SUBMUCOUS FIBROSIS OF THE ORAL CAVITY

HISTOMORPHOLOGICAL STUDIES

P. N. WAHI, USHA K. LUTHRA AND V. L. KAPUR

From the Department of Pathology, S.N. Medical College, Agra, India

Received for publication May 27, 1966

CLINICAL, epidemiological and cytomorphological studies in cases of submucous fibrosis have been the subject of separate communications (Wahi *et al.*, 1966a, b, c, unpublished). The majority of the studies (Sharan, 1959; Rao, 1962; Sirsat and Khanolkar, 1957, 1962) have described the histological changes of the subepithelial tissue in submucous fibrosis. Some have more comprehensively dealt with the histochemical and electron microscopic studies of this condition (Sirsat and Khanolkar, 1957, 1960, 1962). Only an occasional report in the literature (Pindborg *et al.*, 1965) describes the epithelial changes in detail in cases of submucous fibrosis.

None of the studies has dealt with the histological changes in cases of submucous fibrosis relating them to the severity and the extent of involvement of the lesion, i.e. according to the clinical groups of these cases (Wahi *et al.*, 1966a, unpublished). Factors concerned in the aetogenesis of submucous fibrosis are not clearly understood. The use of tobacco, with associated mechanical or thermal trauma, acting in conjunction with vitamin deficiencies, is considered to play an important role in the development of the lesion (Wahi *et al.*, 1966b, unpublished). The present paper deals with the detailed histomorphological features in 104 cases of submucous fibrosis in relation to the severity and the extent of involvement of the lesion, and according to the tobacco consumption habits.

MATERIAL AND METHODS

A total of 104 punch biopsies were taken under local anaesthesia. According to the site of involvement 53 biopsies were taken from the palate, 46 from the buccal mucosa, 3 from the tongue, and 1 each from gingivae and lip.

All biopsies were fixed in 10 per cent formol saline, embedded in paraffin and cut in serial sections. Sections were stained with haematoxylin and eosin, a modified Mallory's connective tissue stain (Weinmann and Meyer, 1959), periodic acid Schiff reagent and toluidine blue stain. Sections were evaluated in detail for changes in the epithelium, juxta-epidermal region and the corium.

OBSERVATIONS

Detailed histomorphological studies are presented in 104 cases of submucous fibrosis. Histochemical studies were carried out in some of these cases. Histomorphological changes in cases of submucous fibrosis have been dealt under the following headings:

- (1) *Changes in epithelium*
- (2) *Changes in subepithelial tissue*

Changes in epithelium (see Table I).

The majority of cases show epithelial hyperplasia (Fig. 1), though rarely one may find normal or atrophic epithelium (Fig. 2). Of the 31 cases of submucous fibrosis in clinical group I, 28 cases (90.4 per cent) show hyperplasia, 2 cases (6.4 per cent) atypical epithelial hyperplasia (Fig. 3 and 4) and 1 case (3.2 per cent) atrophic changes. Somewhat similar observations are seen in 60 cases of clinical group II. Here 55 cases (91.6 per cent) show hyperplasia, 4 cases (6.6 per cent) atypical epithelial hyperplasia and 1 case (1.6 per cent) a normal epithelium. However, striking differences in the epithelial changes are observed in group III cases; of the 13 cases encountered the epithelium shows atypical epithelial hyperplasia in 9 cases (69.2 per cent) and the remaining 4 cases (30.7 per cent) show hyperplasia. Four cases of submucous hyperplasia were associated with epidermoid carcinoma; in the areas of submucous fibrosis the epithelium shows atypical epithelial hyperplasia in all these cases.

Interesting observations are discerned on studying the epithelial changes in relation to the tobacco and betel chewing habit of the patients (see Table II). Of the 22 cases with no habit, 20 cases (91.0 per cent) show epithelial hyperplasia and the other 2 show normal or atrophic epithelium. In none of these cases does the epithelium show atypical epithelial hyperplasia. Amongst the 6 cases using betel nuts and betel leaf, 5 cases (83.4 per cent) show epithelial hyperplasia and 1 case atypical epithelial hyperplasia. On the other hand patients using tobacco, as cigarette smoking or tobacco chewing or both habits, showed a gradual decrease in the number of cases showing epithelial hyperplasia, and an apparent increase in the number of cases showing atypical epithelial hyperplasia (Table II). All the 4 cases of submucous fibrosis associated with epidermoid carcinoma were tobacco users, 3 had the combined habit of chewing and smoking, and one only chewed.

