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DENTURE STOMATITIS
IV. AN EXPERIMENTAL MODEL IN MONKEYS

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An experimental candida infection of the palatal mucosa of 6 Macaca irus was produced by inoculation of C. albicans under an acrylic plate. A diffuse erythema was seen involving the palatal mucosa in contact with the acrylic plate. Infection appeared to be associated with a transformation of the yeast form of C. albicans into the hyphal form. However, histological sections did not show intra-epithelial invasion by hyphae. The inflammatory lesion showed spontaneous healing 2—3 weeks after infection, clinically and histologically. Prolonged topical treatment with Demethylchlortetracycline resulted in a continuous proliferation of candida and a sustained inflammatory reaction, more intense than that caused by candida alone. After re-infection a more intense inflammation appeared as compared with the primary lesion, a fact that may indicate that the experimental candida infection is capable of stimulating delayed hypersensitive responses.

The cause of denture stomatitis is considered to be either trauma (*Nyquist*, 1952) or candida infection (*Cawson*, 1963; *Ritchie et al.* 1969; *Budtz-Jørgensen & Bertram*, 1970 a, b). A traumatic injury to the palatal mucosa is probably a predisposing condition to a candida infection (*Henrici*, 1930; *Lyon & Chick*, 1957; *Lehner*, 1965). It is unknown, however, whether an acrylic plate, in passive contact with the palatal mucosa, i.e. non-traumatic, is an essential factor for the initiation of a candida infection.

In both acquired oral mucous candidiasis (*Waisman*, 1955; *Degos*, 1960; *Jepsen & Winther*, 1965; *Cawson*, 1966; *Lehner*, 1966) and in experimental vaginal candidiasis in mice (*Taschdjian et al.*, 1960) infection is associated with intraepithelial invasion by candida. However, this does not seem to be true in candida — induced denture stomatitis (*Cawson*, 1966; *Budtz-Jørgensen*, 1970).

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The present investigation was designed to produce an experimental candida infection of the palatal mucosa in monkeys by inoculating C. albicans under an acrylic plate. The study has three purposes:

1) To record, clinically and histologically the development and regression of the lesion,

2) To study the significance and effect of topical treatment with tetracycline, and

3) To ascertain whether infection is associated with intraepithelial invasion by C. albicans.

MATERIAL AND METHODS

Six adult monkeys (Macaca irus, 2 male, 4 female) were used. Prior to the experimental procedures palatal swabs were obtained for cultural demonstration of C. albicans. Both first maxillary molars were extracted after which alginate impressions of the upper jaw were taken. Heat-cured acrylic plates, covering most of the hard palate, were constructed to be retained mechanically by means of extentions into the edentulous spaces (Fig. 1).

C. albicans, isolated in a patient with denture stomatitis and cultured on Sabouraud's dextrose agar slants at 37°C for 48 hours was used as inoculum.

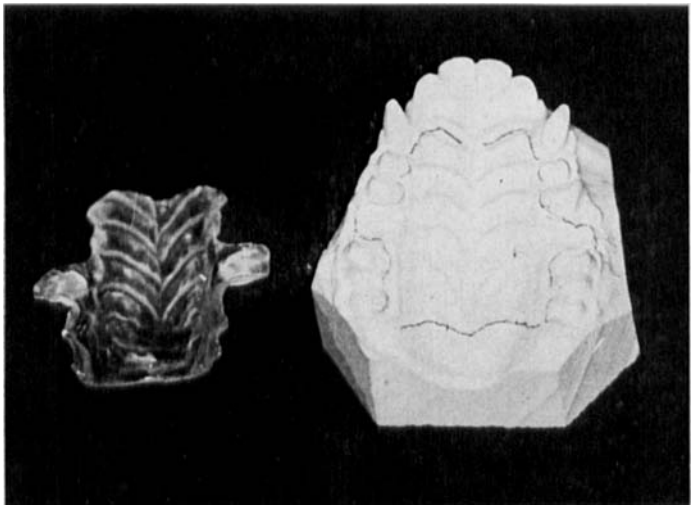


Fig. 1. The acrylic plate used in the experimental inoculation of C. albicans onto the palatal mucosa of monkey (Macaca irus). The dimension of the plate is delinated on the plaster model.

