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STUDIES IN ORAL LEUKOPLAKIAS

VIII. EPITHELIAL CHANGES IN TOBACCO-INDUCED LEUKOPLAKIAS IN INDIA

by

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Although a certain number of oral leukoplakias are idiopathic, the majority of the lesions can be attributed to a local irritant. Only few investigations, however, have tried to analyze the specific effects of the agents causing leukoplakias and to correlate the histological changes with the etiologic agent.

Orr in 1933 reported on leukoplakias caused by "betel-tobacco-chewing". He noted that the superficial layer of the epithelium is thickened and that the cells at the surface show signs of desquamation. In more advanced stages the rete pegs are prolonged, and later they become irregular and the basement membrane less obvious. The epithelial cells then appear swollen and are separated from one another. They have multiple nuclei and show

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signs of rapid division. In 1949, *Balendra* gave a rather detailed description of "betel chewing hyperplasia" of the oral epithelium caused by chewing of betel mixed with tobacco. The two main changes are hyperplasia of the stratum spinosum ("betel acanthosis") and hyperkeratosis. *Marsden* (1960) observed in betel leukoplakias the presence of epithelial hyperplasia, sometimes of papillomatous type, but usually manifested by broad downward growing rete pegs. *Wahi, Arora, Srivastava, Kehar & Bodkhe* (1961) showed three photomicrographs of leukoplakias in smokers and chewers, but they did not correlate the histologic findings with the different habits. *Pindborg & Renstrup* (1963) found in leukoplakias caused by the long-standing use of snuff that is kept in the lower vestibule an increased thickness of the epithelium due to (1) hyperplasia of spinous cells and (2) the presence of a thick layer of vacuolated cells and (3) focal areas of hyperkeratosis.

None of the studies dealing with leukoplakias induced by betel-chewing have analyzed the type of keratinization in these lesions, and no comparison of these leukoplakias with other types of oral leukoplakias have been made. This paper reports on a pilot study analyzing the patterns of keratinization caused by (1) tobacco-chewing with and without betel and (2) different types of smoking.

MATERIAL AND METHODS

The material comprised 39 biopsies from 34 male and three female patients with leukoplakias, selected from an epidemiologic study among patients coming to the Admission Clinic at the Dental College in Lucknow (*Pindborg, Chawla, Misra, Nagpaul & Gupta,* 1964). The average age was 45 years. Leukoplakia was defined as a well-demarcated, elevated white patch of 5 mm or more in diameter that could not be scraped off and could not be attributed to the presence of other disease.

The patients were questioned as to their chewing and smoking habits. Their leukoplakic lesions were recorded and photographed in color.

The biopsies were obtained under local anesthesia from areas within the lesions that had a uniform appearance. The tissues

were fixed in 10 percent formalin, embedded in paraffin, serially cut, and stained with hematoxylin and eosin and a modified Mallory stain.

The sections were evaluated with regard to thickness of epithelium as judged by the width of the spinous cell layer, type of keratinization, mitotic activity and inflammation of the connective tissue.

The patients had the following four types of habit: *Group 1:* Bidi*)-smokers (seven patients); *Group 2:* Cigarette-smokers (five patients); *Group 3:* Tobacco-chewers, chewing either tobacco alone (12 patients) or tobacco included in a pan**) (three patients); *Group 4:* Patients smoking bidis and chewing pan-containing tobacco (10 patients).

RESULTS

The 37 patients had a total of 74 leukoplakic lesions, located as follows: labial commissure: 32, cheek: 28, lower lip: 8, alveolar ridge: 3, gingiva: 2, and tongue: 1. 19 leukoplakias occurred bilaterally.

Group 1: Bidi-smokers. Four biopsies were taken from the cheek and three from the labial commissure. Hyperplasia of the epithelium was seen in four leukoplakias and atrophy in four (Table I). Hyperorthokeratosis was present in four leukoplakias, hyperparakeratosis in two, and unkeratinized epithelium in two. One of the leukoplakias had clinically a speckled appearance (for further explanation, see *Pindborg, Renstrup, Poulsen & Silverman,* 1964). In this lesion an increase in mitotic activity in the

*) Bidi is an Indian form of a cheap cigarette. In Lucknow (Uttar Pradesh) it is made by rolling between the fingers a rectangular dried piece of Temburni (diospyros melanoxylon), also called Tendu leaf, with 0.30 g to 0.36 g of Saurashtra tobacco (from the State of Gujarat) or Nipani tobacco (from the State of Mysore) and finally roped with thread. The size of each "bidi" varies from 6 to 7.5 cm.

