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STUDIES IN ORAL LEUKOPLAKIAS

III. EFFECTS OF VITAMIN A COMPARING CLINICAL, HISTOPATHOLOGIC, CYTOLOGIC, AND HEMATOLOGIC RESPONSES

by

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For the purpose of clearly defining the lesions under investigation in this study, leukoplakias are considered as any white patches or plaques on the oral mucous membranes that: cannot be removed by scraping; cannot be reversed by removing obvious irritants; and cannot be classified clinically or microscopically as another diagnosable disease. Causes for the occurrence of these leukoplakias are unknown. Their rather frequent appearance creates an important problem in management, since leukoplakias are premalignant lesions. The premalignant nature of oral leukoplakias is based on the following findings: a significant number of oral carcinomas are observed with associated areas of leukoplakia (Table 1); and oral leukoplakias do precede some eventual malignant epithelial transformations (*Sturgis* 1934, *Carr* 1948, *Sarnat & Schour* 1950, *Weisberger* 1957, *Silverman & Ware* 1960 and *West* 1962).

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Table 1

Oral carcinoma associated with leukoplakia.

Investigator	Oral carcinoma Patients studied	Patients with associated leukoplakia
Haym (1961)	62	11 %
Archer (1946)	203	16 %
Hobaek (1946)	1,272	16 %
Silverman (1963)	834	19 %
Martin (1940)	103	25 %
Ullman (1935)	67	30 %
Paymaster (1962)	10,580	32 %
Sharp (1947)	81	38 %
Wilkins (1957)	79	40 %
Weisberger (1957)	275	60 %

Evaluations of leukoplakias are at times difficult and misleading because of: inconsistent clinical and microscopic correlations; problems of adequate tissue sampling by biopsy; and unpredictable conversions from benign appearing epithelial maturation to atypias and malignancy (*Fleming* 1947, *Boyle* 1954, *Kollar et al.* 1954, *Renstrup* 1958, *Silverman & Ware* 1960, *Fryer* 1961, *Shafer & Waldron* 1961 and *Chomet et al.* 1962). Complete removal, then, rather than periodic observations appears to be the treatment of choice from a biologic standpoint. Because of the extent and location of many leukoplakias, removal by surgical or cauterization techniques often is not attempted. Therefore, investigations to find an effective biochemical method to control keratinization is most necessary. The purpose of this report is to describe some clinical, microscopic, and hematologic effects of large doses of vitamin A in a series of patients with oral leukoplakia and to discuss the significance of the findings.

HISTORICAL ASPECTS

Wolbach (1925) first adequately showed in vitamin A deficient rats a tendency to keratinization of many epithelial tissues in which keratin formation did not ordinarily occur. *Frazier & Hu* (1931) reported on some similar lesions associated with deficient vitamin A diets in 15 human cases. Responses to diets rich in vitamin A suggested a relationship to vitamin A deficiency rather

than a lack of other nutritional essentials. These early observations stimulated numerous animal and human investigations on the mode of action of vitamin A and the inhibition of keratin formation by vitamin A and other unsaturated lipoid soluble compounds.

Even in the absence of a general vitamin A deficiency it was brought out that large amounts of vitamin A topically had a direct, local and probably non-specific effect on epidermis (*Flesch* 1952, *Flesch* 1953, *Reiss & Campbell* 1954). This hypothesis was based on the observations that several dermatologic conditions characterized by keratin plaques were beneficially treated by vitamin A administered topically. Of additional interest was *Kahn*'s observation (1954) that vitamin A applied topically to vaginal mucosa of castrate rats diminished the expected vaginal cytologic cornification response to estrogen administration. Other epithelial effects of vitamin A were brought out by *Fell & Mellanby* (1953) and *Fell, Mellanby & Pelc* (1954) in tissue culture studies with embryonic chick skin, and *Lawrence & Bern*'s study (1961) on hamster cheek pouch. Both groups demonstrated the capacity of vitamin A to induce mucous metaplasia in normally keratinizing epithelium.

Table 2

Topical vitamin A and oral leukoplakia series.

Investigator	Year	No. pts.	Dosage	Time (weeks)	Disappearance		Toxicity
					Complete	Partial	
Mulay	1958	10	300,000—450,000u	8—26	2	5	0
Zegarelli	1959	25	600,000—900,000u	6—18	2	20	0
Fryer	1961	16	450,000u	7—45	1	3	5a)
Smith	1962	208b)	75,000u	13—26	38	150	0
		121c)	150,000u	13—39	9	41	0
		54d)	300,000—600,000u	13—26	0	14	0
		34e)	300,000—600,000u	13—26	0	0	0

a) "Possible" toxicities. b) minimal hyperkeratosis.

c) moderate hyperkeratosis. d) severe hyperkeratosis. e) dyskeratosis.

It was quite apparent that human clinical trials to investigate the reversal of oral leukoplakias by topical vitamin A were in order. Table 2 summarizes the findings of some studies. There was irregularity as to clinical and histologic diagnosis, removal of irritants, quantitation of improvement, dosages and time factors, use of experimental and control groups and follow-up data. In spite of this, the findings make it evident that in some patients large doses of topical vitamin A are able to alter keratinization and at least temporarily induce partial or complete disappearance of leukoplakia.

PRESENT STUDY

Sixteen patients from the Oral Medicine Clinic, University of California School of Dentistry, were selected for the study on the bases: 1) that the leukoplakic lesions involved more than one intraoral surface; 2) that the lesions had been present or noticed for at least 6 months; and 3) that the lesions displayed no tendencies toward periodic remissions, spontaneous improvements or reversal upon removing local irritants (dentures, tobacco, spicy foods, etc.) or correcting existing systemic disease. The protocol included the following:

1. medical and dental history;
2. observation period after removing any irritants or correcting systemic disease;
3. photograph;
4. exfoliative cytology (stained by a modification of the Papanicolaou method (*Pharr, Wood & Traut,* 1954);
5. biopsy (stained with hematoxylin and eosin);
6. blood sample for serum vitamin A and carotene (vitamin A estimation based on photometric measurement of colored complex formed by vitamin A and activated glycerol dichlorohydrin (*Sobel & Werbin* 1946);
7. vitamin A tablets (Vi Dome A)*, 75,000 units, 8 to 12 daily for at least 5 weeks (to be dissolved while being held against lesion);
8. before discontinuing treatment: cytology, biopsy, blood sample and photographs to be obtained; and

* Vi Dome A tablets donated through the courtesy of Dome Chemicals Inc., N.Y.

9. periodic follow-up observations, with elective option to reinstitute therapy, alter dose and route of administration, administer placebos and follow vitamin A blood levels.

Findings

Table 3 summarizes 27 studies on 16 patients. All of the 16 subjects had lesions that microscopically demonstrated hyperkeratosis; one of these also showed evidence of epithelial atypia (pt. no. 6). Under the conditions of this study, large doses of vitamin A topically in the form of troches were able to induce complete remission of the clinical lesion in 4 patients (Fig. 1). In these cases of complete remission, the leukoplakic mucosa was replaced by an erythematous tissue within 3 weeks. In 3 of these 4 patients upon discontinuing the vitamin A or reducing the daily dose significantly, the lesions returned to their original size, character and location within 2 weeks. In the other patient (no. 15), marked partial remission persisted during a 4 month follow-up observation period (Fig. 2).

