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DENTURE STOMATITIS
III. HISTOPATHOLOGY OF TRAUMA- AND CANDIDA-INDUCED INFLAMMATORY LESIONS OF THE PALATAL MUCOSA

by
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INTRODUCTION

It is generally accepted that the non-denture-covered palatal mucosa is lined with a well developed stratum corneum (*Östlund*, 1953; *Al-Ami et al.*, 1966; *Bowman and Latham*, 1968). *Östlund* (1958), studying the clinically normal denture-covered palatal mucosa on the basis of a histological examination of 118 patients, found that the keratinization of the palatal mucosa diminished in connection with the wearing of dentures. On the other hand, *Kapur and Shklar* (1963) and *Vergeat* (1966) found that well-fitting dentures might have a stimulating effect on the palatal mucosa and result in hyperorthokeratosis.

Trauma (*Nyquist*, 1952) and infection with Candida albicans (*Cahn*, 1936; *Cawson*, 1963) may give rise to inflammatory reactions of the palatal mucosa. According to *Brinch* (1932), *Östlund* (1958) and *Vergeat* (1966) the histological reactions to trauma are parakeratosis or even absence of keratin, achantosis, epithelial proliferation, and chronic subepithelial inflammation. The histopathology of the candida-induced denture stomatitis has been studied only in a few cases (*Cahn*, 1936; *Cawson*, 1966), the main features found being chronic inflammation, intra-epithelial edema and extreme epithelial atrophy. Epithelial invasion by hyphae was not seen. On the other

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hand, denturewearers with thrush did show candida invasion and heavy leucocyte migration (*Cawson*, 1964).

It is generally accepted that the soft connective tissue nodules shown in the granular type of denture stomatitis (inflammatory papillary hyperplasia) are initiated by trauma (*Nyquist*, 1952; *Östlund*, 1958; *Guernsey*, 1965; *Cawson*, 1966), candida infection eventually being a complicating factor (*Neill*, 1965; *Turrell*, 1966). *Cawson* (1966), found invasion of the epithelial surface by hyphal structurs only in a minority of these lesions, and *Flanagan* (1968), using P.A.S. — stain for intra-epithelial localization of glycogen in cases of granular inflammation, did not report on the presence of hyphae.

Recently, *Budtz-Jørgensen and Bertram* (1970a), found that the localized simple inflammation of the maxillary denture-bearing mucosa was caused by trauma, whereas the generalized simple and the granular inflammation more readily could be ascribed to candida infection, as hyphal structures were isolated in palatal smears consistently only among the latter. Furthermore, an anti-inflammatory effect of antifungal therapy was recognized among cases of generalized, simple and granular inflammation and not among cases of localized, simple inflammation, (*Budtz-Jørgensen and Bertram*, 1970b). The decrease in inflammation did not seem to depend on whether single hyphae or mycelium were isolated in pre-treatment palatal smears. Yet, it was significant that an effect of antifungal therapy was seen primarily in patients, who showed moderate or profuse growth of yeasts by cultivation according to the impression method described.

The present investigation was carried out for the following reasons:

1) to study the cytological and histological reactions of the palatal mucosa partly to trauma, partly to infection with Candida albicans.

2) to study the cytological and histological changes of the palatal mucosa following partly antifungal therapy (Mycostatin SQUIBB)®, partly prosthetic treatment.

MATERIAL AND METHODS

The material comprised 64 patients with full dentures and denture stomatitis and 58 controls with full dentures, but without inflammatory changes of the palatal mucosa, evaluated clinically. The distribution according to sex and age is recorded in Table 1.

Except for 6 patients in the denture stomatitis group all patients had previously been investigated for yeast-like fungi by cultivation and by microscopical examination P.A.S.-stained palatal smears, qualitatively as well as quantitatively, (*Budtz-Jørgensen and Bertram* 1970, a and b).

Table I.

Patient distribution according to sex and age

		Age of patients							
		25—34	35—44	45—54	55—64	65—74	75—84	Total	Mean age
Denture stomatitis group	Women	3	4	10	18	8	2	45	57,2
	Men			6	6	6	1	19	58,9
	Total	3	4	16	24	14	3	64	57,6
Control group	Women	2	1	9	16	11	1	40	59,0
	Men		2	3	7	5	1	18	59,6
	Total	2	3	12	23	16	2	58	59,2

All 64 patients with denture stomatitis were biopsied before and one month after termination of antifungal therapy and prosthetic treatment. Five patients were biopsied several times at different intervals after nystatin had been withdrawn. Punch biopsy specimens with a diameter of 5 mm were obtained from a region 1,5 cm anterior to the vibrating line and 1,5 cm lateral to the midline.

The second biopsy was taken in a previously inflamed area, 0,5—1,0 cm from the first biopsy site. Palatal biopsies were obtained from 16 controls. Serial sections, 7μm thick, were stained with hematoxylin-eosin, Van Gieson's stain as well as P.A.S.

All sections were examined for hyphal structures and were evaluated qualitatively for epithelial and connective tissue inflammatory reactions and degree of keratinization. Histologically, the degree of intra-epithelial leucocyte infiltration was graded as:

not infiltrated (0)

slightly infiltrated (+): few leucocytes present intercellularly in the basal epithelial layers.

moderately infiltrated (++): diffuse leucocyte infiltration in all epithelial layers.

profusely infiltrated (+++): diffuse infiltration and dense infiltration in the superficial, epithelial layers with leucocytes. Micro-abcesses present.

The degree of subepithelial, inflammatory cell-infiltration was graded according to the following index:

not inflamed (0): inflammatory cells not present.

slightly inflamed (+): a few, scattered inflammatory cells present.

moderately inflamed (++): diffuse infiltration with inflammatory cells.
severely inflamed (+++): diffuse infiltration alternating with dense infiltration of inflammatory cells.

Palatal smears, obtained from all patients in the experimental group and the control group were evaluated quantitatively for hyphal structures and leucocyte migration and for degree of keratinization of the epithelial cells. As to the quantity of hyphal structures, the following quantitation was used:

no hyphae
single hyphae
mycelium.

The degree of leucocyte migration, determined by palatal smears, was graded as:
no migration (0)
slight migration (+): a few scattered leucocytes present
moderate migration (++): diffusely infiltrated smear
profuse migration (+++): leucocytes present in heavy accumulations.

The relative proportion of epithelial cells with and without intra-cellular glycogen deposits and with and without nuclei was assessed. A relatively high yield of anuclear epithelial cells was related to a high degree of keratinization.

In all patients in the experimental group and the control group the palatal mucosa was examined quantitatively for yeast-like fungi by cultivation using the impression-technique (*Budtz-Jørgensen and Bertram*, 1970a). The following quantitation was used:

none or few (0—10 colonies)
moderate (10—25 colonies)
profuse (> 25 colonies)

According to the criteria previously employed (*Budtz-Jørgensen and Bertram*, 1970a) the degree of inflammation was graded clinically as:
not inflamed (0)
slightly inflamed (+)
moderately inflamed (++)
severely inflamed (+++).

The patient-distribution according to the inflamatory types present among the patients with candidiasis or purely traumatic lesions of the palate is recorded in Table II. The candidiasis group included patients who revealed hyphal structures in palatal smears, and had been responsive to antifungal therapy or unresponsive to prosthetic treatment. Included in the candidiasis group were 2 patients who did not show hyphal structures in the pre-treatment palatal smears, although they had been responsive to antifungal therapy

Table II.

Patient distribution according to the inflammatory types present among patients with candidiasis and traumatic lesions of the palatal mucosa

Etiological factor	Type of inflammation Simple localized	Simple generalized	Granular	Total
Candida infection	0	21	20	41
Trauma	15	4	4	
Total	15	25	24	64

with a permanent result. The trauma group comprised patients who did not reveal hyphal structures in palatal smears, and had been unresponsive to antifungal therapy or responsive to prosthetic treatment.