Considering the epithelial changes in the 28 non-tobacco users, 25 cases (89.2 per cent) show epithelial hyperplasia, 1 case (3.6 per cent) atypical epithelial hyperplasia and 1 case (3.6 per cent) each of atrophic and normal epithelium. However, of the total 76 cases using tobacco in one form or the other, 62 cases (81.6 per cent) show epithelial hyperplasia and 14 cases (18.4 per cent) atypical hyperplasia (Table III). Atypical epithelial hyperplasia is thus an important feature in cases of submucous fibrosis using tobacco as compared to the cases which do not use tobacco.

The squamous epithelium in all the cases of submucous fibrosis shows keratinizing metaplasia—orthokeratosis (Fig. 5) (40 cases), parakeratosis (Fig. 6) (45 cases), or both ortho- or parakeratosis (19 cases). No distinct difference is observed in the type of keratinization in the various clinical groups of cases of submucous fibrosis.

However, interesting observations are discerned when the type of keratinization is correlated with the site of the lesion and the habit of the patients. The two commonest sites involved by submucous fibrosis are the palate and the buccal mucosa (Wahi *et al.*, 1966a, unpublished). The palate is predominantly involved in 53 cases, and buccal mucosa in 46 cases. Of the 53 cases with palatal involvement, 22 cases (41.5 per cent) show orthokeratosis with or without hyperplasia, 11 cases (20.7 per cent) parakeratosis with or without hyperplasia, and the remaining cases a combination of these two types of keratinization. Of the 46 cases involving buccal mucosa, 20 cases (45.2 per cent) show parakeratosis with

or without hyperplasia, 10 cases (21.7 per cent) orthokeratosis with or without hyperplasia, and the remaining cases a combination of these two types of keratinization.

It is thus seen that lesions of palate predominantly showed orthokeratosis, and those of buccal mucosa parakeratosis. The frequent clinical recognition of lesions involving the palate (Wahi *et al.*, 1966a, unpublished) may be aided to some extent by the nature of keratinization involving the different sites of lesion. Of the 4 cases of submucous fibrosis associated with epidermoid carcinoma the epithelium was parakeratotic in 3, and showed a combination of parakeratotic and orthokeratotic epithelium in the remaining one.

The type of keratinization according to habit in cases of submucous fibrosis is shown in Tables II and III. Orthokeratotic epithelium predominates in non-tobacco users, i.e. 17 cases (60.8 per cent) and parakeratotic epithelium in tobacco users, i.e. 39 cases (51.4 per cent).

The rete pegs are more frequently pointed as the cases advance from group I to group III (41.9 per cent to 77.7 per cent) (Table I, Fig. 7). Moreover, pointed rete pegs are also more frequently seen in tobacco users (72.3 per cent) than in non-tobacco users (46.4 per cent) (Table III). Stratum granulosum is either distinctly visualized or is patchy or completely absent; it is more frequently patchy or absent in tobacco users than in non-tobacco users (Table III).

Spongiosis is more frequently observed in cases using tobacco, and comparatively less frequently in non-tobacco users (Tables II and III).

The basement membrane is discernible in the majority of the group I and group II cases, whereas it is focally indistinct in a fair number of group III cases (Table I). Interestingly the basement membrane is distinctly discernible in all the non-tobacco user cases, whereas it is indistinct in 73.8 per cent of tobacco users.

The number of mitotic figures were counted per 10 high power fields in cases belonging to various clinical groups (Table I), and also in cases according to habit (Table II and III). In group I cases the maximum number of cases, 19 (61.3 per cent), reveal 3-4 mitoses/10 high power field and none shows figures above 8 mitoses per 10 high power field, whereas in group II the maximum number of cases, 26 (43.3 per cent), show 5 to 6 mitoses per high power field and 2 cases