Partial remissions, as measured by disappearance of part of a lesion or undisputable thinning of a white patch, were observed in 3 subjects (Fig. 3). In 3 other patients, there was some improvement (Fig. 4), but it was so slight that these instances were recorded as "unchanged". In the other 6 patients, the conditions of this study influenced no beneficial effects in their lesions. Not a single case during treatment became clinically or symptomatically worsened. However, one patient (no. 6) reported with an obvious squamous carcinoma in the leukoplakic area 9 months later (Fig. 5).

Clinical remissions were reflected microscopically by diminution or absence of hyperkeratosis (Fig. 6). There was no apparent influence on the amount or type of inflammatory infiltrate. A mucous metaplasia of the floor of the mouth where the troches were held occurred in patient no. 5 (Fig. 7). Two other patients (nos. 7 and 14), in whom complete remissions were induced, demonstrated end-of-treatment microscopic changes that appeared to be the beginnings of mucous metaplasias. There were no quantitative microscopic changes seen for any patient in the absence of clinical alterations.

Table 3

27 studies on 16 patients with leukoplakic lesions.

Pt.	Age	Sex	Sites	Dose per day (Internat. units)	Time (Weeks)	Serum Vit. A Increase end of treatment	Clinical change	Side effects	Follow-up
1	25	♂	Gingiva	600,000	6	--	None	None	Excised 2 months later
2A				600,000	6	6X	None[1])	None	
2B	58	♀	Tongue, gingiva	675,000	13	—	None	None	
2C				900,000	6	0	None	None	7 months without change
3	49	♂	Cheek, gingiva	600,000	6	4X	None[1])	Rash	2 years without change
4	65	♂	Tongue, lips	600,000	6	2X	None	None	
5A				600,000	6	12X	None	None	
5B	70	♀	Tongue, floor, cheeks	750,000	6	12X	Partial remission	Rash, G. I.	
5C				600,000	15	4X	None	None	Excision, skin graft
6	47	♂	Floor, gingiva	600,000	6	14X	None	None	Sq. ca. 9 months later
7A				600,000	6	7X	None[2])	None	
7B	59	♂	Palate, floor, gingiva	900,000	5	10X	Complete remission	None	Recurred in 2 weeks
7C				600,000	4	—	Complete remission	None	Recurred in 2 weeks
8	45	♂	Gingiva	600,000	6	4X	None	None	
9	56	♀	Floor, gingiva	300,000	6	0	None	G. I.	2 years without change

10A	73	♀	Tongue, cheeks	900,000	6	4X	None[1])	Rash	
10B				600,000	5	4X	None	Rash	5 months without change
11	61	♀	Floor, cheeks, gingiva	600,000	5	—	None	None	1½ years without change
12	46	♂	Palate, gingiva	600,000	12	19X	Partial remission	Rash	Recurred 2 weeks; 4 months without further change
13	57	♀	Cheeks, palate	600,000	5	11X	Partial remission	None	Recurred 2 weeks; 5 months without further change
14A[3])	80	♂	Cheeks, palate gingiva	600,000	5	10X	Complete remission	None	Recurred 2 weeks
14B				600,000	1	—	Complete remission	None	Recurred 2 weeks
14C				300,000	2	—	None	None	
14D				450,000	2	—	Complete remission	None	Recurred 1 week
15	70	♂	Cheeks, floor, tongue	900,000	5	8X	Complete remission	Rash	1 month slight recurrence; 3 months without further change
16A	61	♂	Cheeks	600,000	3	—	Complete remission	G. I.	Recurrence 2 weeks
16B				450,000	6	—	None	G. I.	Excised 2 months later

[1]) slight improvement; [2]) left in dentures; [3]) 4 previous oral carcinomas.

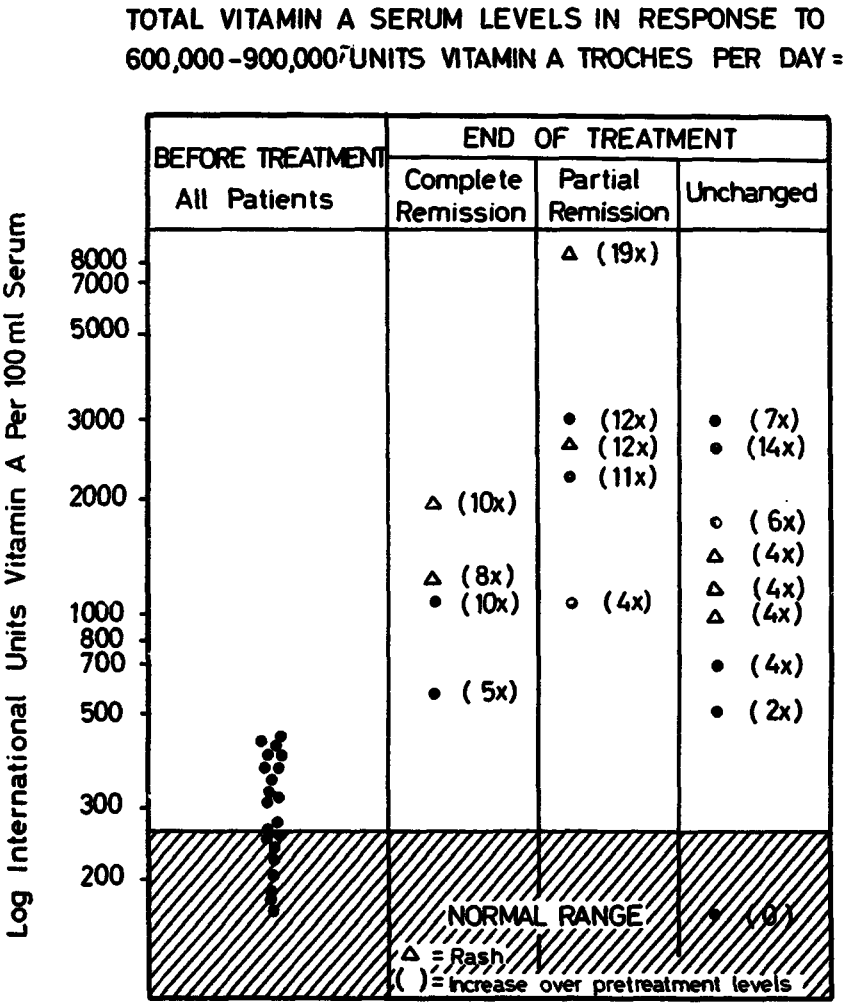

TABLE 4

Table 4 illustrates the blood responses to the vitamin A in troche form. All pretreatment vitamin A blood levels were within the normal range. This is consistent with the clinical findings, which together, then, firmly indicate no evidence of vitamin A deficiencies among this group. During treatment either local or intestinal absorption is highly efficient as reflected by the increased blood concentrations. However, correlations of blood levels and remissions were not apparent. When end-of-treatment

blood levels were low or unchanged, careful interrogations of the patients did not allow any explanation.