Statistical methods. The chi-square test was used for testing the agreement between the values observed (O) and the values expected (E).

$$X^2 = \frac{(E-O)^2}{E}$$

Values of $p \leq 0.05$ were acepted as statistically significant.

RESULTS

Clinically normal mucosa

Palatal biopsies were obtained from 16 controls. Nine specimens exhibited orthokeratosis, 3 parakeratosis and 4 orthokeratosis as well as parakeratosis (Fig. 1). A well developed stratum granulosum was found in 3 patients with orthokeratosis. Subepithelial inflammation, simple epithelial hyperplasia, and intra-epithelial deposits of glycogen localized in the upper prickle cell layers were seen almost exclusively in specimens which displayed parakeratosis. Only one specimen showed intra-epithelial infiltration with leucocytes, but only basally. Epithelial atrophy was not seen in any specimen.

The palatal smears, obtained from 58 controls, showed epithelial cells, partly with, partly without nuclei. In 21 patients the smears displayed predominantly anuclear epithelial cells, in 17 patients anuclear and nucleated cells appeared in approximately equal numbers, whereas in 20 patients

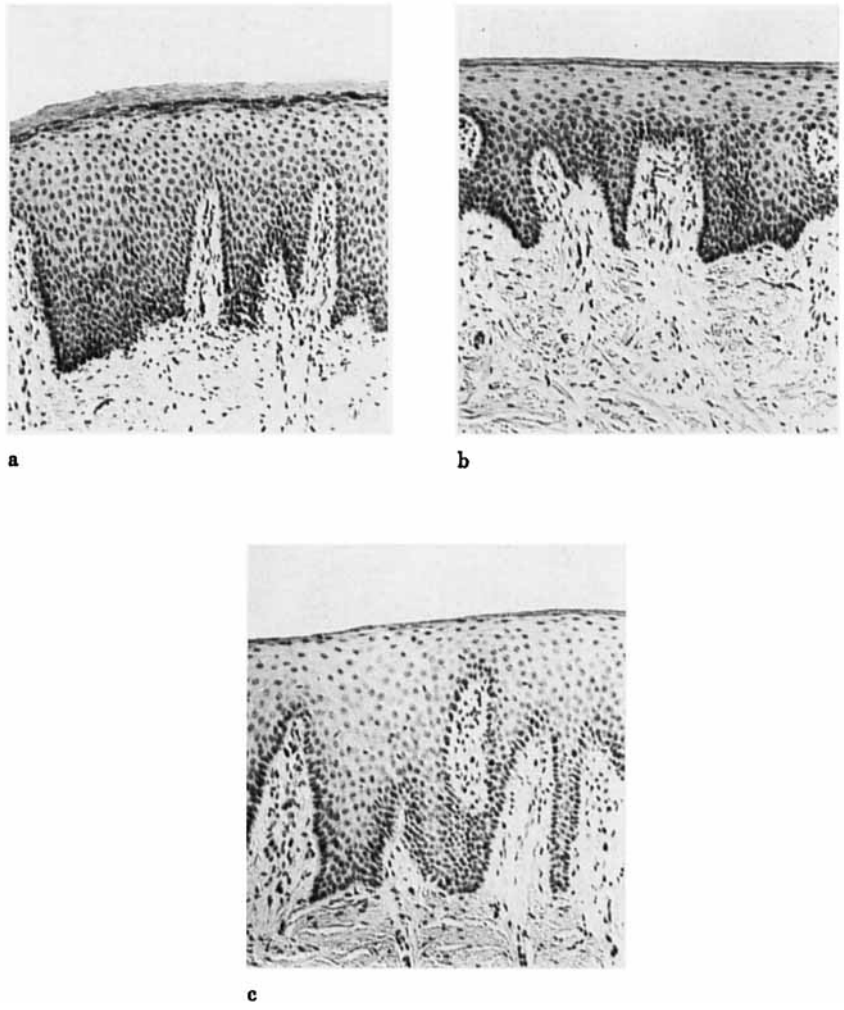


Fig. 1. Photomicrographs showing the histomorphology of non-inflamed denture-bearing palatal mucosae. Hematoxylin-eosin. 100 ×

a) Orthokeratosis. Note distinct granular layers, achantosis, and localized subepithelial inflammation.
b) Orthokeratosis. Only scattered keratohyalin granules are present.
c) Parakeratosis. Note achantosis, epithelial hyperplasia and slight, subepithelial inflammation.

the smears appeared to be dominated by nucleated cells. Single hyphae were isolated in 5 patients. Only in one patient did the smear display any leucocytes.

Whether the smears were dominated by nucleated or anuclear epithelial cells did not seem to depend on denture function (occlusion), quality of the denture-bearing tissues, age of experience with dentures, and denture usage (Tables III, IV, V, VI).

Table III.

The relationship between the degree of keratinization, evaluated by palatal smears, and denture function. Control group

Degree of keratinization	Denture function		
	Traumatic	Non-traumatic	Total
Anuclear	10	11	21
Anuclear/nucleated	9	8	17
Nucleated	11	9	20

Table IV.

The relationship between the degree of keratinization, evaluated by palatal smears, and the quality of the denture-bearing tissues. Control group

Degree of keratinization	Quality of denture-bearing tissues		
	Unsatisfactory	Satisfactory	Total
Anuclear	6	15	21
Anuclear/nucleated	9	8	17
Nucleated	9	11	20

Table V.

The relationship between the degree of keratinization, evaluated by palatal smears, and the years of experience with dentures. Control group

Degree of keratinization	Years of experience with dentures		
	0—20	< 20	Total
Anuclear	14	7	21
Anuclear/nucleated	8	9	17
Nucleated	8	12	20

Table VI.

The relationship between the degree of keratinization, evaluated by palatal smears, and the denture-wearing habits. Control group

Degree of keratinization	Denture-wearing frequency		
	Day	Day and night	Total
Anuclear	6	15	21
Anuclear/nucleated	3	14	17
Nucleated	8	12	20

Localized simple inflammation.

Fifteen patients revealed localized simple inflammation and in most cases the inflammatory lesion was confined to the posterior part of the hard palate. Some of the histological and cytological findings are recorded in Table VII. No specific histopathological features were encountered: Chronic, subepithelial inflammation of varying degree and usually pronounced simple epithelial hyperplasia alternating with slight epithelial atrophy were seen; a distinct parakeratotic layer was usually present (Fig. 2). The leucocyte infiltration was inconspicous, whereas lymphocyte infiltration usually was present in the basal cell layers. Glycogen deposits were localized in the

Table VII.

