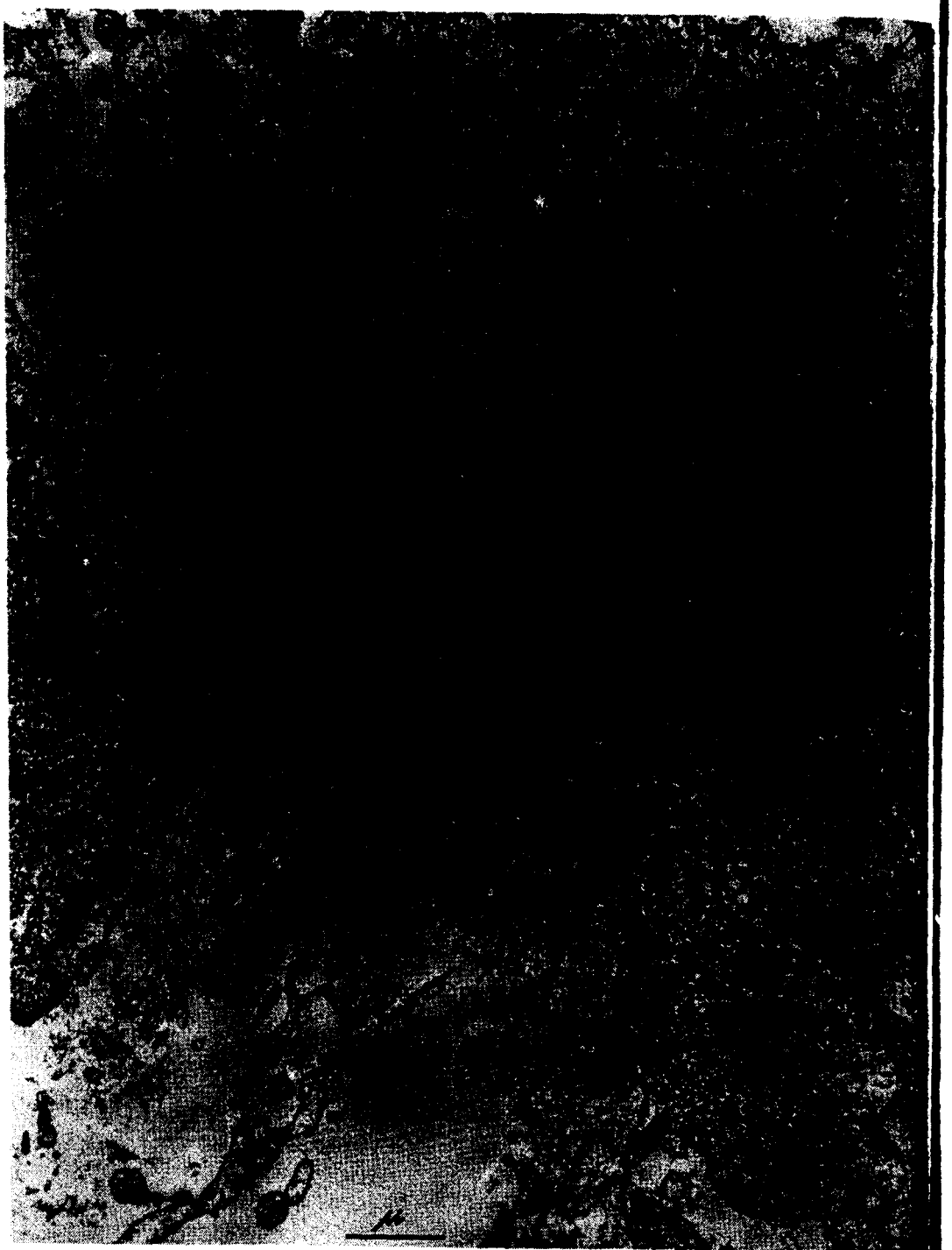

Fig. 8. Vestibular leukoplakia in a 79-year-old man who had used snuff for 60 years. Irregular border betwe epithelium and connective tissue with fragmentation of the basal lamina (BL). Cytoplasma basal cell process (P) extend into the connective tissue. There are connective tissue changes with absence of collagen and presen of amyloid-like aggregates (arrows). Electron micrograph (×18,000.)