The major toxic side effects were those of rash and pruritus, which were occasioned in 5 patients. Upon withdrawl of the troche, the signs and symptoms would disappear within 2 weeks. The regressions coincided with return to normal of the blood levels, which indicates the efficiency of the body to store or excrete abnormally high amounts of circulating vitamin A. Since lower dosages and placebos with the same chemical base would not produce any lesions or complaints in the same patients, it was concluded that the changes were toxic and not allergic manifestations of vitamin A.

Three patients complained of gastrointestinal upsets of minor magnitude periodically during treatment. There were no other side effects during or after treatment.

Serum carotene was essentially unaffected by the vitamin A treatment with "before" and "end-of-treatment" levels being essentially unchanged and all within the normal range (Table 5).

Table 5

Serum carotene levels in response to 600,000—900,000u vitamin A troche per day.

Normal range: 50—300 ug %.

	No. specimen	Range	Average
Before treatment (all patients)	21	76—281 ug %	136 ug %
End of treatment (all patients)	20	58—234 ug %	130 ug %

Cytologic smears obtained from the same area of each lesion before and at the end of treatment were screened for basophilia, acidophilia and presence of nuclei. Reproducible counts per 100 cells were recorded for all patients and compared with clinical responses. The groups that showed partial or complete remissions also reflected this change cytologically with increased basophilia, decreased cornification and a decrease in denucleated cells

Table 6

Cytologic response in patients receiving 600—900,000u vitamin A troche per day.

Result	Patient no.	Site	Before treatment Cells/100				End of treatment Cells/100			
			Blue-green	Pink-red	Yellow-orange	De-nucleated	Blue-green	Pink-red	Yellow-orange	De-nucleated
Partial remission	5A	Floor	0	2	98	94	3	25	72	14
	12A	Palate	5	2	93	88	13	7	80	76
	13	Cheek	32	14	54	48	61	12	27	11
Complete remission	7B	Palate	15	18	67	44	40	11	49	22
	14A	Cheek	11	48	41	0	94	5	1	0
	15	Cheek	35	20	45	25	71	10	19	8
	16	Cheek	55	18	27	11	86	11	3	3

(Table 6). The patients who showed no clinical changes had erratic cytologic fluctuations varying from "no changes" to "slight increases in basophilia". The latter alteration may reflect an influence of vitamin A on keratinization that is present but not of great enough magnitude to change the clinical appearance of a lesion.

All patients were asked about problems of dissolving the troches. Although the times for each individual patient were uniformly consistent, patient to patient dissolution times varied from 7 to 60 minutes. No correlations were evident between this physical characteristic and clinical responses.

Although all 4 of the patients responding with complete remissions were males, the series is far too small for this event to be considered any more than a coincidence.

Histories on the 16 patients revealed: 9 smoked tobacco and one previously took snuff; 3 wore dentures; 2 had mild adult hypothyroidism; 1 had mild adult-onset diabetes; no excess use of alcohol; no luetic history (in addition, serologies were performed and all were negative); and dietary habits were not abnormal. There appeared to be no obvious correlation in any of these patients between the above findings, location of leukoplakia and responses.

DISCUSSION

From this study, as well as from other clinical investigations, it is quite evident that large doses of vitamin A given locally may induce partial or complete remissions of oral leukoplakia. However, there has been no standardized data developed from this or other studies that would allow prediction of those leukoplakias that may respond or a time-dosage schedule necessitated for this event.

Vitamin A Status.

Clinical evaluations in our patients, including blood studies for total vitamin A activity, indicate that none of the subjects were in a vitamin A deficient status. Of interest in support of this observation was the report by *Hume & Krebs* (1949) of an intensive study in which an attempt was made to induce vitamin A de-

ficiencies in 16 volunteers by rigid dietary restrictions. In only 4 subjects, after 12 to 18 months, pronounced drops in blood vitamin A levels were detected. These 4 volunteers demonstrated only mild concomitant clinical changes of hypovitaminosis A and no oral changes were found. *Wahi et al.* (1962), at Agra, India, compared blood vitamin A concentrations of 60 normals to 123 subjects with oral leukoplakia and 555 with oral cancers. The results among the 3 groups were 160 ± 25, 121 ± 19 and 102 ± 14 i.u. vitamin A per 100 ml serum, respectively. Although they observed that deficiencies of vitamin A exist in patients with oral leukoplakias and oral cancers, the data may reflect only patient-group variations not correlated with cause-effect relationships or vitamin A deficiencies.

Route of Action.

Our study indicates that vitamin A in large dosages in troche form effectively increases plasma vitamin A levels by oral and/or intestinal absorption. However, the high vitamin A concentrations attained had no apparent direct relationship to clinical remissions. This raises several points to be considered. First, evaluations performed for vitamin A represent total concentrations and do not necessarily reflect comparable levels of biologically active forms or tissue concentrations (*Harris* 1960). *Dowling* (1961) illustrates one aspect of the unknown nature of active principles in vitamin A metabolism by an experiment on vitamin A deficient rats. In these animals, dosages of vitamin A acid would induce normal growth, but it would not correct the blindness sequelae. Second, *Fitzgerald et al.* (1961) showed variations in blood vitamin A levels within individual volunteers after oral administration of single ampules of either 250,000 units of vitamin A acid or alcohol. They determined the differences to be dependent upon intestinal absorption rates, fasting states and fat content of meals in post-absorptive specimens. Since our patients were on prolonged, high and fractionated dosages of vitamin A, it is felt that the above mentioned physiologic influences cited by *Fitzgerald et al.* may have little affect on our blood level determinations, even though single post-absorptive specimens at irregular time intervals were obtained for each evaluation.

Evidence to support a theory of local tissue accumulation by direct absorption is based on 2 of our patients (nos. 7 and 14), who repeatedly would respond to the troche form, but would experience no remission when given equally large dosages in capsule form as the palmitate. The administration by capsule form, in equal unit dosages to the troches, induced higher total vitamin A blood levels than those attained with absorption from the troche form. Even further support of local action is demonstrated by an observation in patient no. 7: complete remission from the use of troches was precluded when the patient would leave in his upper denture, which insulated his palatal lesions from any local contact with the vitamin. However, *Wulf* (1957) found that peroral administration of 100,000[u] vitamin A daily for 12 weeks to 20 patients with oral leukoplakia induced 2 complete and 16 partial remissions. In 2 recurrences, reinstitution of treatment caused improvement. In comparison with conflicting reports on oral leukoplakia and systemic administration, vitamin A given systemically is therapeutically effective in several types of dermatoses characterized by abnormalities of keratinization (*Burgoon et al.* 1963).

Mode of Action.

Based upon the clinical effectiveness of vitamin A in the troche form and the vitamin A status of the patients, it becomes apparent that high tissue concentrations of vitamin A are necessary to interfere with keratinization and influence remissions. When clinical responses do take place, they are evident within 2 weeks. As a possible explanation, one might speculate that vitamin A or one of its degradation products biologically influences basal and differentiating squamous cells whose nuclei may synthesize keratin (*Pelc* 1959) and therefore, influences future epithelial characteristics. This event combined with an epithelial turnover rate of probably somewhat less than 2 weeks may allow shedding of the hyperkeratotic cells and a return to clinical normalcy.