The severity of the inflammatory reaction, evaluated clinically, histologically, and by palatal smears, and the yield of yeast-like fungi in smears and by cultivation. Simple localized inflammation

Patient	Age	Sex	Years of experience with dentures	Hyphae in smears	Yeasts by cultivation	Inflammation clinically	Inflammation histologically	Leucocyte-infiltration in smears	Leucocyte-infiltration histologically
C.C.	76	F	58	o	+	+	+	+	0
H.K.	58	F	36	s	+	+	+	0	+
A.M.	44	F	24	o	++	++	+	0	+
A.C.	54	F	10	o	0	++	+	0	0
K.N.	65	F	44	o	+	+	++	+	+
I.J.	25	F	3	o	+	++	+	+	+
E.F.	67	F	37	o	0	+	++	0	0
M.L.	63	F	30	o	+	++	++	+	+
A.J.	48	M	10	o	+	+++	++	0	+
B.O.	47	M	20	o	+	+++	+++	+	+
L.P.	61	F	27	o	++	++	++	+	0
R.N.	64	F	13	o	++	++	+++	+	+
E.B.	55	F	7	o	+++	+	++	0	0
V.M.	65	M	15	o	+	++	++	+	+
L.J.	58	F	23	o	+	+	+	0	0

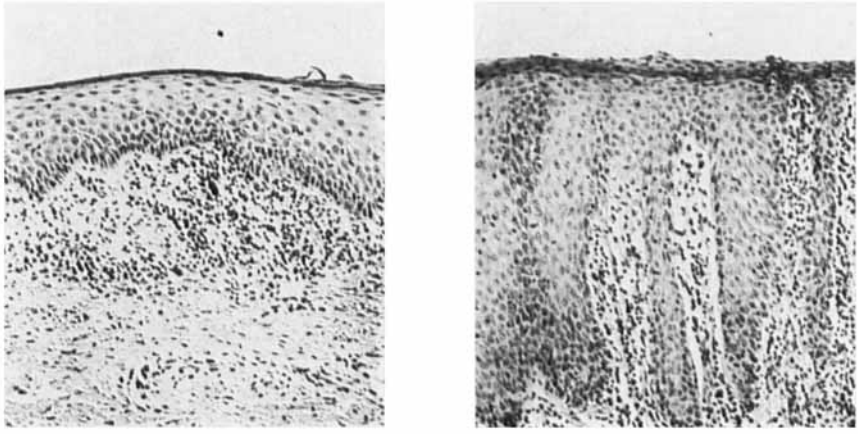
o = no hyphae; s = single hyphae; m = mycelium. As regards the signs: 0, +, ++, +++, see Material and Methods.

upper prickle cell layers and in the parakeratotic cells. Heavy glycogen deposits were seen in sections that showed severe epithelial hyperplasia.

The palatal smears showed hyphal structures (single hyphae) only in one case and the content of leucocytes was usually sparse. The epithelial cells were nucleated and intra-epithelial glycogen deposits were seen relatively frequent.

Generalized simple inflammation

Twenty-four patients revealed generalized simple inflammation of the maxillary denture-bearing tissues. Some of the histopathological and cytological findings are recorded in Table VIII. The following characteristics



a b

Fig. 2. Photomicrographs showing the histomorphology of localized simple inflammation of denture-bearing palatal mucosae. Hematoxylin-eosin. 100 ×.

a) Note moderate, subepithelial, chronic inflammation. Inflammatory changes of the epithelium are insignificant.
b) Note well-developed parakeratotic layers, pronounced epithelial hyperplasia and moderate, subepithelial, chronic inflammation.

were seen in most sections: parakeratosis or complete absence of keratinization, achantosis, alternating simple epithelial hyperplasia and extreme epithelial atrophy, intra-epithelial leucocyte infiltration, lymphocyte infiltration in the basal epithelial layers and chronic subepithelial inflammation of varying intensity (Fig. 3). Some sections showed micro-abcesses in the superficial epithelial layers. In no case did the P.A.S.-stained sections reveal hyphal structures, whereas intra-cellular glycogen deposits were present in all sections in the upper prickle cell layers and in the superficial, parakeratinized and non-keratinized epithelial layers. In all cases the palatal smears showed leucocytes, but in varying quantites. Hyphal structures were recognized in palatal smears in 20 patients (mycelium in 12, single hyphae in 8 patients). Only nucleated epithelial cells were seen with scattered epithelial cells showing intra-epithelial glycogen deposits.

Table VIII.

The severity of the inflammatory reaction, evaluated clinically, histologically, and by palatal smears, and the yield of yeast-like fungi in smears and by cultivation. Simple generalized inflammation

	Patient	Age	Sex	Years of experience with dentures	Hyphae in smears	Yeasts by cultivation	Inflammation clinically	Inflammation histologically	Leucocyte-infiltration in smears	Leucocyte-infiltration histologically
CANDIDIASIS	J.H.	70	M	48	s	++	++	+	++	+
	A.J.	46	F	32	s	++	+++	+	++	++
	L.S.	54	F	29	m	+++	++	+	++	++
	H.R.	70	F	19	s	+++	++	++	++	++
	A.B.	66	F	15	s	+	++	++	++	++
	M.A.	58	F	16	s	++	++	+	+++	+
	E.H.	70	M	27	s	+++	+++	++	++	+++
	C.D.	48	M	27	m	+++	+++	++	++	+++
	H.I.	62	F	41	s	+++	+++	+	+++	+
	E.C.	63	F	42	m	+	++	++	+++	+
	V.B.	65	F	35	m	++	+	++	+	++
	K.S.	60	F	42	m	+++	+++	++	+	+
	E.D.	49	F	35	o	+	++	++	+	+
	F.O.	27	F	11	m	+++	+	+	+	+
	I.J.	60	F	34	m	+++	++	++	+	+
	M.N.	75	F	55	m	+++	+++	++	+++	++
	A.S.	59	F	12	m	++	+	+	++	+
	J.M.	59	F	31	m	++	+	+	++	+
	A.S.	51	F	9	s	+++	+++	++	+++	+++
	M.N.	30	F	17	m	++	+	++	++	++
	L.N.	62	M	26	m	+++	+++	++	++	++
TRAUMA	E.S.	66	F	48	o	+	+++	++	+	+
	K.A.	63	F	5	o	0	+	+	+	+
	D.N.	74	F	20	o	0	++	++	++	+

o = no hyphae; s = single hyphae; m = mycelium. As regards the signs: 0, +, ++, +++, see Material and Methods.

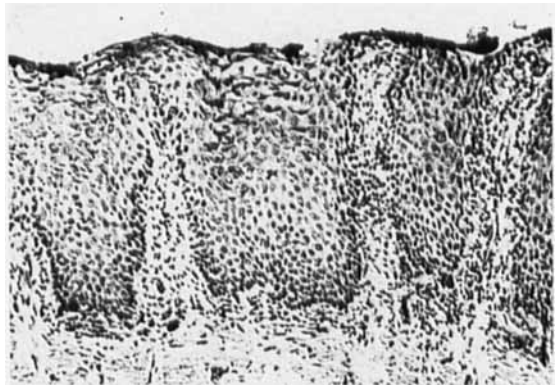


Fig. 3. Photomicrograph showing the histomorphology of a generalized, simple candida-induced inflammation of the denture-bearing palatal mucosa. P.A.S.-hematoxylin. 100 ×. Note extreme, epithelial atrophy, a micro-abcess, epithelial hyperplasia, and moderate, subepithelial, chronic inflammation. Hyphal structures not present.

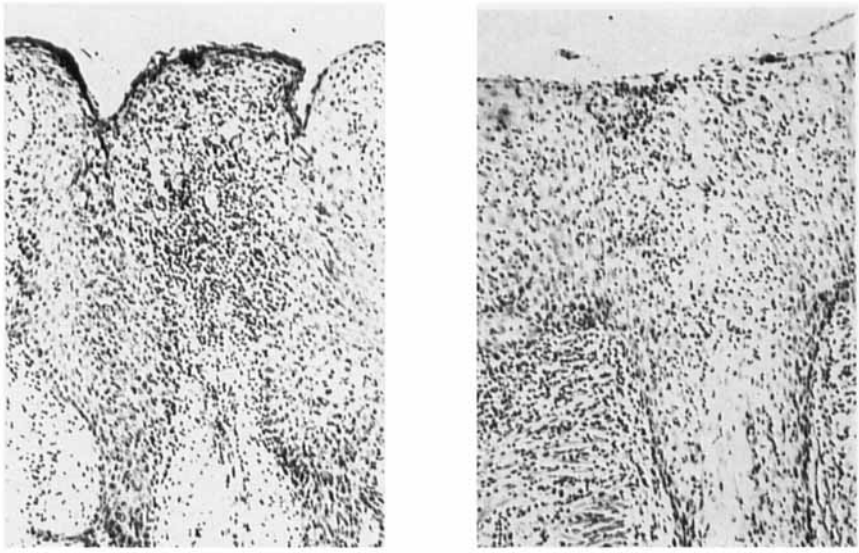


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Fig. 4. Photomicrographs showing the histomorphology of granular inflammation of denture-bearing palatal mucosae associated with candida infection. Note severe, intra-epithelial leucocyte infiltration, absence of keratinizition, epithelial hyperplasia and heavy, subepithelial inflammation. 100 ×

a) P.A.S.-hematoxylin. Note absence of hyphal structures.
b) Hematoxylin-eosin. Note superficial, dense, intra-epithelial accumulation of leucocytes.