Table I.
Duration of the various periods and the clinical procedures employed in the experiment

	Inoculation	Acrylic plate	Scraping	Biopsy	Photograph	Tetracycline	Length of period
Experimental period I	+		+	+	+		1 week
Control period		+	+	+	+		1–9 weeks
Experimental period II	+	+	+	+	+	+	3–12 weeks
Healing period			+	+	+		2–3 weeks
Experimental period III	+	+	+	+	+		1 week

The growth was harvested with a spatula and 100 mg (wet weight) of candida cells in the yeast phase were inoculated onto the palatal mucosa.

The study was divided into three experimental periods with an intervening control period and a healing period (Table I).

At the onset of *experimental period I* C. albicans was inoculated onto the palatal mucosa without the subsequent insertion of the acrylic plate. In the following *control period* the monkeys wore the acrylic plates without concomitant inoculation of C. albicans. In *experimental period II* C. albicans was inoculated under the acrylic plate. After the primary inflammatory reaction had resolved, the effect of tetracycline treatment was studied by coating the fitting surface of the acrylic plate with Ledermycin® (200 mg 0.5 % Demethylchlortetracycline ointment), coincident with a repeated inoculation of C. albicans. Thereafter tetracycline ointment was applied to the plate with weekly or beweekly intervals up to 6 weeks (Table II).

In *the healing period* the acrylic plate was removed.

In *experimental period III* C. albicans was re-inoculated under the acrylic plate in 4 animals in order to study possible differences in primary and secondary lesions.

The duration of the various sequences varied for the individual animals (Table II). Palatal smears, biopsies, and photographs were taken before and at the termination of each sequence and regularily during the *control period* and *experimental period II*. The smears were fixed in a 1:1 ether/alkohol mixture and stained according to the periodic acid schiff method with hematoxylin as counterstain for identification of hyphae and blastospores. Tissue specimens were removed with a 5 mm biopsy punch from a different site every time. The tissue was immediately fixed in 10 % buffered formalin.

Table II.

Duration in weeks of the various periods of the experiment for the individual animals

Animal	Experimental period I	Control period	Experimental period II		Healing period	Experimental period III
			Total	Tetracycline		
237	1	1	10	5	2	
238	1	1	12	6	2	
239	1	9	3	1	2	1
243	1	5	5	3	3	1
244	1	5	5	1	3	1
245	1	4	5	3	3	1

Sections were prepared with a thickness of 7 μm and stained with hematoxylin, Van Gieson's stain and P.A.S. During all clinical procedures the monkeys were under general anaesthesia obtained by injecting 1.4—1.8 ml Nembutal intraperitonally.

RESULTS

C. albicans was isolated by palatal swabs in 2 animals in very few numbers before the experimental inoculation. The palatal smears showed anuclear epithelial cells and no candida cells were detected. The corresponding histological sections showed an orthokeratotic epithelium with an indistinct stratum granulosum and tall and slender rete pegs (Fig. 2).

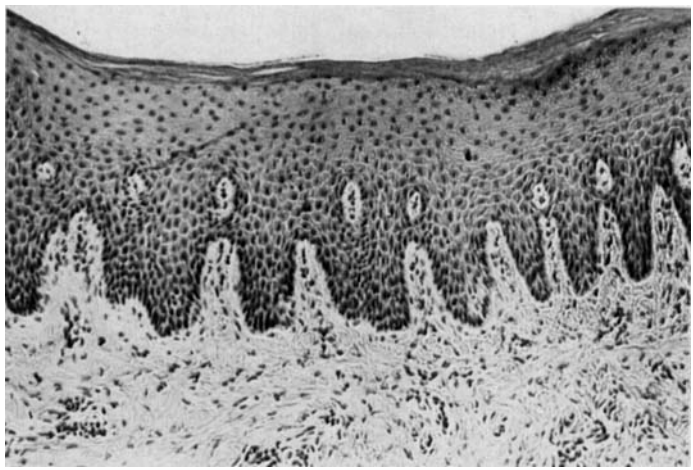


Fig. 2. Photomicrograph showing the histomorphology of the palatal mucosa of Macaca irus. Note orthokeratosis with an indistinct stratum granulosum. Hematoxylin-eosin. 100×.