**) Pan is a preparation of betel leaf, betel nut (raw or cured), slaked lime and catachu. This combination is chewed with or without tobacco. The bolus formed by chewing the preparation is either spat out, swallowed or kept in the mouth for hours, sometimes during sleep also. There are differences in the variety of the betel nut, tobacco, and lime used in different regions of India.

epithelium was demonstrated. In four biopsies, the underlying connective tissue was chronically inflamed.

Table I

Occurrence of epithelial changes in leukoplakias in four different types of habits. A particular biopsy may be registered twice, either if it exhibits both atrophy and hyperplasia or, if it shows hyperorthokeratosis as well as hyperparakeratosis.

Group	Epithelial changes	Thickness of leukoplakic epithelium	*Type of cornification*			Increased mitotic activity	Inflammation in lamina propria
			Hyperorthokeratosis	Hyperparakeratosis	Unkeratinized		
Bidi-smokers (7 patients)		Atrophy 4	4				2
		Hyperplasia 4		2	2	1	2
Cigarette-smokers (5 patients with 6 biopsies)		Atrophy 1	1				1
		Hyperplasia 5	2	4		1	5
Tobacco-chewers (15 patients with 16 biopsies)	Labial mucosa	Hyperplasia 6		6		3	4
		Normal 2		2		1	2
	Cheek mucosa	Atrophy 5	5				4
		Hyperplasia 3	3	1		1	2
		Normal 1	1				
Patients chewing tobacco in pan + smoking bidis (10 patients)		Atrophy 6	6				4
		Hyperplasia 5	1	4		2	4
		Normal 1	1				1

Group 2: Cigarette-smokers. Five biopsies were taken from the labial commissure and one from the cheek. Hyperplasia of the epithelium was observed in five leukoplakias and atrophy in one. Three leukoplakias exhibited hyperorthokeratosis; four were hyperparakeratotic. The hyperorthokeratosis was not completely typical in that the granular cell layer was very narrow and that some nuclei were seen in the keratin layer (Fig. 1). One leuko-

plakia showed increased mitotic activity. Inflammatory changes were present in the connective tissue in all the cigarette-induced leukoplakias.

Group 3: Tobacco-chewers. Eight biopsies were taken from the mucosa of the lower lip and eight from the cheek. The response to tobacco chewing is very different in these two locations and, therefore, they are treated separately in Table I. In the labial mucosa hyperplasia was observed in six leukoplakias and normal