Table IX.

The severity of the inflammatory reaction, evaluated clinically, histologically, and by palatal smears, and the yield of yeast-like fungi in smears and by cultivation. Granular inflammation

	Patient	Age	Sex	Years of experience with dentures	Hyphae in smears	Yeasts by cultivation	Inflammation clinically	Inflammation histologically	Leucocyte-infiltration in smears	Leucocyte-infiltration histologically
CANDIDIASIS	A.P.	57	M	32	m	+	++	++	+++	+
	L.N.	51	F	34	m	+++	+++	+	+++	+
	Å.H.	64	M	17	m	+++	+++	+	+++	+
	C.A.	48	F	26	s	+++	+++	++	+	+
	A.D.	35	F	20	s	+++	+	++	+	++
	K.N.	46	M	28	m	+	++	++	+	+
	E.J.	63	F	30	o	+	++	+	+	++
	C.P.	58	F	35	m	++	+	+	+	+
	B.N.	40	F	14	m	++	+++	+++	++	+++
	W.S.	61	M	32	s	+++	+++	++	+	+
	J.M.	65	F	29	s	++	+++	+++	++	++
	G.A.	48	M	22	m	++	++	++	+	+
	T.P.	71	M	49	m	+++	++	++	++	+
	F.P.	67	M	49	m	++	+++	+++	+++	++
	E.J.	50	F	8	m	+++	+++	+++	+++	+++
	E.N.	45	F	27	m	+++	++	++	+	+
	L.L.	58	F	40	m	+++	+++	++	+++	++
	H.J.	51	F	24	m	+	+	++	+	+
	R.J.	58	M	16	m	+++	+++	++	++	++
	A.S.	61	M	14	m	+++	+++	++	++	++
TRAUMA	O.C.	65	F	17	o	0	++	++	++	+
	G.S.	48	F	20	o	0	++	++	+	+
	K.R.	64	F	20	o	+	+	++	+	+
	V.L.	78	M	54	o	+++	++	+++	++	++
	F.L.	43	F	17	o	+++	+	++	+	+

o = no hyphae; s = single hyphae; m = mycelium. As regards the signs: 0, +, ++, +++, see Material and Methods.

Granular inflammation

Twenty-five patients showed granular inflammation of the palatal mucosa. The histopathological and cytological findings were fairly consistent, some of which are recorded in Table IX. The surface of the sections showed numerous

papillary projections covered by a parakeratinized or non-keratinized epithelium. All sections showed connective tissue hyperplasia and alternating simple epithelial hyperplasia and atrophy. Superficial, intra-epithelial micro-abcesses were seen only in a few sections. In none of the cases reviewed there was evidence of dyskeratosis. Tangentially-cut rete pegs frequently gave the appearance of islands of epithelium below the basement membrane.

A majority of the sections showed heavily vascularized granulation tissue close to the basement membrane. Intra-epithelial leucocyte infiltration and a rather heavy, chronic subepithelial inflammation, infiltrating the basal epithelial cell layers, were present in most sections (Fig. 4). The P.A.S.-stained sections never revealed any hyphal structures, whereas heavy, intra-cellular glycogen deposits were present in all epithelial layers, except for the germinative and lower prickle cell layers.

The P.A.S.-stained smears showed leucocytes in varying, usually great quantities. In 19 patients the palatal smears revealed hyphal structures (mycelium in 15, single hyphae in 4 patients). Bacteria were present in most smears. All the epithelial cells were nucleated, most of them with heavy glycogen deposits.

General histological and cytological comparisons between inflammatory conditions of the palate caused by trauma and candida infection

In 41 patients candida infection was considered the etiological factor of the inflammation of the palatal mucous membrane, in 23 patients trauma was the probable etiological factor (Table II).

Except for a few cases of candida-induced inflammation that showed intra-epithelial micro-abcesses, it was not possible to detect any significant qualitative difference in the inflammatory reaction of the palatal mucosa whether the etiological factor was trauma or candida infection.

Hyphal invasion was never recognized in P.A.S.-stained histological sections. The overall epithelial, inflammatory reactions were parakeratosis, or absence of keratinization, simple epithelial hyperplasia alternating with epithelial atrophy of varying degree, intra-epithelial leucocyte infiltration in most sections and intra-cellular glycogen deposits in all epithelial layers, except for the germinative and lower prickle cell layers. A chronic inflammation of the lamina propria was present in all sections, usually infiltrating the basal, epithelial cell layers.

Table X.

Comparison of the severity of the inflammatory reaction, evaluated clinically, in patients with candida-induced and trauma-induced inflammatory lesions

Degree of inflammation clinically	Candidiasis	Trauma
Slight	8	7
Moderate	14	13
Severe	19	3
$X^2 = 7$;	2 DF;	$0{,}01 < P < 0{,}05$

Table XI.

Comparison of the degree of subepithelial inflammation in patients with candida-induced and trauma-induced inflammatory lesions

Degree of inflammation histologically	Candidiasis	Trauma
Slight	11	6
Moderate	26	14
Severe	4	3

Quantitatively the candida-induced inflammatory reaction was found to differ in some respects from the traumatic inflammatory lesion, however. Evaluated clinically, a severe inflammation was seen more frequently among the patients with candida infection than among the patients with traumatic lesions ($0{,}01 < P < 0{,}05$) (Table X). Histological sections showing a severe subepithelial inflammation were, however, found almost equally frequently in the two categories (Table XI). Pronounced, simple epithelial hyperplasia was seen as well in sections from traumatic lesions as in sections from candida-induced lesions.

Candida-induced and trauma-induced inflammatory reactions were found to differ in degree of leucocyte migration. Evaluated histologically, sections showing pronounced intra-epithelial leucocyte infiltration were seen more frequently in the group of patients with candida infection ($0{,}01 < P < 0{,}05$) (Table XII).

Table XII*.

Comparison of the degree of leucocyte infiltration in histological sections in patients with candida-induced and trauma-induced inflammatory lesions

Degree of leucocyte infiltration	Candidiasis	Trauma
None	1	4
Slight	20	16
Moderate	15	3
Profuse	5	
$X^2 = 6,4$	1 DF	$0,01 < P < 0,05$

Table XIII*.

Comparison of the degree of leucocyte migration, determined by palatal smears, in patients with candida-induced and trauma-induced inflammatory lesions

Degree of leucocyte migration	Candidiasis	Trauma
None	0	9
Slight	16	11
Moderate	14	3
Profuse	11	0
$X^2 = 11,6$	1 DF	$P < 0,01$

Table XIV*.

The relationship between the leucocyte infiltration in histological sections and the yield of yeast-like fungi by cultivation

Degree of leucocyte infiltration	Quantity of yeast colonies		
	0—10	10—25	> 25
None	4	1	0
Slight	16	9	11
Moderate	4	5	9
Profuse	0	1	4
$X^2 = 7,2$	2 DF	$0,01 < P < 0,05$	

Table XV*.