EXPLANATION OF PLATES

- FIG. 1.—Epithelium showing hyperplasia. H. & E. $\times 85$.
 FIG. 2.—Epithelium showing atrophy. H. & E. $\times 360$.
 FIG. 3.—Epithelium showing atypical epithelial hyperplasia. H. & E. $\times 85$.
 FIG. 4.—Epithelium showing atypical epithelial hyperplasia. H. & E. $\times 360$.
 FIG. 5.—Epithelium showing orthokeratosis. H. & E. $\times 85$.
 FIG. 6.—Epithelium showing parakeratosis. H. & E. $\times 85$.
 FIG. 7.—Epithelium showing blunt and pointed rete pegs. H. & E. $\times 360$.
 FIG. 8.—Epithelium showing parakeratosis, rete pegs predominantly blunt. Juxta-epithelial tissue and superficial corium showing dense hyalinization fibrosis and constriction of blood vessels. H. & E. $\times 85$.
 FIG. 9.—Juxta-epithelial tissue and superficial corium showing dense and fibrillar connective tissue. H. & E. $\times 85$.
 FIG. 10.—Juxta-epithelial tissue and superficial corium showing dense hyalinization, fibrosis and constriction of blood vessels with slight endothelial cell proliferation. H. & E. $\times 360$.
 FIG. 11.—Juxta-epithelial tissue and superficial corium with fibrillar connective tissue showing dilated and congested blood vessels. An occasional blood vessel shows slight medial thickening. H. & E. $\times 360$.

5

6

7

8

9

10

11

(3.3 per cent) above 8 mitoses per 10 high power field. However, in group III although the maximum number of 6 cases (46.1 per cent) show 3-4 mitoses per 10 high power field, a considerable number, 3 (23.0 per cent) of the total 13, show mitoses above 8 per 10 high power field (Table I). In the 4 cases of submucous fibrosis associated with epidermoid carcinoma the mitotic count was between 6 and 13 per 10 high power field.

Considering the number of mitotic figures according to habit, a higher mitotic figure count is found in tobacco users than in non-tobacco users. Of the tobacco users, higher mitotic counts (above 8 per 10 high power field) are more frequently encountered in patients with the combined chewing and smoking habits.

Changes in subepithelial tissue

In most of the cases of submucous fibrosis, the juxta-epithelial tissue shows dense hyalinization and fibrosis (Fig. 8). There is a distinct increase in the frequency and severity of this observation from cases of clinical group I to group III. It is observed in 12 cases (38.7 per cent) of group I, 43 cases (71.6 per cent) of group II and in 12 cases (92.3 per cent) of group III cases. The remaining cases show combination of the other types of juxta-epithelial tissue—i.e. dense and fibrillar (Fig. 9), loose and fibrillar and loose and hyalinized. In all the 4 cases of submucous fibrosis associated with epidermoid carcinoma the juxta-epithelial tissue is dense and hyalinized. According to the habit, dense and hyalinized type of juxta-epithelial tissue is present in 12 cases, i.e. 92.3 per cent of cases with the habit of tobacco chewing and smoking, and in 12 cases, i.e. 75.0 per cent of those with the habit of chewing alone (Table IV).

In the majority of cases the blood vessels in juxta-epidermal connective tissue are constricted (Fig. 10). This observation is seen in increasing frequency from group I to group III cases (Table V). This feature is also commoner in tobacco users, i.e. 59 of 76 cases (77.7 per cent) than in non-tobacco users, i.e. 11 of 28 cases (39.2 per cent) (Table VI). In the remaining few cases the blood vessels are either dilated (Fig. 11) or normal. In all the 4 cases of submucous fibrosis associated with epidermoid carcinoma, there is marked constriction of the blood vessels in the juxta-epithelial connective tissue. Inflammation of the juxta-epidermal connective tissue was a rare feature in cases of submucous fibrosis.

Toluidine blue metachromasia was studied in a total of 58 cases of submucous fibrosis. The results of the reactions are expressed as negative, slight metachromasia $\pm$, moderate metachromasia $+$, and marked metachromasia $++$. The maximum number of group I cases, 10 (45.4 per cent), gave a negative reaction, whereas the maximum number of group III cases, 4 (50.0 per cent) gave a markedly positive metochromasia (Table VII). All 4 cases of submucous fibrosis associated with epidermoid carcinoma gave a markedly positive toluidine blue metachromasia.