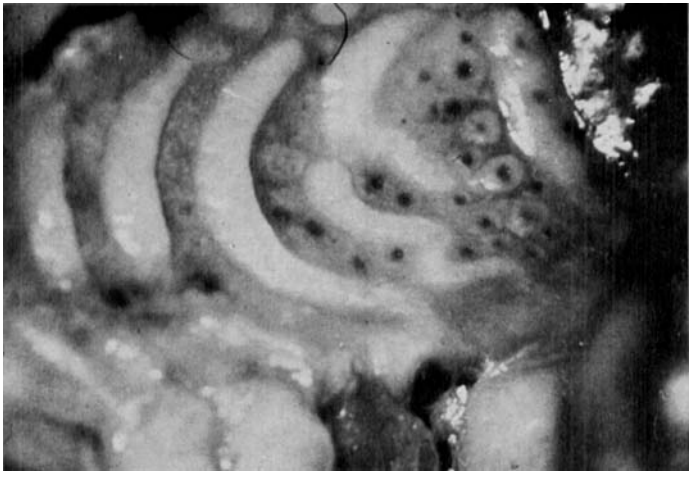


Fig. 3. An inflammatory reaction is seen, localized to the ducts of the palatal mucous glands. It is probably caused by occlusion of the duct orifices. Control period.

Experimental period I

Inoculation with C. albicans onto the non-covered palatal mucosa did not result in inflammatory changes, neither clinically nor histologically. One week after inoculation candida cells were absent in smears.

Control period

Uninoculated acrylic plates were worn for a period varying for the individual animals between 1 week and 2 months (Table II). The monkeys were examined regularily during this period, and there were no inflammatory changes apparent by clinical and histological examination, except for one case which showed elevated and inflamed orifices of the mucous salivary glands (Fig. 3). The corresponding histological sections showed a chronic inflammatory cell-infiltrate around a tangentially cut salivary duct (Fig. 4). During the control period the palatal smears showed anuclear epithelial cells. After 2 to 3 weeks a small number of blastospores were detected but no hyphae.

Experimental period II

One week after inoculation with C. albicans under the acrylic plate a diffuse erythema was seen involving the palatal mucosa in contact with the plate (Fig. 5). Scraping lightly with a spatula produced bleeding of the palatal

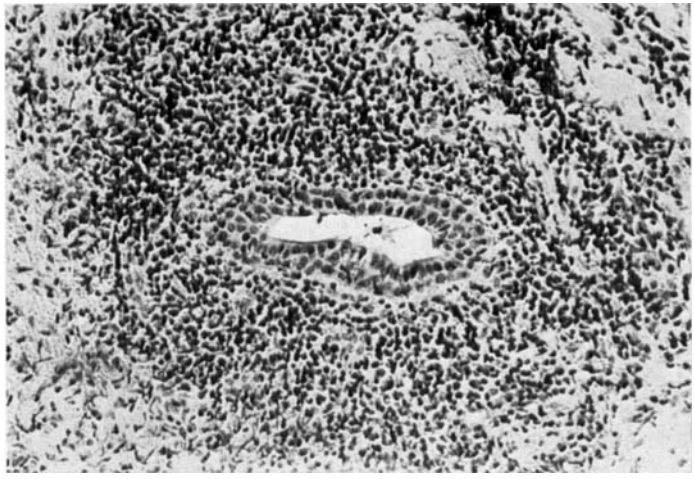


Fig. 4. Photomicrograph showing a chronic inflammatory cell-infiltrate around a tangentially cut salivary duct. Control period. Hematoxylin-eosin. 160×.