Fig. 9. Vestibular l melanin-like granul

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Fig. 9. Vestibular leukoplakia after 15 years of use of snuff. There is an unusual accumulation of round, dense melanin-like granules (g) less than 0.1 μm in diameter. BL = basal lamina. Electron micrograph. (× 60,000.)

in USA, 1751 were investigated by cytology (22). Of these, 1502 were classified as normal and 237 as 'benign hyperkeratosis'. 157 biopsies were performed, all without any sign of malignancy. More than 75% of the patients were recalled during 5½ years. There was no aggravation of the lesions during this period. In a hospital material of 500 snuff users the same authors found 12 lesions with dyskeratosis and 2 with carcinoma.

In a material of 114 biopsies from snuff dippers in Sweden no cellular atypia or epithelial dysplasia was found (2).

The result of the histological examination

in the present study supports the statement presented by Waldron & Shafer (24) that the clinical appearance of oral leukoplakia never gives any information about the presence of atypical cellular changes of premalignant significance.

According to Renstrup (19), the frequency of cellular atypia is higher in snuff-induced lesions than in other types of leukoplakias in the oral cavity. This finding and the mild epithelial dysplasia observed in the present study suggest that attention has to be paid to the snuff-induced lesions both from a clinical and therapeutic point of view (18–20). It is, however, important to emphasize that the premalignant significance of the mild dysplasia found in this study is questionable. The epithelial dysplasia might for instance be a reactive change due to inflammatory infiltration.

Electron microscopy

The results of the electron-microscopical examination also indicated that attention has to be paid to the snuff-induced lesions. The ultrastructural changes in the basement lamina region such as defects of the basement lamina and the occurrence of nude basal cell processes in the connective tissue are difficult to distinguish from changes observed in inflammatory reaction as well as carcinoma in situ and invasive cancer (8). Similar cytoplasmic processes extending into the connective tissue were demonstrated in 7 out of 9 tobacco-chewing patients from India and were classified as early invasive cancer (7).

The high concentration of tonofilaments and tonofibrils in the cells of stratum basale is a characteristic of a keratinized epithelium (10). The increased degree of keratinization in the epithelial surface layer, which clinically appears as a whitish, less translucent lesion, is preceded by a marked increase of cellular activity in the basal layer including an increased concentration of ribosomes and an increased production of tonofilaments.

Lamellated Odland bodies (15), which in the present study were found in 9 out of 11 specimens, are rarely found in the normal oral vestibular mucosa but are regularly observed in keratinized epithelium (11). The function of the Odland bodies is still obscure. They contain enzymes (25) and a substance structurally similar to phospholipids (9).

The nature of the filamentous material, which was observed in the subepithelial connective tissue, was unknown. The ultrastructure of this material was to a certain degree similar to the amyloid structure demonstrated by Cohen et al. (6). The observation might have a biological significance as Lyon et al. (14) showed that amyloid structures occurred in the subepithelial connective tissue in snuff-induced lesions.

Differential diagnosis

Differential diagnostic considerations mainly deal with the premalignant or malignant character of the snuff-induced lesions. The clinical appearance of the lesions does not give any indication about their histological character. A histological examination is therefore always necessary for an adequate differential diagnosis.

Gingival atrophy in connection with use of snuff is of differential diagnostic interest from a periodontal point of view. The localization of the atrophy, the brownish discolouration of the root surface, the tooth brushing habits, the oral hygiene and the periodontal condition of the rest of the dentition are guiding factors to be considered in the analysis of the possible cause of the gingival recession.

Etiology

Even if the lesions studied undoubtedly were induced by the longstanding use of snuff, little is known about whether the chemical or the mechanical irritation is the main inducing factor.