Human as well as animal studies indicate that vitamin A in either systemic or topical form, up to certain toxic ranges, will increase the mitotic index (*Lawrence & Bern* 1958 and *Sherman* 1961). The assumption has therefore been made that this phenomenon increases the turnover rates, which in turn precludes

cornification because of lack of adequate time for keratin formation. The mechanism of this postulated growth promoting affect is unknown.

Studies on humans and different animal species indicate that other unsaturated fat soluble compounds may achieve the therapeutic effects of vitamin A (*Flesch* 1952 and *Flesch* 1953). Yet, many compounds with similar structure to vitamin A do not possess this activity. Based on animal and *in vitro* observations, *Balakhovsky & Drozdova* (1957) showed that carotenoid polyenes antagonize the catalytic action of copper in the oxidation of cysteine to cystine. Therefore, it has been assumed that a yet unidentified intermediary metabolic degradation product of vitamin A may interfere with sulfhydryl metabolism, thus impairing the formation of keratin.

To illustrate the variety of potential explanations, *Dingle* (1961) demonstrated the possibility of how an enzyme may act at a subcellular level to affect the composition of extracellular material. He promoted *in vitro* the release of a bound protease from the liver of young adult rats by the action of vitamin A. This protease possessed the capacity to break down the protein-polysaccharide complex of cartilage matrix. *Wolf* (1961) showed by an *in vitro* model using S^{35} the dependence of mucopolysaccharide biosynthesis on vitamin A. Further, he traced this effect to an enzyme fraction. Comparing this finding with Dingle's, there is an indication that differential effects of vitamin A are reflections of distinctive patterns of enzyme distribution.

Hypervitaminosis A.

As already has been indicated, keratinization can be interrupted at least temporarily, and in an unpredictable fashion, by high dosages of vitamin A locally. It might be further speculated, that if the dosages would have been raised to produce even higher local concentrations, keratin formation in all patients with leukoplakia possibly could have been controlled. Because of the high dosages used, toxic manifestations must be considered. Reports by *Raaschou-Nielsen* (1961) and *Stimpson* (1961) relate chronic intoxications with vitamin A in 9 patients ranging in ages from 18 to 67. Dosages varied from 100,000 to 600,000 units per day

through treatments that covered from 2 to 102 months. Signs and symptoms included bone and joint pains, fatigue and insomnia, dryness and fissuring of the lips, anorexia, weight loss, skin lesions, pruritus, loss of hair, bleeding and hepatomegaly. Onset of signs and symptoms after starting vitamin A therapy varied within these patients from 6 weeks to 3½ years; relief was effected from 3 to 17 days after discontinuing vitamin A.

In the present study, the most serious side effect was that of a rash and associated pruritus. This would only persist until the blood concentrations were lowered to certain levels. This was efficiently accomplished within 2 weeks after withdrawing the drug either by liver storage or urinary and intestinal excretion of metabolic breakdown products (*Wolf & Johnson*, 1960). There were no complaints or signs of eye changes or bone tenderness from the patients in this study. Other oral leukoplakia studies referred to earlier in this paper (see Table 2) reported no well defined toxic manifestations associated with administration of vitamin A. In *Burgoon*'s study (1963), adults were given 400,000 to 1,000,000u vitamin A daily for 1 to 8 months. The only toxic manifestation was a pruritus in 1 patient.

Several studies (see Table 2) used lower dosages over a longer period of time with some apparent reversal of leukoplakia. This again may indicate that the active mechanism, whether by enzyme inhibition or production, may depend upon critical local tissue levels of a specific biologically active form or degradation product. In the patients who responded in our group, none showed remission with dosages under 450,000u per day. However, this involved only short periods of administration and observation.

Cytology.

Cytology at present serves two purposes: first, as a screening procedure for abnormal cellular morphologic changes; and second, as a potential sensitive indicator of the degree of keratinization of a tissue in question. This latter evaluation is based upon the relative numbers of shedding surface cells that demonstrate cytoplasmic basophilia or acidophilia and nucleation. The differentiations seen in cytologic smears are not similarly reflected in studying histomorphology of tissue sections. This

raises two questions. First, for any particular intraoral site, what cytologic ratio of keratinized to unkeratinized cells is necessary to clinically produce a white patch? And second, do cytoplasmic staining reactions to acidophilic dyes in exfoliative cytologic techniques accurately or necessarily reflect formation of keratin that may be the basis of a leukoplakia? The physical and chemical properties of oral keratins must be analyzed completely before answers are forthcoming.

Histopathology.

Leukoplakias are clinical manifestations primarily due to hyperkeratoses. However, histologic studies demonstrate various types of cornification: hyperparakeratosis, hyperorthokeratosis and incomplete hyperparakeratosis. Some differences in these histomorphologic types indicate the importance of careful classification. In the Department of Oral Pathology at the Royal Dental College in Copenhagen, it was found that: hyperparakeratotic lesions exhibited a definite higher mitotic activity than hyperorthokeratotic lesions; acid phosphatase was found in hyperorthokeratoses and not in hyperparakeratoses; and epithelial atypia was demonstrated only in cases of hyperkeratoses classified as hyperparakeratosis.

The presence or absence of inflammation showed no characteristic patterns in relation to a hyperkeratosis either before or at the end of treatment. This agrees with *Renstrup*'s observation (1958) of an inconclusive relationship between hyperkeratosis and inflammation after histologically examining 80 patients with leukoplakia. It appears that inflammation and keratinization may be coincidental and not have a cause-effect relationship. Two further points of interest to support this assumption are: observations of a marked absence of inflammation in the subepithelial tissue of keratinizing cysts (*Pindborg et al.* 1962); and a contrasting finding in lichen planus of hyperkeratosis and a subepithelial accumulation of lymphoid cells.

It has not been reported previously that topical vitamin A induced a human oral epithelial mucous metaplasia. The significance of this change in relationship to carcinogenesis, biochemical alterations and histochemistry is not known. However, it further indicates the biologic importance of vitamin A and re-

lated compounds in the treatment of a variety of diseases whose pathogenesis appears to be centered about keratinization and dehydration.

Significance.

The extreme variabilities in types, extent and location of oral leukoplakias preclude adequate clinical studies to evaluate the therapeutic effectiveness of vitamin A on keratinization or establish with any certainty time-dosage schedules. Of importance, however, is the fact that a substance is available that in some manner at least temporarily interferes with keratinization. This biologic property furnishes a model for further basic investigations of this poorly understood biochemical mechanism by permitting detailed studies during stepwise controlled development and prevention of hyperkeratosis. As the data on leukoplakias, keratinization and vitamin A accumulates and is systematized, the enigmatous metabolic pathways and controls will unfold. This understanding may eventually allow drug control of this process. The significance from a public health point of view may be the preventive aspects of oral carcinoma, if the statistical association between these two pathological processes reflects a true cause-effect relationship.

