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LACTATE AND MALATE DEHYDROGENASE ACTIVITIES IN NORMAL ORAL MUCOSA AND IN HOMOGENEOUS LEUKOPLAKIA[1]

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Abstract. Biopsies were obtained of homogeneous leukoplakias and clinically healthy oral mucosa in nine patients. One-half of each biopsy was used for the assay of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) employing the Lowry technique, and the other half of mitotic counts. Material for the enzymatic assays was obtained from the upper third of the cellular layer. LDH activity increased by 73% in hyperortho-, and by 39% in hyperparakeratinized epithelia of leukoplakias. MDH activity decreased by approximately 32% in both types of keratinization. Regional differences in enzymatic activity were found between buccal and labial sites in normal mucosa and hyperparakeratotic leukoplakia. The ratio of LDH and MDH activity increased from 0.95 in normal mucosa to 1.97 in hyperpara- and 2.51 in hyperorthokeratinized areas in leukoplakias. Changes in the levels of enzymatic activity could not be correlated with the mitotic frequency or the epithelial ridge height.

Follow-up studies have shown that 5% or less of oral leukoplakias undergo a malignant transformation (6, 28). It is difficult, however, to predict a cancerous change in these lesions. If the leukoplakias are grouped on the basis of their clinical appearance, as described by Pindborg et al. (27, 29), the speckled type appears to show a higher incidence of malignant transformation than does the homogeneous type (28). Other approaches for predicting the behaviour of oral leukoplakias include computer-aided analysis or grading of histological changes (12, 33) and immunological studies (5, 16).

Biochemical investigations in oral leukoplakia are, however, few (13, 14). In other tissues, the precancerous cell exhibits increased glycolysis and lactate production during its conversion to the malignant state (1, 3, 31, 32, 35). The present investigation was therefore undertaken as a preliminary step to elucidate enzymatic alterations in homogeneous leukoplakia. LDH and MDH were chosen to provide information on anaerobic and aerobic metabolism. Frequency of mitosis and epithelial ridge height were also studied in order to relate these to enzymatic activity.

MATERIAL AND METHODS

In 9 patients biopsies were obtained after prior local anesthesia (1% Lidocain) of the leukoplakia and clinically normal oral mucosa of the same patient. Two leukoplakias displayed a hyperorthokeratotic epithelium, three showed hyperparakeratosis, and in 4 of the patients the lesions consisted of alternate hyperortho- and hyperparakeratotic areas. The material is summarized in Table I.

Cultures for oral candidosis taken at the same time as the biopsy were negative in 8 of the 9 patients. The patient with candidosis had not received any treatment. Two patients had a history of candida infection and had received topical gentian violet prior to but not on the day of the biopsy procedure at which time their mucosa showed no discoloration from the dye.

Preparation of the material. One-half of each biopsy was frozen in liquid nitrogen and used for enzyme assays and the other half was fixed in 10% neutral formalin and used for mitotic counts. The frozen material was sectioned and lyophilized. Every 4th or 5th section was retained for hematoxylin and eosin staining. These sections served as a guide in the micro-dissection procedure (18, 19) and were of particular aid in those leukoplakias where both ortho- and parakeratinized areas were present. The enzyme assays in leukoplakias were carried out on dissected specimens containing the superficial third of the subcorneal epithelium. In controls, the most superficial 1 to 3 rows of cells were discarded. This was done in order to eliminate a contaminated

[1] Lactate dehydrogenase, EC 1.1.1.27 (LDH), malate dehydrogenase, EC 1.1.1.37 (MDH).

Table I. *Material of normal oral mucosa and homogeneous leukoplakia, divided according to biopsy site, age of patient and degree of inflammation*

Inflammatory infiltrate in submucosa is graded from slight to severe (1.0–4.0). Means are indicated and, if necessary, ranges are also included within parentheses

Material	No. of patients	Age	Inflammation
Labial			
Normal mucosa	4	63 (49–73)	0.5
Hyperorthokeratotic leukoplakia	1	65	1.0
Hyperparakeratotic leukoplakia	2	66 (65–67)	0.7 (0.5–1.0)
Buccal			
Normal mucosa	4	60 (47–69)	0.5
Hyperorthokeratotic leukoplakia	4	66 (59–73)	1.4 (0.5–3.0)
Hyperparakeratotic leukoplakia	5	61 (49–73)	1.2 (0.5–3.0)
Tongue			
Patient I			
Normal mucosa	1	72	0.5
Hyperorthokeratotic leukoplakia	1	72	4.0

surface and a possibly parakeratinized surface layer. Care was taken to use the central part of the rete ridges only.