Both chemical and mechanical factors may be contributory. The fact that inveterate snuff dippers show a leathery, thickened lesion with hyperplasia and a hyperkeratotic epithelium suggests that the type and degree of irritation permits a protective tissue response. It may be assumed that the irritation also is a contributory factor to the malignization of snuff-induced lesions. It has been noted that the majority of the ora

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cancer patients with the snuff habit also wore dentures and that their oral hygiene was poor (13).

It cannot be excluded that studies on habitual snuff users include patients with a number of other common local and general factors in addition to the snuff habit. In some cultures the snuff habit is a part of a social pattern that also frequently includes undernourishment, vitamin deficiencies, iron deficiency, habitual use of alcohol, and irregular daily life pattern (12, 16, 18, 23).

Treatment

No case of carcinoma in situ or squamous cell carcinoma was found in the present study. The results of other investigations, however, have shown that, if the snuff habit is continued for a long period of time, certain patients undoubtedly risk developing a squamous carcinoma within or close to the region where the snuff is situated (2, 3, 21). The majority of patients with carcinoma in the mucobuccal fold habitually use snuff (1, 4, 21).

A higher mortality rate and incidence rate of oral cancer was reported (23) on white women in the southern USA, where the snuff habit is much more pronounced among women than in the rest of the country (4).

In a study based on the Swedish Cancer Registry 1962–1971, in the cases of 33 patients with oral carcinoma there was found a documented or probable connection between the localization of the tumor and snuff habit (3). All patients were more than 50 years old, and 20 of them were above the age of 70. In Sweden 3–4 cases of oral carcinoma per year among men can be connected with the snuff habit. That is an incidence of 0.5 cases per 100,000 male snuff users per year (3).

The treatment of the snuff-induced lesion aims at prevention of further irritation. This is achieved if the patient could be induced to break the habit. The treatment should not lead to the patient starting to smoke to satisfy his demand for nicotine. Smoking is obviously a far more serious health hazard than snuffing (3).

Lesions in which dysplasia or cellular atypia has been revealed after biopsy should be excised in toto. The patient should be informed about the hazards and urged to break the habit. Regular follow-up with 3 to 6-month interval is justified.

Prognosis

Clinical healing of the snuff-induced lesions in the present material occurred after two to three weeks when the patient could break the habit, even when the anamnestic data suggested decades of habitual snuff-use. Pindborg & Renstrup (17) also observed healing after 3 weeks of abstinence from snuff. Differences in habits and in composition between brands of snuff dominating the local market of different countries makes if difficult to assess the general probability of malignification of snuff-induced lesions.

As to the prognosis of a carcinoma within or close to a snuff-induced lesion, it was found in a study of 394 oral carcinomas that the 5-year survival was higher in the group of 78 snuff users than in the non-user group (4). Rosenfeldt & Callaway (21) made similar observations on 525 patients with oral carcinoma of which 154 were snuffers. The high degree of differentiation and the low metastatic potential were mentioned as the most important factors contributing to the comparatively high 5-year survival. Landy & White (13) reported 25 cases of carcinoma in snuff users, collected during 9 years. They were usually highly differentiated and when metastases occurred, they were limited to the upper cervical nodes.

Conclusions

The daily use of snuff in a limited area of the mucobuccal fold results in a characteristic lesion. Obviously, many individuals can use snuff for more than 40 years or more without any carcinoma developing.

According to the tumour statistics, however, 3–4 cases of carcinoma develop every year among Swedish snuff users in the area where the snuff is placed.

According to the morphological literature, very few and mostly slight dysplasias are seen even in large materials of snuff-induced lesions and rarely any malignancies.

The conclusion is that the malignant lesion appears suddenly, and only in some individuals, and without any long-standing morphological signs of warning that we are capable of recognizing.

To the dentist and clinician who is eager to contribute to the health of his patients there is only one alternative: to diagnose the lesions; to inform the patients in order to make them quit the habit; and to follow up regularly the remaining lesions.

To the scientist there remains the problem of early diagnosis: to find among 200,000 snuff users the single person who will eventually develop a carcinoma.

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