SUMMARY

For the purpose of clearly defining the lesions under investigation in this study, leukoplakias are considered as any white patches or plaques on the oral mucous membranes that: cannot be removed by scraping; cannot be reversed by removing obvious irritants; and cannot be classified clinically or microscopically as another diagnosable disease. Twenty seven studies on 16 patients with oral leukoplakias were performed, giving daily fractionated dosages of vitamin A acetate troches, totalling 300,000 to 900,000 units per day, for durations varying from 1 to 15 weeks. Four patients had complete remissions and 3 had only partial disappearance of their lesions. In all instances, after withdrawl of vitamin A, there were partial or complete recurrences of the leukoplakic lesions.

Clinical remissions were reflected by microscopic changes of diminution of hyperkeratoses and/or mucous metaplasia. Cyto-

logic scrapings appeared to reflect the influence of vitamin A on keratinization by showing increased basophilia and nucleation at the end of treatment as compared to pretreatment counts.

Serum levels of total vitamin A were markedly increased at the end of treatment, but there were no apparent correlations between these concentrations and clinical remissions. Five patients demonstrated skin lesions and pruritus, which disappeared within 2 weeks after withdrawing the vitamin.

These results are discussed in regard to mode and routes of action, vitamin A status of the patients, and investigative and clinical significance.

RÉSUMÉ

RECHERCHES SUR LES LEUCOPLASIES BUCCALES

III. EFFETS DE LA VITAMINE A. COMPARAISON DES RÉACTIONS CLINIQUES, HISTOPATHOLOGIQUES, CYTOLOGIQUES ET HÉMATOLOGIQUES

Dans le but de définir clairement les lésions étudiées dans ce travail, est considérée comme leucoplasie toute plaque blanche sur la muqueuse buccale ne pouvant être enlevée par grattage, ne pouvant rétrocéder par suppression d'irritants manifestes, et ne pouvant, ni du point de vue clinique, ni du point de vue microscopique être classée comme étant une autre affection susceptible d'un diagnostic. Vingt-sept études ont été faites sur 16 patients présentant des leucoplasies buccales, en administrant des doses journalières fractionnées de tablettes d'acétate de vitamine A totalisant 300.000 à 900.000 unités par jour pendant une durée variant de 1 à 15 semaines. Quatre des patients ont présenté une guérison totale, et 3 des patients ont seulement présenté une disparition partielle des lésions. Dans tous les cas, il s'est produit, après arrêt de la vitamine A, une récidive partielle ou totale des lésions leucoplasiques.

Aux améliorations cliniques répondaient des modifications microscopiques consistant en une diminution des hyperkératoses et des métaplasies muqueuses, ou de l'un de ces processus. Au point de vue cytologique, les frottis par grattage reflétaient l'influence de la vitamin A sur la kératinisation, en montrant une

augmentation de la basophilie et de la formation nucléaire à la fin du traitement par comparaison avec les numérations faites avant traitement.

Les taux de vitamine A totale dans le sérum étaient notablement augmentés à la fin du traitement, mais il n'y avait pas de corrélation apparente entre ces concentrations et les améliorations cliniques. Cinq des patients ont présenté des lésions cutanées et du prurit, disparaissant en l'espace de 2 semaines après la suppression de la vitamine.

Les auteurs discutent ces résultats en ce qui concerne le mode d'action, le bilan du patient en vitamine A et en ce qui concerne leur signification expérimentale et clinique.

ZUSAMMENFASSUNG

UNTERSUCHUNGEN ÜBER LEUKOPLAKIEN DER MUNDSCHLEIMHAUT

III. VERGLEICHENDE KLINISCHE, HISTOPATHOLOGISCHE, CYTOLOGISCHE UND HÄMATHOLOGISCHE UNTERSUCHUNGEN ÜBER DIE WIRKUNG DES A-VITAMINS

Um die in dieser Arbeit untersuchten Krankheitszustände klar zu definieren, so werden alle weissen Flecke oder Plaques auf der Mundschleimhaut als Leukoplakien betrachtet, die: nicht abstreifbar sind, nicht durch Beseitigung von deutlichen Irritamenten geändert werden können, und nicht klinisch und mikroskopisch als andere diagnostizierbare Krankheitszustände klassifiziert werden können. Es wurden bei 16 Patienten mit Leukoplakien der Mundschleimhaut 27 Untersuchungen ausgeführt. Im Laufe von 1—15 Wochen wurden täglich in fraktionierten Dosen Vitamin A Acetat-Lutschtabletten gegeben, jeden Tag 300.000—900.000 Einheiten. Bei 4 Patienten trat eine vollständige Remission ein, bei 3 Patienten nur eine teilweise. In allen Fällen sah man nach der Unterbrechung der A-Vitaminbehandlung teilweise oder vollständige Rezidive der Leukoplakien.

Klinische Remissionen spiegelten sich im Mikroskop durch Verminderung der Hyperkeratosen und/oder der Schleimhautmetaplasie wieder. Cytologische Abstriche scheinen den Einfluss des A-Vitamins auf die Verhornung zu zeigen, indem eine stärkere Basophilie und Nukleation am Ende der Behandlung im Vergleich mit den Befunden vor der Behandlung beobachtet wurden.

Der totale A-Vitaminspiegel im Blutserum war bedeutend höher am Ende der Behandlung; aber scheinbar gab es keinen Zusammenhang zwischen Serumkonzentration und klinischer Remission. 5 Patienten zeigten Hautaffektionen und Pruritus, welche 2 Wochen nach der letzten Vitamineingabe wieder verschwanden.

Diese Ergebnisse werden mit Bezug auf Wirkungsmodus und Wirkungswege, A-Vitaminstatus der Patienten, und Bedeutung für Forschung und Klinik diskutiert.