The relationship between the leucocyte migration, determined by palatal smears, and the yield of yeast-like fungi by cultivation

Degree of leucocyte migration	Quantity of yeast colonies		
	0—10	10—25	> 25
None	7	1	1
Slight	12	8	7
Moderate	3	5	9
Profuse	2	2	7
$X^2 = 10,2$	2 DF		$P < 0,01$

Table XVI**.

The relationship between the subepithelial inflammation and yield of yeast-like fungi by cultivation

Degree of inflammation histologically	Quantity of yeast colonies		
	0—10	10—25	> 25
Slight	6	7	4
Moderate	17	6	17
Severe	1	3	3
$X^2 = 3,4$	2 DF		$0,1 < P < 0,5$

* Tables XII, XIII, XIV and XV: When using the chi square test the four groups, designated none, slight, moderate and profuse, were combined to form two: none-slight and moderate-profuse.

** Table XVI: When using the chi square test the groups designated moderate and severe were combined.

Determined by palatal smears, a corresponding association between pronounced leucocyte migration and candida infection could be established ($P < 0,01$) (Table XIII).

Finally, the degree of subepithelial inflammation and the intra-epithelial leucocyte infiltration, evaluated histologically, and the degree of leucocyte migration, determined by smears, were correlated with the quantity of

yeast colonies found by cultivation, using the impression method for isolation of yeasts, (*Budtz-Jørgensen & Bertram*, 1970a). The quantitative cultivating data are recorded along with the clinical, histological and cytological findings in Table VII, VIII and IX. The following results were found:

1) A statistically significant association could be established between the degree of leucocyte infiltration, evaluated histologically, and the quantity of yeast colonies ($0{,}01 < P < 0{,}05$) (Table XIV).

2) A statistically significant association was found between the degree of leucocyte migration, determined by palatal smears and the quantity of yeast colonies ($P < 0{,}01$) (Table XV).

3) It was not possible to find a statistically significant association between the degree of inflammation, evaluated histologically, and the quantity of yeast colonies ($0{,}1 < P < 0{,}5$) (Table XVI).

Effect of antifungal therapy and prosthetic treatment

In a previous report the anti-inflammatory effect of antifungal therapy and prosthetic treatment was evaluated clinically and determined by palatal smears (*Budtz-Jørgensen and Bertram*, 1970b). In this study the histological changes following antifungal therapy and prosthetic treatment will be reported along with the exfoliative cytological changes.

Antifungal therapy. Thirty-seven patients received nystatin tablets, (Mycostatin SQUIBB), three times a day for 14 days. Twenty-seven patients, of whom 25 showed hyphal structures in palatal smears, responded to antifungal therapy, whereas 10 patients without a verifiable candida infection did not respond. One month following withdrawal of nystatin all 37 patients were biopsied.

Fourteen out of the 27 patients, who initially had been responsive to antifungal therapy, showed relapse of the candida-induced inflammation determined both clinically and by palatal smears. Compared with the microscopical findings prior to treatment these cases showed nearly unchanged inflammatory reactions in the lamina propria and in the epithelium. Of the 13 patients who did not show relapse 6 patients revealed discrete, circumscribed, probably trauma-induced lesions of the palatal mucosa, whereas in 7 patients the palatal mucosa was clinically normal. In comparing the first and the corresponding second specimen in 9 patients, who prior to treatment demonstrated a generalized simple inflammation, the histological examination revealed the following changes (Fig. 5): The epithelial atrophy the intra-cellular edema of the cells of stratum spinosum and the leucocyte infiltration had disappeared. The epithelial surface revealed a distinct

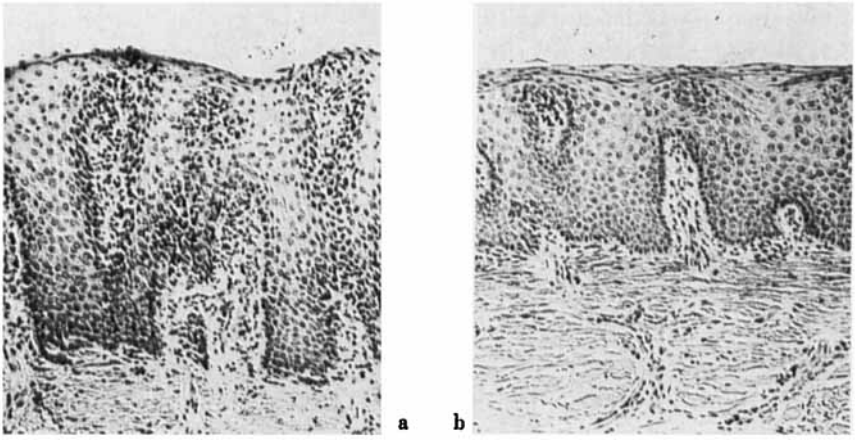

Fig. 5. Photomicrographs showing the histomorphological changes following anti-fungal therapy. Generalized, simple inflammation. Hematoxylin-eosin. 100 ×

a) Before treatment. Note epithelial atrophy, incomplete keratinization, superficial, intra-epithelial accumulation of leucocytes, epithelial hyperplasia and moderate, subepithelial, chronic inflammation.

b) One month after treatment. Note reappearance of flattened, parakeratinized, epithelial cells and reduced, epithelial atrophy and hyperplasia.

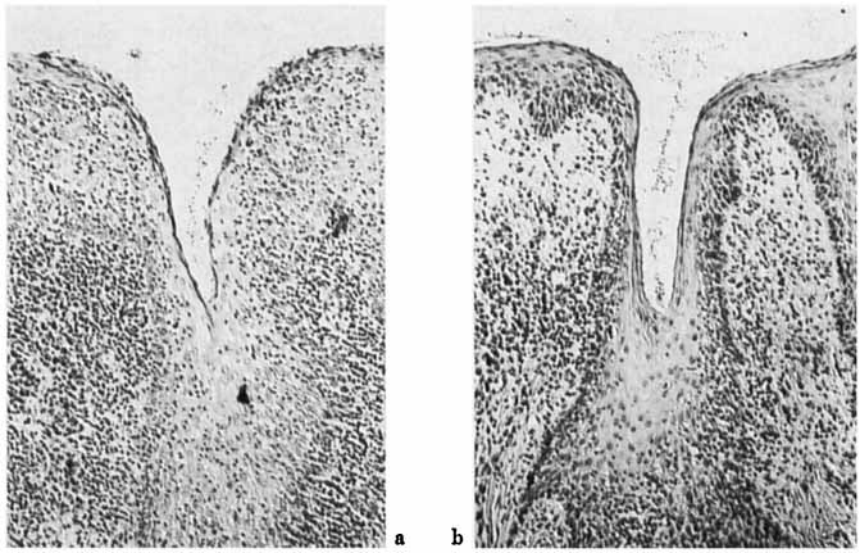

Fig. 6. Photomicrographs showing the histomorphological changes following antifungal therapy. Granular inflammation. Hematoxylin-eosin. 100 ×

a) Before treatment. Note incomplete keratinization, intra-epithelial leucocyte infiltration, epithelial atrophy and hyperplasia, indistinct stratum basale and heavily, inflamed granulation tissue of the lamina propria.

b) One month after treatment. Note lack of leucocyte infiltration and reappearance of flattened, parakeratotic cells and a distinct stratum basale. A subepithelial, chronic inflammation has persisted.

parakeratotic or orthokeratotic layer and the simple epithelial hyperplasia showed a regressive tendency. In 2 patients the palatal mucosa histologically appeared completely normal.

Most of the P.A.S.-stained sections showed decreased intra-cellular deposits of glycogen. The P.A.S.-stained palatal smears displayed predominantly nucleated epithelial cells, frequently with a glycogen-rich cytoplasma. Leucocytes were never seen.