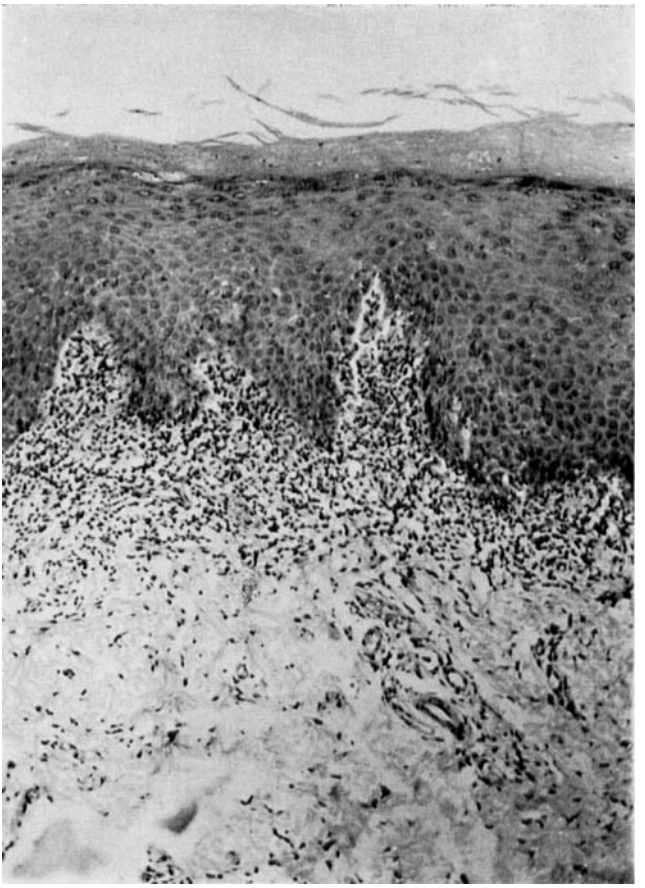

Fig. 1. Photomicrograph of leukoplakia of buccal mucosa caused by cigarette-smoking. Note narrow granular cell layer and presence of nuclei in keratin layer. Magnification: 8 × 10.

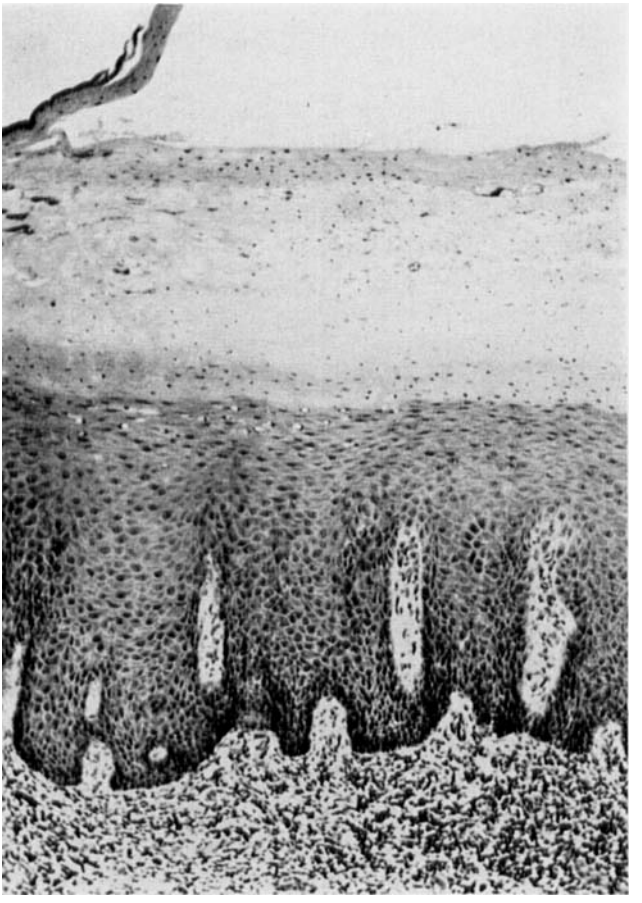

Fig. 2. Photomicrograph of leukoplakia of lower labial mucosa caused by tobacco-chewing. Note hyperplasia of spinous cell layer and marked atypical hyperparakeratosis. Magnification: 8 × 10.

thickness in two. All eight lesions exhibited hyperparakeratosis and four showed markedly increased mitotic activity. None of the lesions had a speckled appearance. The hyperparakeratosis was not typical, because a number of cells in the peripheral layers were not flat, but showed vacuolization instead (Fig. 2). The nuclei did not flatten either, but exhibited karyorrhectic changes (Fig. 3). The Mallory-stain did not show keratinization throughout the outer layer in all lesions; sometimes the impression was one of