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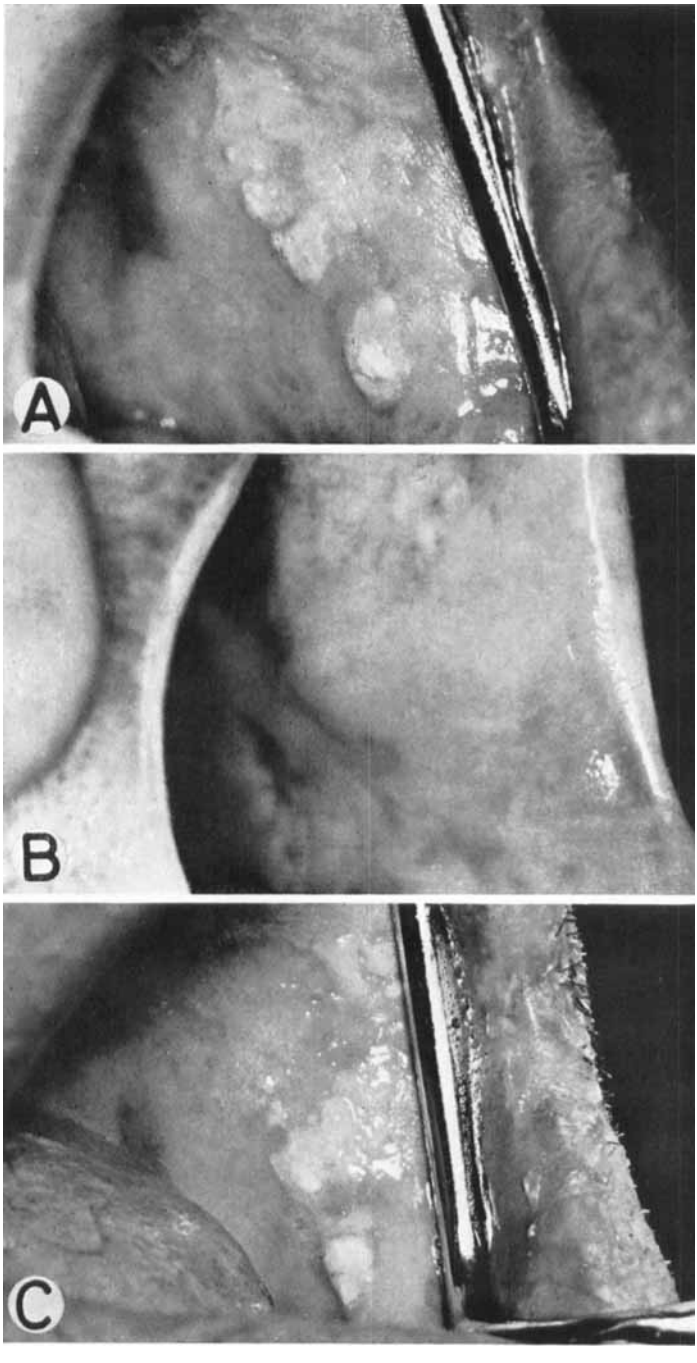
PLATES

Figure 1.

A. Leukoplakia of left buccal mucosa, before vitamin A administration (patient no. 14).

B. Same patient, demonstrating complete remission of his lesion, 2 weeks after 600,000 i. u. vitamin A daily in fractionated troche dosages.

C. Same patient, 2 weeks after discontinuing vitamin A, demonstrating recurrence of lesion with similarity to original appearance.


A
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C

Figure 2.

A. Leukoplakia, right buccal mucosa, before vitamin A administration (patient no. 13).

B. Same lesion, demonstrating partial remission after 600,000 i.u. vitamin A daily for 5 weeks.

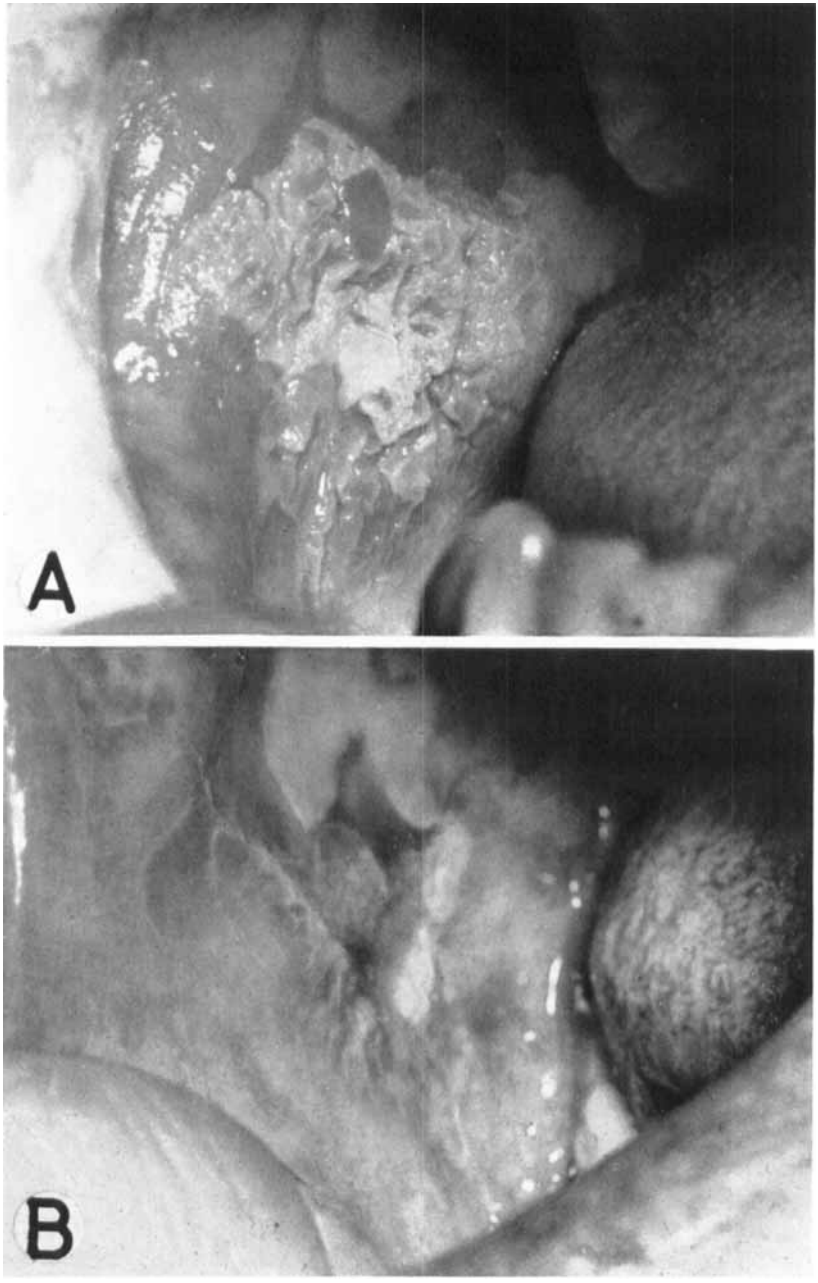
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Figure 3.