Interestingly, 9 cases (90.0 per cent) of submucous fibrosis with no habit, and all 4 cases using betal nut leaf gave a negative reaction. On the other hand, the metachromasia increased in severity and frequency from tobacco smokers to tobacco chewers being maximum in those with the combined habit of chewing and smoking (Table VIII). Histochemical tissue staining reactions for maturation of the collagen were carried out in some cases. There is greater evidence of maturation defect of connective tissue in cases of clinical group III, than in cases of clinical groups I and II. Moreover, the non-tobacco users did not show any

TABLE I.—*Epithelial Changes in Cases of Submucous Fibrosis in the Various Clinical Groups (Expressed as Percentage of the Number of Cases)*

Clinical groups	No. of cases	Epithelium										Mitosis/10 H.P. Field								
		Rete pegs																		
		Normal	Hypertlasia	Atypical epithelial hypertlasia	Atrophy	Pointed	Blunt	Flat	Orthokeratosis	Parakeratosis	Ortho- and para-keratosis	Stratum granulosum present	Stratum granulosum patchy	Stratum granulosum absent	Spongiosis	Pigment	Basement membrane distinct H. & E.	Basement membrane indistinct H. & E.		
I	31	90.4	6.4	—	3.2	41.9	49.4	9.7	58.1	35.5	6.4	51.6	19.4	29.3	61.3	35.3	93.6	6.4	6.4	—
II	60	91.6	6.6	—	—	75.0	18.4	6.6	28.3	50.0	21.6	25.0	28.3	30.0	43.4	20.0	93.4	6.6	—	7-8
III	13	30.7	69.2	—	—	77.1	15.3	7.6	38.4	30.7	30.7	38.4	38.4	23.0	69.1	61.5	53.9	46.1	—	Above 8

TABLE II.—*Epithelial Changes in Cases of Submucous Fibrosis According to Habit (Expressed as Percentage of the Number of Cases)*

Habit	No. of cases	Epithelium										Mitosis/10 H.P. Field												
		Rete pegs																						
		Normal	Hyperplasia	Atypical epithelial hyperplasia	Atrophy	Pointed	Blunt	Flat	Orthokeratosis	Parakeratosis	Ortho- and para-keratosis	Stratum granulosum present	Stratum granulosum patchy	Stratum granulosum absent	Spongiosis	Pigment	Basement membrane distinct H. & E.	Basement membrane indistinct H. & E.						
No habit	22	4.5	91.0	—	4.5	45.4	45.4	9.1	63.7	22.7	13.6	50.0	22.7	27.3	22.7	22.7	9.1	100.0	—	—	—	—	—	—
Betal nut and betel leaf	6	—	83.4	—	—	50.0	16.6	38.4	50.0	16.6	33.4	50.0	16.6	33.4	16.6	83.4	100.0	—	—	—	—	—	—	—
Tobacco smoking	47	—	91.7	8.3	—	68.1	25.5	6.3	25.5	63.9	10.6	29.8	31.8	38.4	76.6	34.0	95.8	4.2	—	—	—	—	—	—
Tobacco chewing	16	—	75.0	25.0	—	75.0	18.7	6.2	31.2	31.2	37.5	31.2	18.7	50.0	37.5	18.7	68.8	31.2	—	—	—	—	—	—
Tobacco chewing and smoking	13	—	46.2	53.9	—	84.7	15.3	—	46.2	30.7	23.1	23.1	30.7	46.2	53.7	38.4	61.6	38.4	—	—	—	—	—	—
																				0.2	3.4	5.6	7.8	Above 8
																				16.6	66.6	16.6	—	—
																				77.2	22.7	—	—	—
																				—	—	—	—	—
																				2.1	19.3	63.8	12.7	—
																				50.0	6.2	37.5	6.2	—
																				38.4	7.6	30.7	23.1	—

TABLE III.—*Epithelial Changes in Non-Tobacco Users and Tobacco Users in Cases of Submucous Fibrosis (Expressed as Percentage of the Number of Cases)*

	No. of cases	Epithelium										Mitosis/10 H.P. Field							
		Rete pegs																	
Habit		Normal	Hyperplasia	Atypical epithelial	Atrophy	Pointed	Blunt	Flat	Orthokeratosis	Parakeratosis	Ortho- and para-keratosis	Stratum granulosum present	Stratum granulosum patchy	Stratum granulosum absent	Spongiosis	Pigment	Pigment membrane distinct H. & E.	Pigment membrane indistinct H. & E.	
Non-tobacco users	28	3.6	89.2	3.6	3.6	46.4	39.2	14.4	60.8	21.4	17.8	50.0	21.4	28.6	21.4	25.0	100.0	—	Above 8
Tobacco users	76	—	81.6	18.4	—	72.3	22.3	5.2	30.2	51.4	18.4	28.9	28.9	42.2	64.4	42.2	26.2	73.8	5.2