In comparing the first and the corresponding second specimen in 4 patients, who prior to treatment demonstrated a granular inflammation, the histological examination revealed the following changes: The degree of intra-epithelial leucocyte infiltration and epithelial atrophy had significantly decreased; the intra-cellular edema of the cells of stratum spinosum and the subepithelial, heavily vascularized granulation tissue had disappeared, Yet, the epithelial crevices, the simple epithelial hyperplasia and the chronic subepithelial inflammation had persisted almost unchanged (Fig. 6). The P.A.S.-stained sections showed intra-cellular deposits of glycogen in the upper prickle cells and the parakeratotic strata in almost unchanged amounts. The P.A.S.-stained smears showed nucleated epithelial cells, partly with, partly without glycogen deposits. Leucocytes were never present.

Five patients with candida infection were biopsied before antifungal therapy, immediately after and one week as well as one month following withdrawal of nystatin. Significant histological changes were apparant, even immediately after withdrawal of nystatin, the most striking being reappearance of flattened parakeratotic cells concomitant with disappearance of epithelial atrophy and superficial, intra-epithelial leucocyte infiltration.

Prosthetic treatment. The prosthetic treatment group originally comprised 27 patients. One patient did not complete the treatment, whereas 26 patients had new well-functioning dentures constructed. In 11 of these candida infection was the cause of the inflammatory condition, whereas in 15 trauma was the probable etiological factor. The final impression for the new dentures was taken after the patients had been using their original dentures, relined with tissue-conditioning material and equilibrated occlusally, for 1 week. The 26 patients were biopsied after having used the new set of dentures for 1 month.

In the 11 patients with a verifiable candida infection the inflammatory conditions did not change significantly following prosthetic treatment in comparison with the situation prior to treatment, as evaluated clinically, by palatal smears and histologically.

Six out of 11 patients with a simple inflammation, caused by trauma, were responsive to prosthetic treatment, evaluated histologically. The overall

changes were found to be reduced inflammation of the lamina propria, usually accompanied by an apparent decrease in degree of simple epithelial hyperplasia and in amount of intra-epithelial glycogen deposits. In 2 patients there was a change from parakeratosis to orthokeratosis.

Four patients with a probable trauma-induced granular inflammation were responsive to prosthetic treatment showing the same overall histological changes as the patients with a simple inflammation. The epithelial crevices invariably persisted following prosthetic treatment.

As the epithelial inflammatory reactions were less severe in trauma-induced inflammatory lesions, the histological changes in the epithelium were less striking following prosthetic treatment than those seen after anti-fungal therapy of candida-induced inflammatory lesions.

DISCUSSION

Rather unexpectedly hyphal structures were never encountered in histological sections in cases of candida-induced simple or granular denture stomatitis. This lack of correlation in the microscopical picture between the candida-induced denture stomatitis and other types of chronic muco-cutaneous candidiasis may be explained by the fact that the maxillary denture provides outstanding ecological conditions for yeast-like fungi.

Taschdjian (1960) produced experimental vaginal candidiasis in mice. The microscopical picture showed yeasts in the mycelial phase only profoundly in stratum corneum, i.e., in sulphydryl-rich epithelial layers with a low oxygen tension. *McClary* (1952) found that the optimal Ph for the mycelial phase would be five. A maxillary denture most likely deprives the palatal mucous membrane from the buffering effect of saliva and free accession of oxygen, hence yeasts in the mycelial phase would find optimal conditions in the most superficial shedding epithelial cells. Moreover, *Davenport* (1969) has reported on the isolation of mycelium in larger quantities in smears from the fitting surface of the denture than in smears from the mucosa.

Cawson (1966) observed that the hyphal structures isolated in cases of denture stomatitis sometimes were very long and appeared to have grown over the surface between denture and mucosa.

It seems realistic to conclude that the artificial conditions produced by the denture make the histological picture of candida-induced simple or granular inflammation differ from that of chronic, mucous candidiasis in non-denture-covered areas, as mycelium was never found intra-epithelially in the former.

The general histomorphological features of the chronic, hyperplastic candidiasis has been described as hyperparakeratosis with hyphal invasion in the parakeratotic layer, but not beyond the junction with the stratum spinosum, intra-epithelial leucocyte infiltration, micro-abcesses localized between the stratum spinosum and the parakeratotic layers, simple epithelial hyperplasia and chronic subepithelial inflammation (*Waisman,* 1955; *Degos,* 1960; *Jepsen & Winther,* 1965; *Cawson,* 1966; *Lehner,* 1966). In this study the candida-induced simple and granular denture stomatitis was found to display the same histomorphological changes, except for hyperparakeratosis and intra-epithelial candida invasion, which were never seen, and micro-abcesses, which were seen only in a few sections. On the contrary, epithelial atrophy and thinning or even absence of the superficial parakeratotic layers were characteristic features of the candida-induced denture stomatitis, hence the term chronic atrophic candidiasis (*Lehner,* 1963) would seem to be the appropriate histopathological diagnosis of these inflammatory lesions.

Maibach and Kligman (1962) were able to produce experimental cutaneous candidiasis by applying living Candida cells as well as a sterile extract of disintegrated Candida cells and a sediment of ruptured cells to normal skin covering the testsite with tape. In 36 to 72 hours small pustules emerged on an erythematous background. Microscopically, fungi were seen incidentally superficially in stratum corneum, but never in the living epidermis nor in the subcorneal pustules. It was concluded that the pathologic reactions in cutaneous moniliasis is mediated by endotoxin-like substances, released by the organisms, and is a biological contact dermatitis of the primary irritant type. The candida-induced denture stomatitis is possibly generated in the same way, i.e., through endotoxin-like substances released by yeast-like fungi, haboured in the fitting surface of the denture and superficially in the palatal mucosa.

Even though the degree of inflammation, evaluated clinically, was found to be more severe among patients with candida-induced denture stomatitis than among the patients with purely traumatic lesions (*Budtz-Jørgensen & Bertram,* 1970a) the microscopical examinations did not reveal a corresponding difference in degree of subepithelial inflammation. The explanation may be that extreme epithelial atrophy and incomplete keratinization, almost exclusively present in sections from patients with candida-induced denture stomatitis, would make an even moderate, chronic subepithelial inflammation appear clinically very intense.

Kapica and Blank (1957) have produced evidence of the keratolytic properties of candida, hence the continuous shedding of epithelial cells

may to some extent be due to keratolytic enzymes produced by the fungi.

In this study a statistically significant difference in degree of leucocyte migration could be established between patients with candida-induced and purely traumatic inflammatory lesions of the palatal mucosa by microscopical examination of histological sections and smears. Furthermore, a statistically significant correlation was found between degree of leucocyte migration and the quantity of yeast colonies cultivated according to the impression method. In Maibach and Klingman's experimental candida lesions, intra-epithelial leucocyte infiltration was a significant finding, too. *Børglum Jensen* (1967), applying as little as 0,2 μg veillonella endotoxin every second hour in a skin-window, was able to produce a persistent leucocyte migration. The sustained migration of leucocytes in association with chronic candida-induced denture stomatitis may be ascribed to even small amounts of endotoxin released continously by autolysis of yeast-like fungi localized in the denture base or in the superficial epithelial layers.

Hurley (1964) was able to demonstrate a factor in saline extracts of leucocytes and in serum after incubation with leucocytes, which promoted leucocyte migration. *Page* (1964) found a marked deficit in mononuclear response to inflammation during periods of neutropenia. Furthermore, he was able to show that local injection of leucocytes in neutropenic animals would stimulate lymphocytes to appear in the injection site.

Accordingly, it may be suggested that the continuous accumulation and death of leucocytes under a maxillary denture base may induce, primarily, an increased leucocyte migration, secondly, lymphocyte infiltration subepithelially and in the basal epithelial cell layers.