mucosa. Microscopically, the palatal mucosa showed a parakeratotic epithelium, intra-epithelial leucocyte infiltration, formation of microabscesses in the subcorneal and prickle cell layers, epithelial atrophy, and a slight, chronic inflammation of the lamina propria, (Fig. 6 a and b). Invasion by hyphae was not detected in P.A.S.-stained sections. The P.A.S.-stained smears

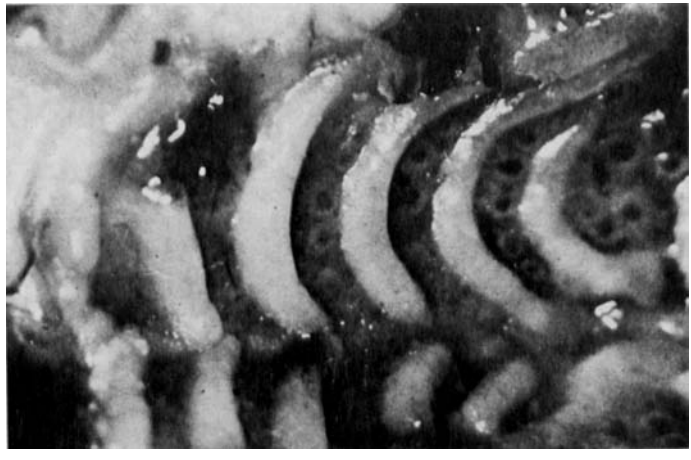
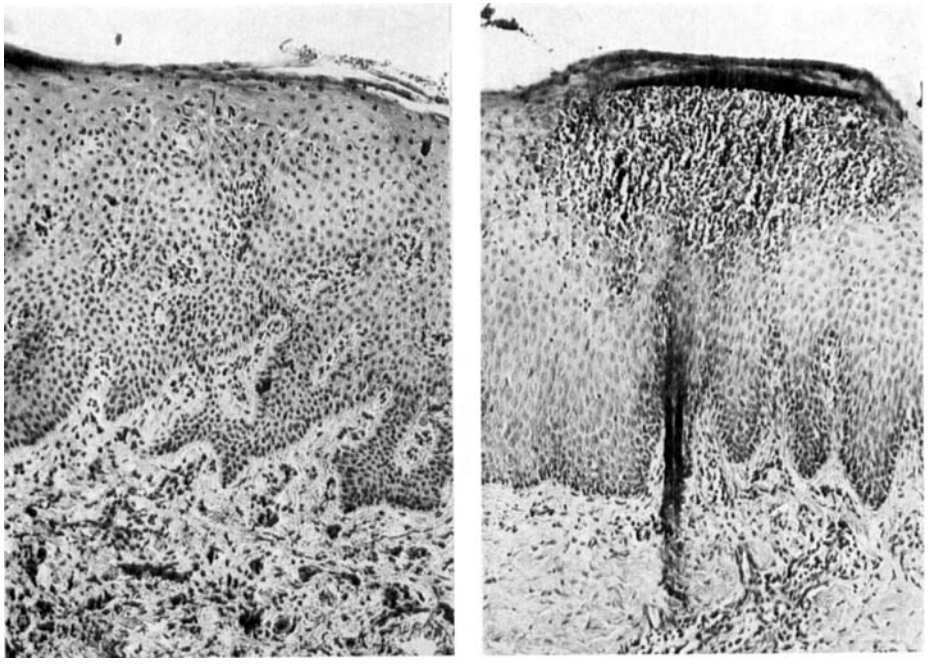


Fig. 5. One week after inoculation with C. albicans the mucosa in contact with the acrylic plate is diffusely inflamed. Inflamed duct orifices are seen as well. Experimental period II.



a b

Fig. 6. Photomicrographs showing the inflammatory changes one week after inoculation with C. albicans.

a) Note parakeratosis and thinning of stratum corneum, epithelial atrophy, and intraepithelial infiltration with leucocytes. Hematoxylin-eosin. 100×.

b) Note formation of an intraepithelial abscess. Invasion by candida is not seen. P.A.S.-hematoxylin.

showed scattered, usually very large hyphae, small numbers of blastospores, leucocytes and nucleated epithelial cells.