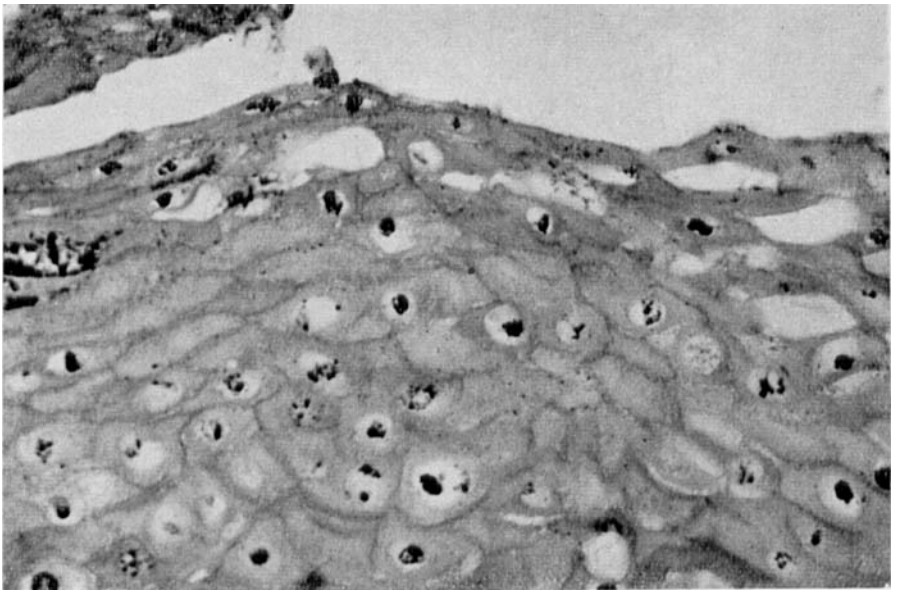

Fig. 3. High power of the surface layer from Fig. 2. Note karyorrhectic changes. Magnification: 8 × 40.

incomplete hyperparakeratosis (*Weinmann & Meyer,* 1959). Inflammation was present in six lesions.

The cheek mucosa by contrast exhibited atrophy in five leukoplakias, hyperplasia in three and normal thickness in one. Hyperorthokeratosis was the predominant type of keratinization and occurred in atrophic as well as in hyperplastic lesions. It was observed in eight leukoplakias, hyperparakeratosis in one. One leukoplakia revealed increased mitotic activity, and inflammation was present in six lesions.

Group 4: Patients chewing tobacco in pan+bidi-smoking. Six biopsies were taken from the labial commissure and four from the cheek. Atrophy was seen in six leukoplakias, hyperplasia in five and normal thickness in one. Hyperorthokeratosis (Fig. 4) was seen in eight leukoplakias, and hyperparakeratosis in four. Two of the leukoplakias were of the speckled type and exhibited increased mitotic activity (Fig. 5). Inflammation of varying degree was seen in nine leukoplakias.

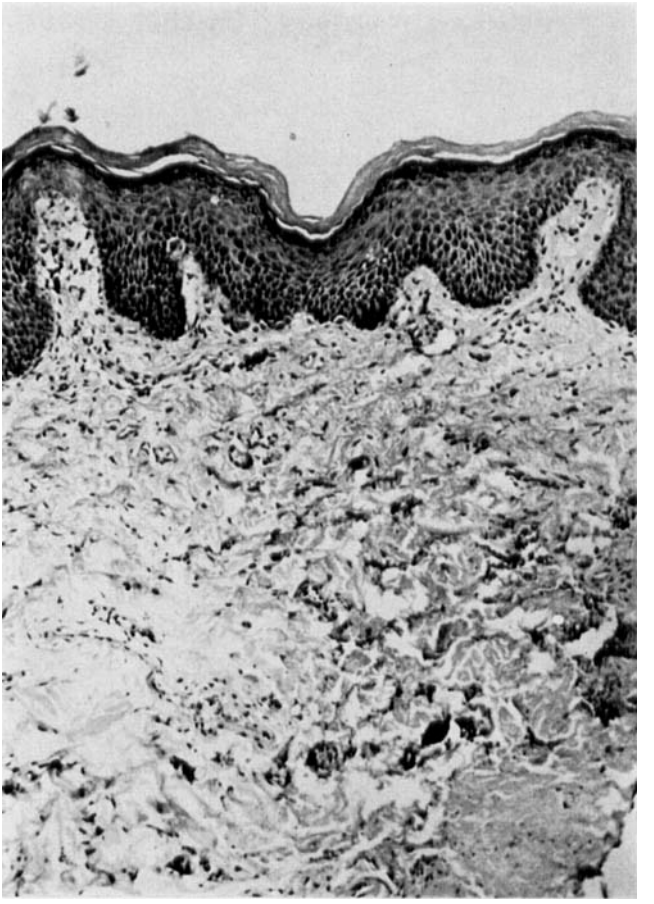

Fig. 4. Photomicrograph of leukoplakia of buccal mucosa caused by chewing tobacco in pan and bidi-smoking. Note atrophy of epithelium and hyper-orthokeratosis. Magnification: 8 × 10.