Although other micro-organisms in addition to fungi were revealed rather frequently in P.A.S.-stained palatal smears antifungal therapy was followed by a rather striking decrease in inflammatory reaction. Microscopically, it was a characteristic histopathological feature that the extreme epithelial atrophy and the leucocyte infiltration disappeared and a distinct parakeratotic or even orthokeratotic stratum reappeared. It is difficult to explain why a chronic subepithelial inflammation persisted in cases of granular inflammation following antifungal therapy, even though the inflammation clinically had resolved. The connective tissue inflammation may probably be sustained by micro-organisms other than yeast-like fungi, harboured in the crevices between the papilloferous outgrowths.

Much controversy exsists regarding the significance of intra-epithelial glycogen-deposits in keratinizing oral epithelium. *Falin* (1961) found that the epithelium of the hard palate contained very little if any glycogen, a finding that corresponds well with the histological and cytological picture

in samples taken from non-inflamed palates in this study. Intra-epithelial glycogen deposits in oral keratinizing mucosa has been demonstrated in association with chronic inflammation and parakeratosis (*Doyle et al.*, 1968; *Falin*, 1961; *Meyer & Medak*, 1962; *Weiss et al.*, 1959).

According to *Bradfield* (1951) glycogen is not accumulated in cornifying epithelium because of its rapid utilization in keratin synthesis. The presence of intra-epithelial glycogen deposits in inflamed keratinizing oral mucosa is explained as due to a more active glucose-supply following an increased blood-circulation (*Weiss et al.*, 1959) and a decreased glucose-consumption as the need of energy is reduced in virtue of the inhibition of the cornification process (*Fasske et al.*, 1967); according to *Firket* (1951) epithelial glycogen-accumulation is the result of decrease in oxygen-supply.

Flanagan and Porter (1968), studying the microscopical and histochemical picture of the granular inflammation of the denture-bearing palatal mucosa, found that glycogen stained most intensely in the achantotic fissured areas although some glycogen was present in the thinner epithelium overlying the papillae. In this study glycogen deposits in sections showing granular inflammation were found concentrated in the same areas as reported by Flanagan; in sections showing simple inflammation glycogen was primarily accumulated in the corresponding areas, i.e., superficially in stratum spinosum in areas with simple epithelial hyperplasia and intracellular edema and accordingly probably insufficient oxygensupply. That the most heavy glycogen-staining was seen in sections showing granular inflammation may be explained by the fact that the heavily vascularized subepithelial granulation tissue is providing excess glucose.

In Flanagan's study there appeared to be a close relationship between the degree of subepithelial inflammation and the amount of glycogen in the epithelium. This relationship could not be established in the present study as some histological sections, showing intense subepithelial inflammation, only revealed minor amounts of glycogen intraepithelially. On the other hand, heavy glycogen deposits were seen in sections, slightly inflamed, but with pronounced simple epithelial hyperplasia. From the present findings heavy intra-epithelial glycogen deposits seemed to be associated primarily with pronounced, simple epithelial hyperplasia and heavily vascularized, subepithelial granulation tissue.

As the biopsy specimens were comprising only an extremely limited part of the total maxillary denture-bearing mucous membrane some caution is indicated in evaluation of differences in the histological picture between patients and between groups of patients. However, the following may be concluded:

1) Mycelium was not recognized intra-epithelially in any patients showing candida-induced denture stomatitis. All the biopsies were obtained in inflamed areas that did not show actual thrush.

2) Candida-induced inflammation of the palatal mucosa was differing from trauma-induced inflammation in extreme epithelial atrophy, incomplete keratinization and intra-epithelial leucocyte infiltration being more pronounced among the former.

3) Sufficient prosthetic treatment of a trauma-induced inflammation of the palatal mucosa was frequently resulting in a normalisation of the histological picture.

4) Antifungal treatment of a generalized simple candida-induced inflammation usually resulted in a normalisation of the histological picture.

5) Antifungal treatment of a candida-induced granular inflammation was resulting to a certain extent in a normalisation of the histological picture. From the present study it is not possible to explain why a chronic inflammation of the lamina propria persisted even though the inflammation had resolved clinically.

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SUMMARY

Sixty-four patients with denture stomatitis and 58 denture wearers with a clinically normal, palatal mucosa were studied histologically and by smears for the degree of inflammation, keratinization, and glycogen content of the palatal mucosa. All patients in the experimental group and the control group were investigated quatitatively and quantitatively by cultivation and quantitatively by a smear-technique for occurrence of yeast-like fungi.

Epithelial atrophy, absence of keratinization, and intra-epithelial leucocyte infiltration were more pronounced in candida-induced inflammatory lesions than in inflammatory lesions caused by trauma; yet, hyphal structures were never recognized intra-epithelially in the former. The amount of intra-epithelial deposits of glycogen appeared to be related to the degree of simple, epithelial hyperplasia.

A correlation was established between the yield of yeast colonies by cultivation and the degree of leucocyte migration, as evaluated by smears and histologically.

Prosthetic treatment and antifungal therapy were performed in cases of candida-induced as well as in cases of trauma-induced denture stomatitis. Palatal biopsies were obtained in all patients one month after treatment. The most pronounced histological changes were seen in candida-infected patients after antifungal therapy. In cases of granular inflammation the hyperplasia and a chronic subepithelial inflammation persisted even though the inflammation had resolved clinically.

It was discussed why candida-induced denture stomatitis differs histomorphologically from chronic mucous candidiasis not associated with dentures.

RÉSUMÉ

STOMATITE PROTHÉTIQUE. III. HISTOPATHOLOGIE DES LÉSIONS INFLAMMATOIRES DE LA MUQUEUSE DU PALAIS PROVOQUÉES PAR LES TRAUMATISMES ET PAR LES CANDIDA

Une étude par frottis et par examen histologique destinée à déterminer le degré d'inflammation, la kératinisation et la teneur en glycogène de la muqueuse du palais a été effectuée chez 64 patients présentant une stomatite prothètique et chez 58 porteurs de prothèses présentant une muqueuse palatine normale. Chez tous les patients du groupe expérimental et du groupe témoin, il a été procédé à une étude qualitative et quantitative par culture et une étude quantitative par une méthode de frottis, pour la recherche des champignons levuriformes.

L'atrophie épithéliale, l'absence de kératinisation et l'infiltration leucocytaire intra-épithéliale étaient plus prononcées dans les lésions inflammatoires provoquées par l'infection à Candida que dans les lésions inflammatoires causées par traumatisme; cependant, il n'a jamais été décelé de structures filamenteuses dans ces premières. La quantité des dépôts intra-épithéliaux de glycogène paraissait être liée au degré de l'hyperplasie épithéliale simple.

Une corrélation a été démontrée entre la production de colonies de levure par culture et le degré de migration leucocytaire, d'après l'évaluation par frottis et par examen histologique.

Un traitement prothétique et un traitement antifungique ont été effectués, tant dans des cas de stomatite prothétique provoquée par les Candida que dans des cas où elle était provoquée par traumatisme. Des biopsies du palais

ont été prélevées sur tous les patients un mois après le traitement. Les modifications histologiques les plus marquées ont été vues après traitement antifungique chez les patients atteints d'infection à Candida. Dans les cas d'inflammation granuleuse, l'hyperplasie et une inflammation chronique sous-épithéliale persistaient malgré la guérison clinique de l'inflammation.

La raison de la différence histo-morphologique existant entre les stomatites prothétiques provoquées par Candida et les candidoses muqueuses chroniques non associées à des prothèses a fait l'objet d'une discussion.