TABLE IV.—*Subepithelial Changes in Cases of Submucous Fibrosis According to Habit (Expressed as Percentage of the Number of Cases)*

	No. of cases	Juxta-epithelial tissue										Deeper corium									
		Dense and hyalinized	Loose and fibrillar	Dense and fibrillar	Loose and hyalinized	Blood vessels normal	Blood vessels constricted	Blood vessels dilated	Inflammation +	Pigment +	Deeper corium present—number of cases	Dense and hyalinized	Loose and fibrillar	Dense and fibrillar	Loose and hyalinized	Blood vessels normal	Blood vessels constricted	Blood vessels dilated	Inflammation	Pigment +	
Habit																					
No habit	22	59.1	18.2	22.7	—	63.7	31.8	4.5	9.1	4.5	19	15.7	36.8	47.5	68.4	15.8	—	15.8	—	—	
Betal nuts and betel leaf	6	50.0	33.4	16.6	—	16.6	66.6	16.6	50.0	33.4	2	—	50.0	50.0	100.0	—	—	—	50.0	—	
Tobacco smoking	47	59.5	19.3	12.7	8.5	10.6	78.7	10.6	8.5	12.7	42	40.5	28.3	32.2	54.7	38.7	6.6	6.6	4.7	—	
Tobacco chewing	16	75.0	—	18.7	6.2	6.2	76.0	18.7	25.0	6.2	10	60.0	30.0	30.0	20.0	50.0	30.0	50.0	50.0	—	
Tobacco chewing and smoking	13	92.3	7.6	—	—	15.3	77.1	7.6	23.1	7.6	9	88.8	11.2	11.2	11.2	77.6	11.2	11.2	22.4	—	

TABLE V.—Subepithelial Changes in Cases of Submucous Fibrosis in the Various Clinical Groups
(Expressed as Percentage of the Number of Cases)

Clinical group	No. of cases	Juxta-epithelial tissue										Deeper corium present—number of cases	Deeper corium									
		Dense and hyalinized	Loose and fibrillar	Dense and fibrillar	Loose and hyalinized	Blood vessels normal	Blood vessels constricted	Blood vessels dilated	Inflammation +	Pigment +	Dense and hyalinized		Loose and fibrillar	Dense and fibrillar	Loose and hyalinized	Blood vessels normal	Blood vessels constricted	Blood vessels dilated	Inflammation +	Pigment +		
I	31	38.7	35.3	19.4	6.4	45.1	45.1	9.7	16.1	9.7	24	29.2	12.6	58.2	79.2	3.3	12.5	12.5	12.5	12.5		
II	60	71.6	10.0	15.0	3.3	15.0	75.0	10.0	16.6	10.0	49	42.8	34.8	33.4	48.9	40.9	10.2	12.2	12.2	12.2		
III	13	92.3	—	—	7.6	—	84.6	15.3	7.6	15.3	9	66.6	—	—	—	22.4	55.6	22.2	22.2	22.2		

TABLE VI.—Subepithelial Changes in Non-Tobacco User and Tobacco User in Cases of Submucous Fibrosis
(Expressed as Percentage of the Number of Cases)

Habit	No. of cases	Juxta-epithelial tissue										Deeper corium present—number of cases	Deeper corium									
		Dense and hyalinized	Loose and fibrillar	Dense and fibrillar	Loose and hyalinized	Blood vessels normal	Blood vessels constricted	Blood vessels dilated	Inflammation +	Pigment +	Dense and hyalinized		Loose and fibrillar	Dense and fibrillar	Loose and hyalinized	Blood vessels normal	Blood vessels constricted	Blood vessels dilated	Inflammation +	Pigment +		
Non-tobacco user	28	59.2	21.4	21.4	—	53.6	39.2	7.2	17.8	10.7	21	14.2	38.1	47.7	71.4	14.3	14.3	14.3	4.8	—		
Tobacco user	76	68.4	13.3	11.8	6.5	10.5	77.7	11.8	14.4	10.5	61	50.4	24.5	24.5	42.6	45.9	11.5	11.5	10.9	—		

TABLE VII.—*Toluidine Blue Metachromasia in Cases of Submucous Fibrosis According to the Clinical Group.*

Group	Total No. of cases	Reaction					
		- ve		±		+	
		No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage
I	22	10	45.4	5	22.7	6	27.2
II	28	7	25.0	4	14.3	14	50.0
III	8	1	12.5	1	12.5	2	25.0
						1	4.5
						3	10.7
						4	50.0