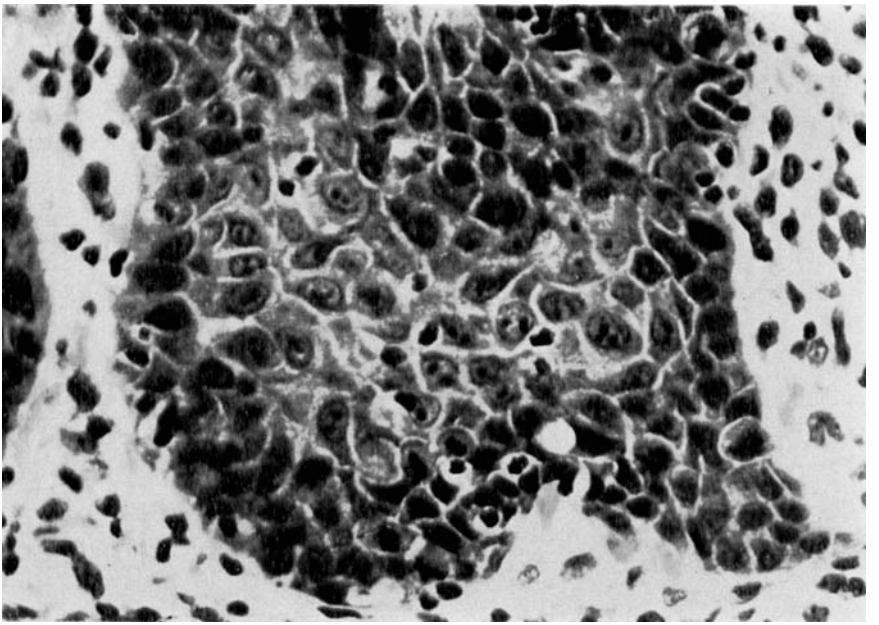

Fig. 5. Photomicrograph of speckled leukoplakia of labial commissure caused by chewing tobacco in pan and bidi-smoking. Note the increased mitotic activity. Magnification: 8 × 40.

DISCUSSION

Although the material in each group is not large, it suggests that the various habits tend to have different effects upon the oral epithelium.

In bidi-smokers, the epithelium may be atrophic or hyperplastic. In the atrophic lesions the cornification is hyperorthokeratosis; in hyperplastic lesions one finds either hyperparakeratosis or unkeratinized epithelium. In cigarette-smokers, by contrast, the predominant change is hyperplasia of the epithelium associated with a hyperparakeratosis and a chronic inflammation in the lamina propria.

The labial mucosa of tobacco chewers shows an interesting change which was never observed in the cheek. Instead of becoming flattened, the outer epithelial cells undergo vacuolization, the nuclei show karyorrhexis, and the outermost layers assume

a loose appearance. These changes are somewhat similar to those described by *Pindborg & Renstrup* in 1963 in the epithelium of the lower labial mucosa exposed to snuff over many years (Fig. 6). Thus, it appears that the direct, longstanding contact of the labial mucosa with tobacco produces epithelial changes different from those produced by smoking.

In 1962, *Pindborg & Poulsen* reported on the deposit of a PAS-positive diastase-resistant substance in the submucosal tissue in patients with hyperparakeratosis caused by longstanding use of