ZUSAMMENFASSUNG

STOMATITIS PROTHETIKA. III. HISTOPATHOLOGIE VON TRAUMATISCHEN UND CANDIDAINDUZIERTEN ENTZÜNDLICHEN SCHÄDIGUNGEN DER GAUMENSCHLEIMHAUT

Bei 64 Patienten mit Stomatitis prothetika und bei 58 Prothesenträgeren mit klinisch gesunden Gaumenschleimhaut wurden Entzündungsgrad, Keratinizirung und Glykogeninhalt histologisch und durch Abstriche untersucht. Alle Patienten in der experimentellen Gruppe und in der Kontroll-Gruppe wurden qvalitativ und qvantitativ mittels Kultivierung und qvantitativ mittels Abstrichtechnik von hefeähnlichen Pilze untersucht.

Epithelatrophie, fehlende Keratinizierung und intraepitheliale Leukozytinfiltration waren mehr ausgesprochen in den candidainduzierten Läsionen als in den traumatisch entwickelten Entzündungsläsionen. Doch — intraepithelial gelargerte Pilzfäden waren nie in der candida-Gruppe entdeckt. Die Menge von intraepithelialen Glykogenablagerungen scheinten in Relation zum Grad von einfachen epithelialen Hyperplasie zu sein.

Es war histologisch und durch Abstriche berechnet, dass eine Korrelation zwischen dem Ertrag der gezogenen Pilzkolonien und der Abwanderungsgrad der Leukozyten hergestellt war. Prothetische und pilztötende Therapie wurden sowohl in Fällen der candidainduzierten als in Fällen der trauma-induzierten Stomatitis prothetika verwendet. Gaumenbiopsien wurden von allen Patienten ein Monat nach dem Behandlung genommen. Die schwersten histologischen Wandlungen waren bei candidainfizierten Patienten nach pilztötender Therapie gesehen. In Fällen granulärer Entzündung dauerte die Hyperplasie und eine kronische subepitheliale Entzündung — trotzdem die Entzündung rein klinisch nicht kennbar war.

Man diskutiert warum candidainduzierten Stomatitis prothetika histomorphologisch sich von — nicht-prothetischen — kronischen, mukösen Soor unterscheiden.

REFERENCES

Al-Ami, S., G. Shklar & A. A. Yurkstas, 1966: The effect of dentures on the exfoliative cytology of palatal and buccal mucosa. J. prosth. Dent. *16:* 513—21.

Bowman, A. J. & R. A. Latham, 1968: The differential development and structure of keratinizing mucosa of upper denture-bearing area. Dent. Practit. *18:* 349—52.

Bradfield, J. R. G., 1951: Glycogen of vertebrate epidermis. Nature, (London) *167:* 40—41.

Brinch, O., 1932: Histologiske undersøgelser af ganevævet fra protesebærende patienter. Tandlægebl. *36:* 281—94.

Budtz-Jørgensen, E. & U. Bertram, 1970a): Denture stomatitis. Part I. The etiology in relation to trauma and infection. Acta odont. scand. (In press).

—»— 1970b): Denture stomatitis. Part II: The anti-inflammatory effect of antifungal and prosthetic treatment. Acta odont. scand. (In press).

Cahn, L. R., 1936: The denture sore mouth. Ann. Dent. *3:* 33—36.

Cawson, R. A., 1963: Denture sore mouth and angular cheilitis. Brit. dent. J. *115:* 441—49.

—»— 1964: Yeasts and mycelial phases of candida in oral infections. J. dent. Res. suppl. *43:* 951—52.

—»— 1966: Chronic oral candidosis. In: Symposium on candida infections. Ed. H. I. Winner and R. Hurley. E. & S. Livingstone. Edingburgh and London.

Davenport, J. C., 1969: The distribution of candida in denture stomatitis. (In press).

Degos, M. M. R., 1960: Granulomes moniliasiques. Moniliases papillomateuses et hyperkeratosiques. Bull. Soc. franc. Derm. Syph. *67:* 701—05.

Doyle, J. L., J. H. Manhold & E. Weisinger, 1968: Study of glycogen content and »basement membrane» in benign and malignant oral lesions. Oral. Surg., Oral Path. & Oral Med. *26:* 667—73.

Falin, L. I., 1961: Glycogen in the epithelium of mucous membranes and skin and its significance. Acta anat. (Basel) *46:* 244—76.

Fasske, E., K. Morgenroth & H. Themann, 1967: Cytochemische Untersuchungen zum Kohlenhydratstoffwechsel im Deckeepithel der Mundschleimhaut. Dtsch. zahnärstl. Z. *21:* 580—85.

Firket, H., 1951: Recherches sur la régénération de la peau de mammifères. II^e Partie. Etude histochimique. Arch. Biol. (Liege) *62:* 335—50.

Flanagan, V. D. & K. Porter, 1968: Histochemical studies on papillary hyperplasia of the palate. Oral Surg., Oral Path. & Oral Med. *26:* 660—67.

Guernsey, L. H., 1965: Reactive inflammatory papillary hyperplasia of the palate. Oral Surg., Oral Path. & Oral Med. *20:* 814—27.

Hurley, J. V., 1964: Substances promoting leukocyte migration. In: The acute inflammatory response. Ann. N. Y. Acad. Sci. *116:* 918—35.

Jepsen, A. & E. Winther, 1965: Mycotic infection in oral leukoplakia. Acta odont. scand. *23:* 239—56.

Jensen, S. B., 1967: The influence of endotoxin from oral bacteria on host resistance. Thesis. Århus.

Kapica, L. & F. Blank, 1957: Growth of Candida albicans on keratin as sole source of nitrogen. Dermatologica (Basel) *115:* 81—105.

Kapur, K. & Shklar, 1963: The effect of complete dentures on alveolar mucosa. J. prosth. Dent. *13:* 1030—37.

Lehner, T., 1963: Oral Candidiasis, Oral Pathology in the Child. International Academy of Oral Pathology. New York.

Lehner, T. 1966: Classification and clinico-pathological features of candida infections in the mouth. In: Symposium on candida infections. Ed. H. I. Winner and R. Hurley. E. & S. Livingstone. Edingburgh and London.

Maibach, H. I. & A. M. Kligman, 1962: The biology of experimental human cutaneous moniliasis (Candida albicans). Arch. Derm. *85:* 233—57.

McClary, D. O., 1952: Factors affecting the morphology of Candida albicans. Ann. Mo. Bot. Gard. *39:* 137—65.

Meyer, J. & H. Medak, 1962: Keratinization of the oral mucosa. In: Fundamentals of keratinization. Amer. Ass. Advanc. Sci. Washington.

Nyquist, G., 1952: Denture sore mouth. Acta odont. scand. *10:* suppl. 9.

Neill, D. J., 1965: Symposium denture sore mouth. An etiological review. Dent. Practit. *16:* 135—38.

Page, A. R., 1964: Lymphocyte response. In: The acute inflammatory response. Ann. N. Y. Acad. Sci. *116:* 950—63.

Taschdjian, C. L., 1960: Experimental vaginal candidiasis in mice; its implications for superficial candidiasis in humans. J. invest. Derm. *34:* 89—94.

Turrell, A. S. W., 1966: Ætiology of inflamed upper denture-bearing tissues. Brit. dent. J. *118:* 542—46.

Vergeat, M. P., 1966: Les modifications de la muqueuse buccale sous les prothèses adjointes. Ann. odonto-stomat. *23:* 45—48.

Waisman, M., 1955: Submucous thrush: chronic moniliasis of the buccal mucous membrane. Sth. med. J. *48:* 694—700.

Weiss, M. D., J. P. Weinmann & J. Meyer, 1959: Degree of keratinization and glycogen content in the uninflamed gingiva and alveolar mucosa. J. periodont. *30:* 208—18.

Östlund, S. G., 1953: Om förekomsten av hornskikt hos den dorsale hårda gommens slemhinna. Odont. Revy *4:* 291—96.

—»— 1958: The effect of complete dentures on the gum tissues. Acta odont. scand. *16:*1—43.

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