TABLE VIII.—*Toluidine Blue Metachromasia in Cases of Submucous Fibrosis According to Habit*

Habit	No. of cases	- ve		±		+		++	
		No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage
No habit	10	9	90.0	1	10.0	—	—	—	—
Betal nut and betal leaf	4	4	100.0	—	—	—	—	—	—
Tobacco smoking	25	4	16.0	6	24.0	14	56.0	1	4.0
Tobacco chewing	10	1	10.0	2	20.0	6	60.0	1	10.0
Tobacco chewing and smoking	9	—	—	2	20.0	1	11.1	6	66.7

defect of connective tissue maturation whereas this was more often observed in tobacco users.

Similar histologic and histochemical observations were seen in the deeper corium although of less severity.

COMMENTS

In most of our cases the epithelium showed hyperplasia and less frequently atrophy. Sirsat and Khanolkar (1957, 1962) also described a thickened epithelium with deep invagination into subjacent lamina propria. Sharan (1959) described hypertrophy with occasional areas of atrophy. In a large percentage of our cases (69.2 per cent) of clinical group III, the epithelium showed atypical epithelial hyperplasia (Table I). The epithelium in all the 4 cases of submucous fibrosis associated with epidermoid carcinoma showed atypical epithelial hyperplasia. Cellular atypism has been described in the epithelium by Desa (1957), although this was not correlated with the severity or extent of the lesion.

Pindborg *et al.* (1964) on the other hand, described atrophy of the epithelium as the predominant feature. Although epithelial atrophy was an infrequent finding in our cases, it is felt that it may follow hyperplasia as in skin carcinogenesis where proliferative epithelial changes precede atrophy (Stewart, 1959).

In our cases using tobacco smoking, chewing or both habits in this order, there was a greater frequency of atypical epithelium compared to the non-tobacco users (Table II). Tobacco tar paintings on the skin have been described to induce progressive epithelial hyperplasia followed by areas of cellular atypism (Reddy, Reddy and Rao, 1960). This observation helps in the understanding of the high frequency of atypical epithelial hyperplasia in cases with the tobacco habit as compared to non-tobacco users.

The more advanced clinical cases had an increased frequency and severity of epithelial hyperplasia and atypism. Various views have been put forward as to the development of epithelial changes. The alterations in connective tissue are described to precede the development of epitheliomas in sailors and ranchers exposed to intense sunlight excessively (Stewart, 1959). It has been suggested that stromal changes occur in preneoplastic sites that affect the epithelium by interference with metabolic exchange, so that the epithelium remains viable but deprived of essential organisational control (Orr, 1963). Further, altered connective tissue is thought to be responsible for epithelial hyperplasia and invasiveness by liberation of hyaluronic acid from degenerate connective tissue or ground substance (Gillman and Roux, 1955). Varoni (1951) postulated that the products of degeneration have growth promoting properties.

Interesting observations were discerned when the type of keratinizing metaplasia was correlated with the site of the lesion and the habit of the patients. Lesions involving the palate predominantly showed orthokeratosis, and those of buccal mucosa parakeratosis. The frequent clinical recognition of the lesions involving the palate (Wahi *et al.* 1966a, unpublished) may be facilitated to some extent by the nature of keratinizing metaplasia, i.e. orthokeratosis.

A purely orthokeratotic type of epithelium was predominantly seen in non-tobacco users in contrast to purely parakeratotic type of epithelium in tobacco users. A parakeratotic type of epithelium was seen in 3 of our cases of submucous fibrosis associated with carcinoma and a combination of para and orthokeratotic

types of epithelium in the fourth case. The type of keratinizing metaplasia in relation to the habit has not been reported by any worker in cases of submucous fibrosis. Pindborg *et al.* (1965) describe keratinization in less than 50 per cent of cases of submucous fibrosis, with a high frequency of orthokeratotic and hyperorthokeratotic type of change.

A high mitotic count in parakeratotic epithelium as compared to orthokeratotic epithelium and also an association of some parakeratotic leukoplakia with epithelial atypia, carcinoma *in situ* or invasive carcinoma has been reported by Renstrup (1963). Pindborg and Renstrup (1963) have described hyperplasia and parakeratosis of epithelium in snuff induced leukoplakia. Acanthosis and hyperparakeratosis of palatal epithelium have been described in pipe smokers by Chapman and Redish (1960). It is interesting to observe that the parakeratotic or hyperparakeratotic epithelium has been correlated with tobacco use in one way or another. Further, the association or co-existence of this type of epithelium to atypia and neoplasia is more frequently recognized.