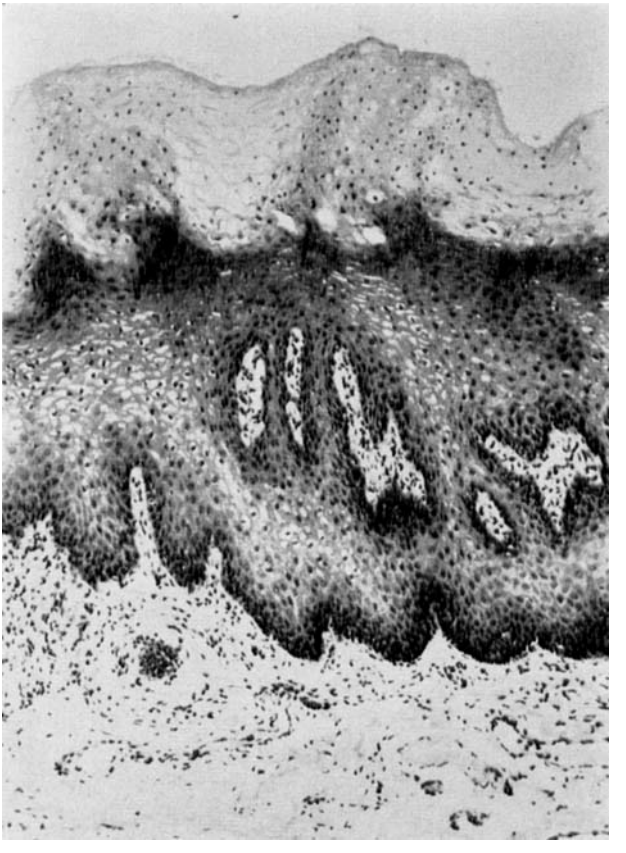

Fig. 6. Photomicrograph of snuff-induced leukoplakia of lower labial mucosa. Note the marked thickness of the epithelium.
Magnification: × 85.

snuff. Such material could not be found in the connective tissue of tobacco chewers.

When tobacco is chewed in a pan and the patient is also a bidi-smoker, the histologic changes show an equal distribution of atrophy and hyperplasia. Hyperorthokeratosis predominates, but in some of these leukoplakias, the granular cell layer is very sparse.

In none of the groups could a relation between length or intensity of habit and the histologic changes be demonstrated. Atrophic epithelium was always associated with hyperorthokeratosis. Hyperplastic epithelium was predominantly associated with hyperparakeratosis.

The findings reported in this paper are not in disagreement with previous histologic descriptions of betel-tobacco induced leukoplakias (*Orr,* 1933, *Balendra,* 1949, *Marsden,* 1960, and *Wahi, Arora, Srivastava, Kehar & Bodkhe,* 1961). However, in these descriptions, the various ways of tobacco consumption were not distinguished as was done in the present investigation. Such a distinction seems necessary in order to single out what type of etiologic agent is the most likely to produce leukoplakias showing potential malignant transformation.

One of the clinical features of malignant transformation is a speckled (nodular) appearance of the leukoplakia, *(Pindborg, Renstrup, Poulsen & Silverman,* 1964). Such a change was observed in three leukoplakias in this series, all located at the labial commissure, two caused by pan with tobacco + bidi-smoking and one by bidi-smoking alone. In all three cases the histologic examination revealed one of the features of epithelial atypia, *viz.,* an increased mitotic activity. Interesting was the finding of high mitotic activity in five non-speckled leukoplakias caused by tobacco-chewing.

The present study suggests that various habits of tobacco consumption, although creating a similar clinical picture of leukoplakia, cause microscopically different changes of the oral epithelium and that the oral epithelium may react differently in different locations. These differences, which may be of significance for our understanding of the precancerous nature of certain leukoplakias, will be examined in detail in future studies by means of larger series of cases for each habit.

SUMMARY

39 biopsies from leukoplakias in 37 East-Indians were examined histologically. The material was divided into four groups according to tobacco-habits. In *bidi-smokers* the epithelium may be atrophic or hyperplastic and the predominating cornification is hyperorthokeratosis. In *cigarette-smokers* the predominating change is hyperplasia of the epithelium associated with a hyperparakeratosis. In *tobacco-chewers* the labial mucosa exhibits hyperplasia with an atypical hyperparakeratosis, sometimes associated with increased mitotic activity, whereas the buccal mucosa shows atrophy and hyperorthokeratosis. In patients *chewing tobacco in pan + smoking bidi,* the epithelium reacts with either atrophy or hyperplasia with hyperorthokeratosis as the most common hypercornification.