A. Leukoplakia of the tongue before vitamin A administrations (patient no. 10).

B. Same lesion, demonstrating slight regression (encircled area) after 900,000 i. u. vitamin A daily for 6 weeks.

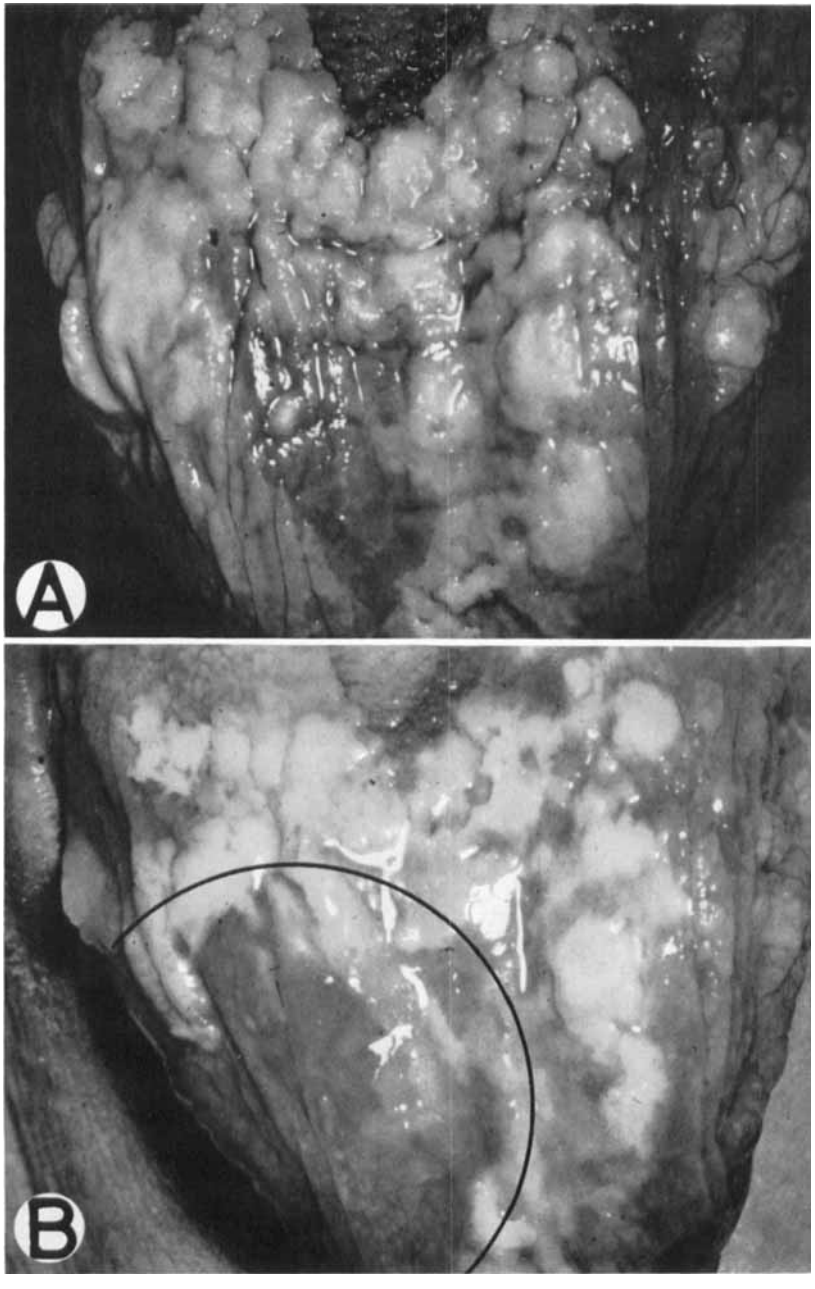
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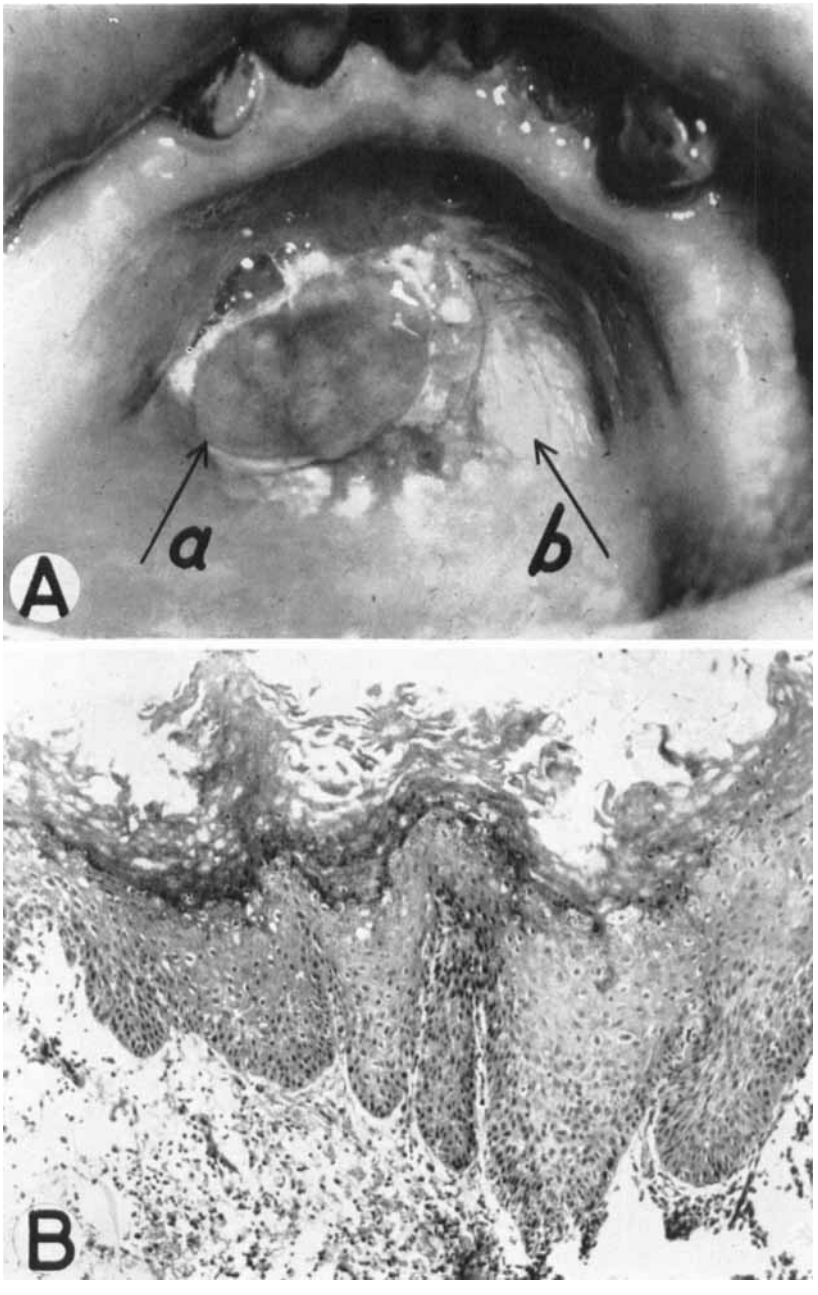
Figure 4.

A. Carcinoma (arrow a), floor of the mouth, occurring in a leukoplakic area (arrow b) 9 months after discontinuing vitamin A administration to which there was no response (patient no. 6).

B. A representative microscopic section of this patient's leukoplakia before vitamin A administration, showing epithelial atypia. Original magnification x 115.


a
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A
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Figure 5.

A. A representative microscopic appearance of leukoplakia from the buccal mucosa of patient no. 15 before vitamin A administration. Note hyperorthokeratosis. Original magnification x 400.

B. A representative microscopic appearance of the buccal mucosa from an area of complete remission in the same patient. Note the parakeratotic condition of the superficial epithelial strata. Original magnification x 400.