The stratum granulosum was more frequently patchy or absent in tobacco users than in non-tobacco users (Table III). The stratum granulosum was seen in areas of orthokeratotic epithelium, while it was absent or indistinct in the parakeratotic type of epithelium. Spongiosis of the stratum malpighi was frequently observed in tobacco smokers while it was a rare feature in non-tobacco users (Table III).

The rete pegs were more frequently pointed as one considered cases from group I to group III. Moreover, they were more frequently pointed in tobacco users (Table III). Deep invagination of epithelium into subjacent lamina propria in cases of submucous fibrosis has been described by Sirsat and Khanolkar (1962). Liquefaction in basal cell layer was not observed in our cases as reported by Sharan (1959) and Pindborg *et al.* (1965).

Basement membrane was distinctly discernible in a majority of the group I and group II cases, and focally indistinct in a fair number (46.1 per cent) of group III cases (Table I). Interestingly, the basement membrane was distinctly discernible in all non-tobacco users whereas, it was indistinct in 12 cases, i.e. 15.8 per cent of tobacco users. Loss of sharp definition of the basement membrane has been described in leukoplakia of the palate in women smoking chutta (Reddy and Rao, 1957). Stewart (1959) described the ability of proliferating epithelial cells during skin carcinogenesis to dissolve the basement membrane.

It has been observed in recent studies (Ashworth, Stembridge and Leibel, 1961) on the basement membrane of cervix in intraepithelial and invasive carcinoma that the basement membrane visualized by electron microscopy is a true membrane and the cytoplasmic penetration during invasion occurs only at the sites where the basement membrane is defective. It has been suggested that malignant cells in some way mechanically disrupt or destroy the membrane or they produce a metabolite which may depolymerize or otherwise destroy the basement membrane, or the connective tissue may fail in the perpetual reconstruction of basement membrane.

The number of mitotic figures in the epithelium increased from cases of clinical group I to clinical group III and reached a maximum in 4 cases of submucous fibrosis which were associated with epidermoid carcinoma, i.e. 6 to 13 per 10 high power fields. Considering the number of mitotic figures according to the habit and the type of keratinization, these were more frequent in tobacco users who

predominantly showed a parakeratotic epithelium in contrast to non-tobacco users who frequently revealed an orthokeratotic type of epithelium. High mitotic activity in parakeratotic epithelium as compared to orthokeratotic type of epithelium has been reported in cases of leukoplakia (Renstrup, 1963). Reddy *et al.* (1960) reported definite evidence of increased mitotic and amitotic division in interscapular skin of mice treated with tobacco tar and heat, suggesting the influence of these two factors in causing increased and rapid epithelial proliferation.

Striking alterations were discerned in the juxta-epithelial tissue and less so in the deeper corium. In most of the cases of submucous fibrosis, the juxta-epithelial tissue showed dense hyalinization and fibrosis and only infrequently a loose fibrillar type of subepithelial tissue.

Connective tissue changes in cases of submucous fibrosis have been variably interpreted. Sirsat and Khanolkar (1957 and 1962), Sharan (1959), and Rao (1962) have described marked increase in dense collagen in the sub-epithelial tissue. Sirsat and Khanolkar (1957 and 1962) and Sharan (1959) have also described hyalinization of the connective tissue. The changes in connective tissue have been interpreted as fibrinoid degeneration by Sharan (1959) and elastotic degeneration by Sirsat and Khanolkar (1962). Rao (1962), on the other hand, did not find elastotic or fibrinoid degeneration.

A distinct increase in the frequency of dense hyalinized juxta-epithelial tissue was observed in cases from group I to group III. The majority of the patients with the habit of tobacco chewing and smoking and of tobacco chewing alone showed dense and hyalinized type of juxta-epithelial tissue.

In the majority of cases of submucous fibrosis, the blood vessels in the juxta-epithelial connective tissue were constricted. Interestingly, the number of constricted blood vessels in the juxta-epithelial connective tissue was more in group III cases than in group I cases. This feature was also more common in tobacco users as compared to non-tobacco users.