RÉSUMÉ

ÉTUDES SUR LES LEUCOPLASIES BUCCALES
VIII. MODIFICATIONS ÉPITHÉLIALES DANS LES LEUCOPLASIES PROVOQUÉES PAR LE TABAC DANS L'INDE

39 biopsies de leucoplasies prélevées sur 37 Indiens ont été soumises à un examen histologique. L'ensemble a été divisé en quatre groupes suivant l'usage du tabac. Chez *les fumeurs de bidi,* l'épithélium peut être atrophique ou hyperplastique, et la transformation cornée prédominante est une hyperorthokératose. Chez *les fumeurs de cigarettes,* l'altération prédominante est l'hyperplasie de l'épithélium associée à une hyperparakératose. Chez *les chiqueurs de tabac,* la muqueuse des lèvres présente une hyperplasie avec une hyperparakératose atypique, associée parfois à une augmentation de l'activité mitotique, tandis que la muqueuse des joues présente une atrophie et une hyperorthokératose. Chez les patients *masticant le tabac dans le bétel + fumant le bidi,* l'épithélium réagit soit par une atrophie, soit par une hyperplasie où l'hyperkératinisation la plus fréquente est une hyperorthokératose.

ZUSAMMENFASSUNG

STUDIEN IN ORALEN LEUKOPLAKIEN

VIII. EPITHELIALE ÄNDERUNGEN IN LEUKOPLAKIEN — VON TABAK VERURSACHT — IN INDIEN

39 Biopsien von Leukoplakien bei 37 Ostindern wurden untersucht. Das Material wurde in vier Gruppen je nach Tabakgewohnheit geteilt.

Bei *Bidi-Rauchern* mag das Epithel atrophisch oder hyperplastisch sein, und die vorherrschende Verhornung ist Hyperorthokeratose.

Bei *Zigaretten-Rauchern* ist die vorwiegende Änderung Hyperplasie des Epithels mit Hyperparakeratose verbunden.

Bei *Tabak-Käuern* zeigt die Labialmucosa Hyperplasie mit atypischer Hyperparakeratose, zuweilen mit vergrösster, mitotischer Aktivität verbunden, wogegen die Bukkalmucosa Atrophie und Hyperorthokeratose zeigt.

Bei Patienten, die *Tabak kauen sowie Bidi rauchen,* reagiert das Epithel entweder mit Atrophie, oder mit Hyperplasie mit Hyperorthokeratose als die meist gewöhnliche Hyperkeratose.

REFERENCES

Balendra, W., 1949: The effect of betel chewing on the dental and oral tissues and its possible relationship to buccal carcinoma. Brit. dent. J. *87:* 83—87.

Marsden, A. T. H., 1960: Betel cancer in Malaya. Med. J. Malaya *14:* 162—165.

Orr, I. M., 1933: Oral cancer in betel nut chewing in Travancore. Lancet *225:* 575—580.

Pindborg, J. J., T. N. Chawla, R. K. Misra, R. K. Nagpaul & V. K. Gupta, 1964: Frequency of oral carcinoma, leukoplakia, leukokeratosis, leukoedema, submucous fibrosis, and lichen planus in 10,000 Indians in Lucknow, Uttar Pradesh, India. Preliminary report. J. dent. Res.: In press.

Pindborg, J. J. & H. E. Poulsen, 1962: Studies in oral leukoplakias I. The influence of snuff upon the connective tissue of the oral mucosa. Preliminary report. Acta path. microbiol. scand. *55:* 412—414.

Pindborg, J. J. & Grete Renstrup, 1963: Studies in oral leukoplakias II. The effect of snuff on the oral epithelium. Acta derm.-venereol. *43:* 271—276.

Pindborg, J. J., Grete Renstrup, H. E. Poulsen & S. Silverman, 1963: Studies in oral leukoplakias. V. Clinical and histologic signs of malignancy. Acta odont. scand. *21:* 407—414.

Wahi, T. N., Arora Shashi, M. C. Srivastava, Usha Kehar & R. R. Bodkhe, 1961: Leukoplakia of oral cavity. Indian J. Path. Bact. *4:* 189—202.

Weinmann, J. P. & Julia Meyer, 1959: Types of keratinization in the human gingiva. J. invest. Derm. *32:* 87—93.