During the following 1 to 2 weeks the inflammation subsided even though the animals continued wearing the acrylic plates. An orthokeratotic stratum corneum usually reappeared. The palatal smears revealed hyphae and blastospores in small numbers.

Effect of tetracycline. One week following application of tetracycline ointment to the acrylic plate coincident with a repeated inoculation of C. albicans an intense inflammation was seen (Fig. 7). The palatal smears showed hyphae, blastospores, as well as leucocytes in large numbers, the epithelial cells were nucleated (Fig. 8 a and b). Histologically, extreme epithelial atrophy, microabscesses in the subcorneal and prickle cell layer, and profuse intraepithelial leucocyte infiltration were seen (Fig. 9). The epithelium was slightly hyperplastic and achantotic and there was a moderate chronic inflammation of the lamina propria. Hyphae were not seen invading the epithelium.

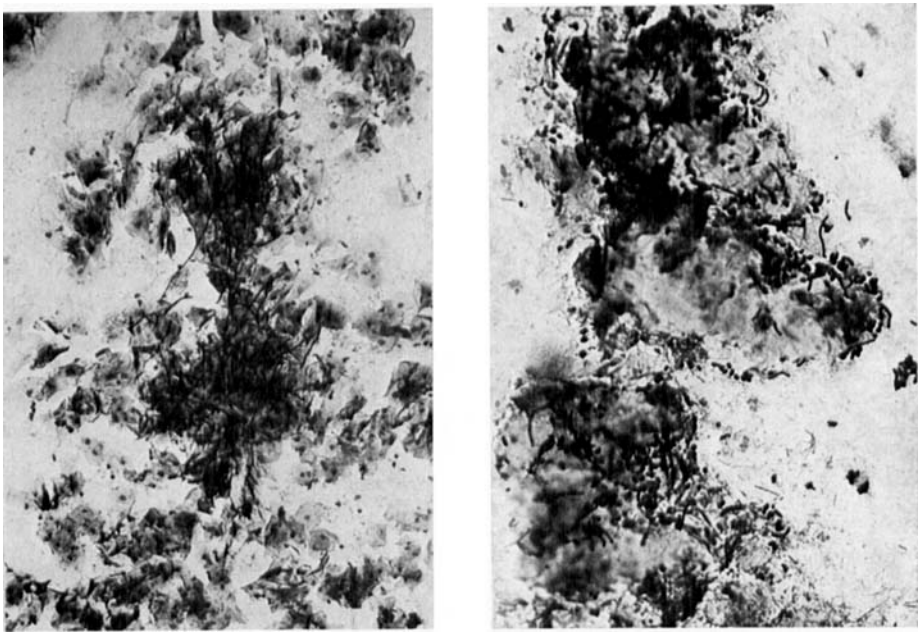
Fig. 7. An intense erythema is seen one week after tetracycline ointment was applied to the acrylic plate, coincident with a repeated inoculation of C. albicans. Experimental period II.

The candida infection was sustained by weekly or beweekly applications of tetracycline ointment without any significant change in the intensity of the inflammatory reaction.

During experimental period II the biopsy wounds healed very slowly. Two or three weeks after the acrylic plate was removed the palatal mucosa showed almost complete healing, clinically and histologically.

Experimental period III

After healing of the mucosa C. albicans was reinoculated under the acrylic plate. One week after inoculation a more intense erythema appeared as compared with the primary lesion. Histologically, a more dense infiltration with chronic, inflammatory cells was seen in the lamina propria (Fig. 10). The palatal smears showed scattered hyphae and blastospores.



a b

Fig. 8. Segments of a palatal smear showing growth and proliferation of C. albicans influenced by treatment with tetracycline ointment. P.A.S.-hematoxylin.

a) Colony of hyphae. 100×.
b) Blastospores and pseudohyphae. 160×.