The majority of the cases of clinical group III and those with the habit of tobacco chewing and smoking gave a markedly positive metachromasia with alcoholic toluidine blue indicative of acid mucopolysaccharides (Pearse, 1960). An increase in the mucopolysaccharide content of connective tissue in general has been reported in guinea-pigs after inhalation of tobacco smoke (Lupu and Velikan, 1962). Accumulation of mucopolysaccharides in the dermis in carcinogen treated rabbits has been described by Maltoni and Prodi (1960).

It is suggested that the connective tissue changes may precede any epithelial anomaly or may be concomitant. The epithelial and connective tissue changes seem to depend on the combined effect of tobacco on tissues preconditioned by vitamin deficiencies (Wahi *et al.*, 1966*b*, unpublished). However, the epithelial changes may be aggravated by the abnormalities of underlying connective tissue and blood vessels, which may act by interference with metabolic exchange or the direct effect of the products of degeneration or altered metabolism. The products of degeneration have been considered to have growth promoting properties (Varoni, 1951), and thus the epithelial changes may be secondary to the connective tissue changes.

SUMMARY

A detailed histomorphological study in 104 cases of submucous fibrosis is described. These observations are presented with special reference to the severity

and extent of the lesion and the habits (tobacco smoking, tobacco and betel nut chewing) of the patients. A close correlation was found between the clinical severity of the cases and the histopathological changes in the biopsy material. Patients using tobacco in one form or the other i.e. smoking, chewing or both, showed more marked histopathological changes. Four cases of submucous fibrosis were associated with epidermoid carcinoma.

REFERENCES

- ASHWORTH, C. T., STEMBRIDGE, V. A. AND LEIBEL, F. N.—(1961) *Acta cytol.*, **5**, 369.
 CHAPMAN, I. AND REDISH, C. H.—(1960) *A.M.A. Archs Path.*, **70**, 133.
 DESA, J. V.—(1957) *Ann. Otol. Rhinol. Lar.*, **66**, 1143.
 GILLMAN, T., PENN, J., BRONKS, D. AND ROUX, M.—(1954) *Nature, Lond.*, **174**, 789.
 GILLMAN, T. AND ROUX, M.—(1955) *A.M.A. Archs Path.*, **59**, 733.
 LUPU, N. G. AND VELIKAN, K.—(1962) *Ark. Patol.*, **24**, 2.
 MALTONI, C. AND PRODI, G.—(1960) in 'Recent contribution to Cancer research in Italy' edited by Bucalossi, P. and Veronesi, U., Milan (Casa editrice ambrosiana) Vol. 1, p. 49.
 ORR, J. W.—(1963) in 'Conference on biology of cutaneous cancer' *Natn. Cancer Inst. Monogr.* No. 10, edited by Urbach, F., Bethesda, 14, Maryland (U.S. Department of Health, Educational and Welfare) p. 531.
 PEARSE, A. G. E.—(1960) 'Histochemistry—Theoretical and applied', 2nd. edition, London (Churchill).
 PINDBORG, J. J., CHAWLA, T. N., SHRIVASTAVA, A. N. AND DAYA GUPTA—(1964) *Acta odont. scand.*, **22**, 679.—(1965) *Acta odont. scand.*, **23**, 277.
 PINDBORG, J. J. AND RENSTRUP, G.—(1963) *Acta dermat.-vener.*, **43**, 271.
 RAO, A. B. M.—(1962) *Br. J. Surg.*, **50**, 23.
 REDDY, D. G. AND RAO, V. K.—(1957) *Indian J. med. Sci.*, **11**, 791.
 REDDY, D. G., REDDY, D. B. AND RAO, P. R.—(1960) *Cancer, N.Y.*, **13**, 263.
 RENSTRUP, G.—(1963) *Acta odont. scand.*, **4**, 333.
 SHARAN, J.—(1959) *Indian J. Path. Bact.*, **2**, 150.
 SIRSAT, S. M. AND KHANOLKAR, V. R.—(1957) *J. Path. Bact.*, **73**, 439.—(1960) *J. Path. Bact.*, **79**, 53.—(1962) *Indian J. med. Sci.*, **16**, 189.
 STEWART, H. L.—(1959) in 'The physiopathology of Cancer', 2nd edition, edited by Homberger, F., New York (Hoeber & Harper) p. 3.
 VARONI, C. L.—(1951) *Scientia med. ital.*, **2**, 273.
 WEINMANN, J. P. AND MEYER, J.—(1959) *J. invest. Derm.*, **32**, 87.