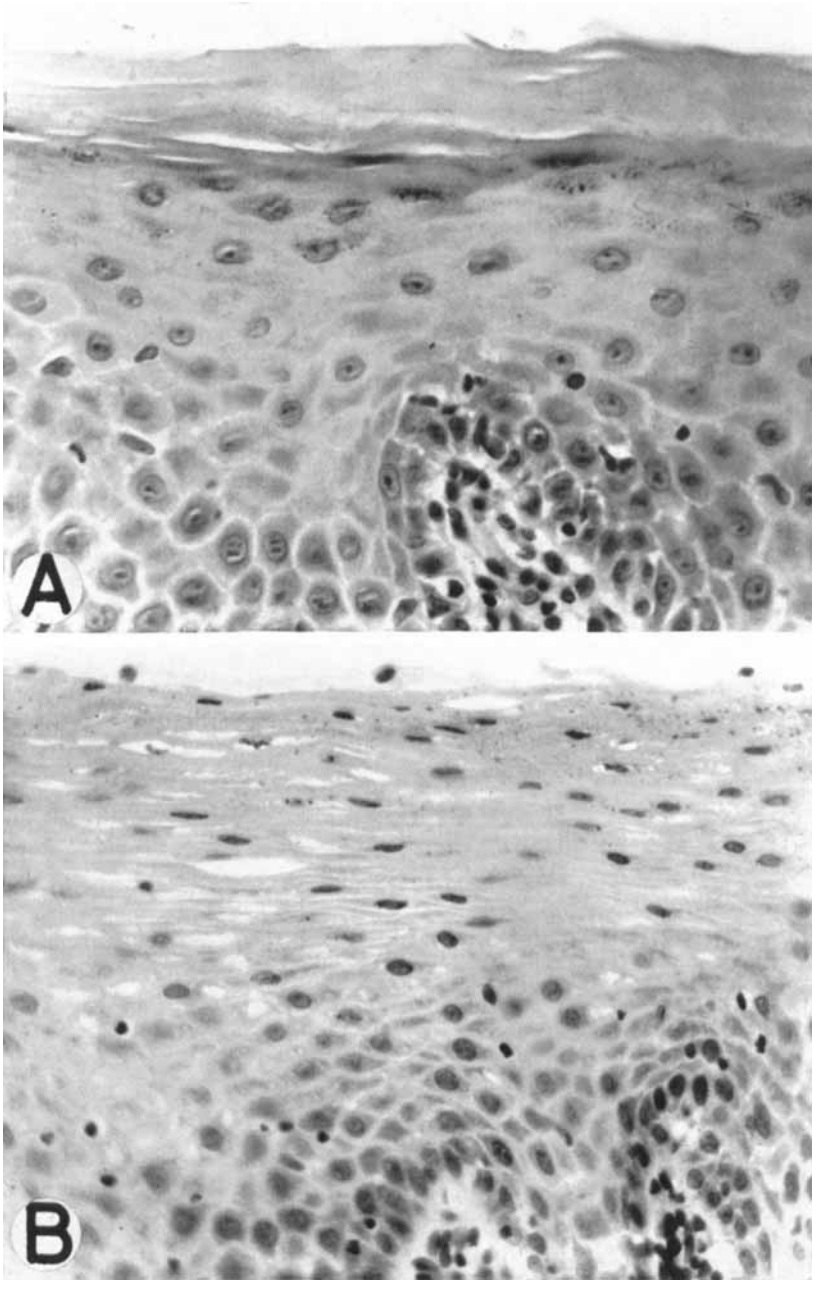
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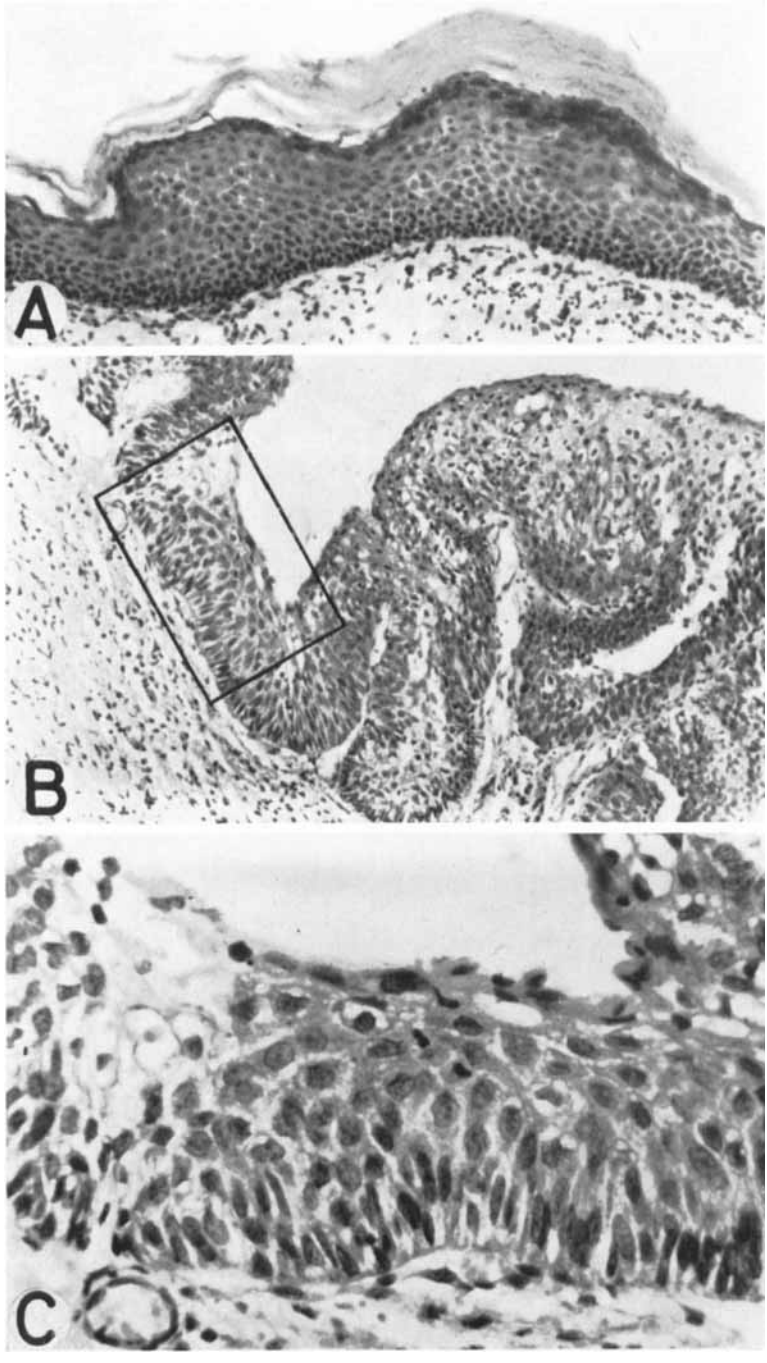
Figure 6.

A. A representative microscopic area from a leukoplakia of the floor of the mouth before vitamin A administration (patient no. 5). Note the hyperorthokeratosis. Original manification x 170.

B. Mucous metaplasia induced in the epithelium of the mouth in the same patient after 600,000 i. u. vitamin A daily for 6 weeks. Original magnification x 130.

C. Higher power of area marked in plate 5 B, illustrating columnar appearance of basal cells and mucous-containing vacuoles. Original magnification x 400.


A
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