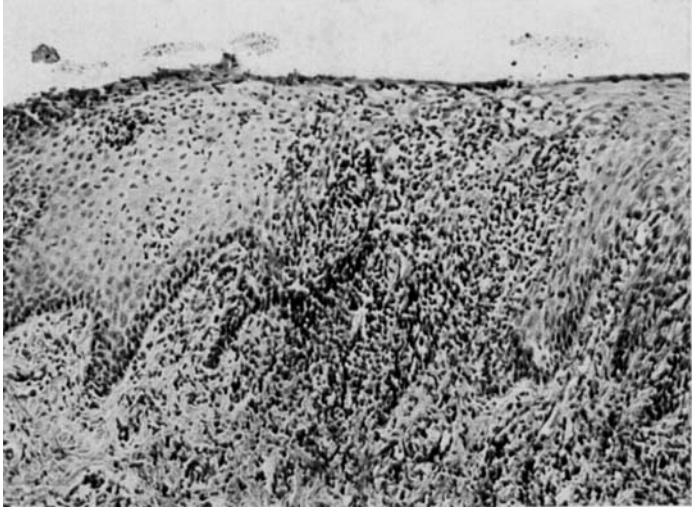


Fig. 9. Photomicrograph showing pronounced inflammatory changes after tetracycline treatment. Note extreme epithelial atrophy and a moderate, chronic inflammation of the lamina propria. Invasion by candida is not seen. P.A.S.-hematoxylin. 100×.

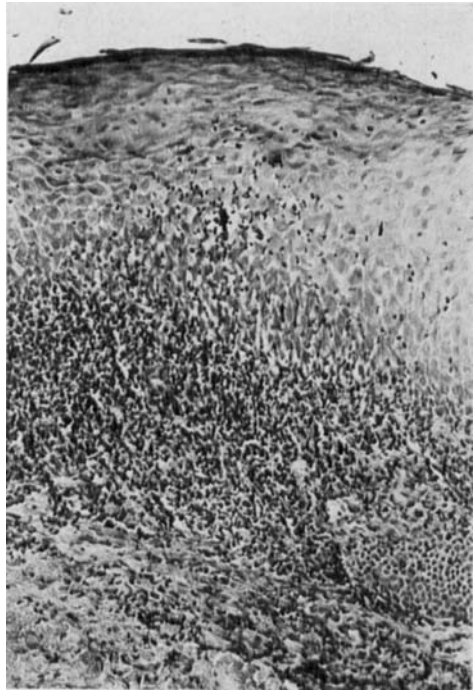


Fig. 10. Experimental period III. Photomicrograph showing the inflammatory changes one week after re-inoculation of C. albicans. Note dense accumulation of chronic inflammatory cells in the lamina propria infiltrating the basal epithelial cell layers. P.A.S.-hematoxylin 100×.

DISCUSSION

Macaca irus was chosen for this study for the following reasons:

1) It was found that this animal is able to wear and tolerate an acrylic plate in the oral cavity,

2) According to *Bowen* (1963, 1965) the oral microflora of M. irus is qualitatively as well as quantitatively very similar to the human one, and

3) Candida albicans is a frequent saprophyte of the oral cavity of the monkey (*Ruch*, 1959; *Bowen*, 1963), monkeys are susceptible to candida infection (*Stockdale et al.*, 1964; *Fiennes*, 1967), and no significant variations have been found in the pathogenicity of candida isolated in humans and monkeys (*Al-Doory*, 1968).

The experimentally-induced palatal candida infection in monkeys showed the same non-specific clinical and histological inflammatory changes as described in acquired candida-induced denture stomatitis (*Budtz-Jørgensen & Bertram*, 1970; *Budtz-Jørgensen*, 1970). Clinically, the experimental

lesion was similar to the generalized simple type of denture stomatitis, showing a diffuse erythema confined to the mucosa in contact with the acryclic plate. None of the animals showed the granular type of inflammation. Histologically, epithelial atrophy and intraepithelial leucocyte infiltration were characteristic features of both the experimental and the acquired infections. Hyphae were not seen invading the epithelium, neither in the experimental nor in the acquired infection. In the latter, however, achantosis, epithelial hyperplasia, and the cellular infiltration of the lamina propria were more pronounced, probably due to the chronicity of the inflammatory reaction. On the other hand, micro-abscesses were seen more frequently in the experimentally-induced lesion.

Although overgrowth of candida in the oral cavity is seen following topical treatment with tetracycline *Sklar* (1961), *Johnston et al.* (1967) and *McKendrick* (1967) maintain that a quantitative increase of C. albicans does not necessarily increase the susceptibility to candida infection. However, *Winner* (1958) and *Maibach & Kligman* (1962), have suggested that endotoxin-like substances, released from an increased number of candida cells, may explain local tissue damage. Both *in vivo* and *in vitro* studies have shown that broadspectrum antibiotics favor the invasive hyphal from of C. albicans (*Beemer et al.*, 1954; *Seligmann*, 1952; *Winther et al.*, 1956). Finally, topically applied tetracycline *per se* may cause damage to the tissues at the site of infection, thus, increasing hyphal invasion (*Hazen et al.*, 1955). In the present study inoculation of candida and a prolonged treatment with tetracycline ointment resulted in a continuous proliferation of candida and a sustained inflammatory reaction, more intense than that caused by candida alone. The more intense inflammation may partially be explained as a delayed hypersensitivity reaction the monkey being sensitised at the onset of *experimental period II.*

Most investigators agree that the hyphal form of C. albicans is primarily responsible for its invasion into tissues (*Taschdjian and Kozinn*, 1957; *Gresham & Whittle.* 1961). However, in studies on experimental systemic candidiasis in mice after intravenous infection there was no evidence that invasiveness could be correlated specifically with either the hyphal or the yeast form of C. albicans (*Louria et al.*, 1962). The palatal smears from the experimental candida infection showed candida almost exclusively in the hyphal form as in candida-induced denture stomatitis (*Cawson*, 1963; *Budtz-Jørgensen & Bertram*, 1970). It is possible that a transformation of C. albicans from the yeast form into the hyphal form may result from inhibition of cell division due to the exhausting of a limiting metabolite (*Kemp & Solotrowsky*, 1962), a low pH and a low oxygen-tension (*McClary*, 1952). A close-

fitting acrylic plate is probably depriving the palatal mucosa of the buffering effect of saliva and free accession of oxygen. Hence C. albicans in the hyphal form would find optimal conditions in the superficial, shedding layers of the epithelium.

The absence of intra-epithelial invasion by candida in the experimental lesion of the palate is in contrast with experimental vaginal candidiasis in mice (*Taschdjian et al.*, 1960). In her study blastospores were seen in the superficial epithelial layers whereas hyphae were penetrating into the deeper sulphydryl-rich layers with a low oxygen tension. On the other hand, experimental cutaneous candidiasis in man (*Maibach & Kligman*, 1962) and dog (*Schwartzmann et al.*, 1965) corresponds well with the palatal candida infection, both in its course and clinical and histological appearance. The skin lesion was produced by inoculating C. albicans under tape, i.e. under experimental conditions probably comparable to those used in the present study. The epithelium appears atrophic in the experimental infection of the palate as well as of the skin. It is a question whether the continuous shedding of epithelial cells is caused by keratinolytic enzymes (*Kapica & Blank*, 1957) or the primary irritant effect of endotoxin-like substances (*Maibach & Kligman*, 1962), released from candida cells accumulated beneath the acrylic plate. The continuous shedding of epithelial cells might explain why candida-invasion of the epithelium is not recognized histologically.

After healing of the mucosa re-infection resulted in a more intense sub-epithelial inflammation when compared with the primary lesion. This may indicate that the experimental infection gives rise to delayed hypersensitivity, though the number of cases investigated were too few to give valid information. *O'Grady et al.* (1967), studying the histological reaction of mouse-thigh lesions to reinfection with C. albicans, found supporting evidence for a delayed hypersensitivity reaction.

The present study has demonstrated that it is essential to cover the mucosa in order to produce an experimental candida infection in the palate of a monkey. However, spontaneous healing was seen whether the plate was removed, or not. It is a question whether regression of the infection is caused by an immunological reaction of the host and/or insufficient environmental conditions for the propagation of the fungi. Topical treatment with tetracycline changed the environmental conditions so that the infection progressed.

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