Enzyme assays. LDH and MDH activities were measured according to Lowry et al. (19, 20). The details are given in Table II. Time curves were linear in the interval between 15 and 60 minutes and linearity was also shown between enzymatic activity and tissue weights in the range 150 to 500 ng. The weights of specimens used in the assays ranged between 200 and 450 ng. Activities were calculated from means of quadruplicates and expressed as moles of substrate converted per kg dry weight per hour of incubation (MKH). Statistical significance of differences between means was established by Student's *t*-test or analyses of variance (30, 34). The experimental error was measured on quadruplicates as coefficients of variation. It was 16% for LDH and 14% for MDH.

Mitotic frequency and epithelial thickness. The formalin fixed material was sectioned at 5 μm thickness and every 5th section stained with hematoxylin and eosin. Tracings of epithelium were then obtained by projecting the sections. In leukoplakias where both hyperortho- and hyperparakeratotic ridges were present, such areas were delineated on the tracings. Surface and basement membrane lengths were measured with a map measure calibrated at 1 mm intervals. The mitotic index represented the number of mitoses per mm surface length. The ratio of surface and basement membrane length served as an index of epithelial ridge height.

RESULTS

The duration of the lesions ranged from 6 months to 30 years. The median was 1 year. Each biopsy was graded for inflammatory cell infiltrate (Table I). In most cases inflammation was slight except in patient I, who exhibited epithelial atypia and an intense inflammatory cell infiltrate in the submucosa (Table I).

Fig. 1 shows how LDH activity increased by 73% in hyperorthokeratotic and 39% in hyperparakeratotic leukoplakia as compared with normal mucosa ($P < 0.001$). The difference between the two types of keratinized epithelia in the leukoplakia was also significant ($P < 0.01$).

MDH activity decreased 32 and 33% in leukoplakias as compared with normal epithelium ($P < 0.005$). No difference was evident between the two types of keratinized epithelia in the leukoplakias (Fig. 1).

The LDH/MDH ratio increased from 0.95 ±

Table II. *Assay conditions*

BPA = Bovine plasma albumin, LDH = Lactate dehydrogenase, MDH = Malate dehydrogenase, NAD, NADH = Oxidized, reduced nicotinamide adenine dinucleotide

Enzyme	Buffer	Substrate	Other additives	Incubation volume (μl)	Time of incubation at 38° (min)	Final volume (ml)	Product measured
LDH	0.02 M Imidazol pH 7.0	1.0 mM pyruvate	0.5 mM NADH 0.02% BPA	10.4	30	0.15	NAD
MDH	0.1 M Tris-(hydroxy-methyl)-aminomethane, pH 8.7	0.7 mM oxalacetate	0.4 mM NADH 0.02% BPA	10.4	45	0.19	NAD

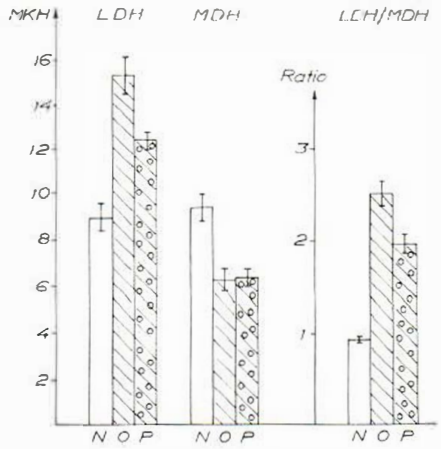

Fig. 1. Enzyme activities of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) together with their ratios in normal oral mucosa (N), hyperorthokeratotic (O) and hyperparakeratotic (P) leukoplakia. Activities are expressed as moles of substrate converted per kg dry weight per hour of incubation at 38° (MKH). Standard errors of the mean are indicated at each bar.

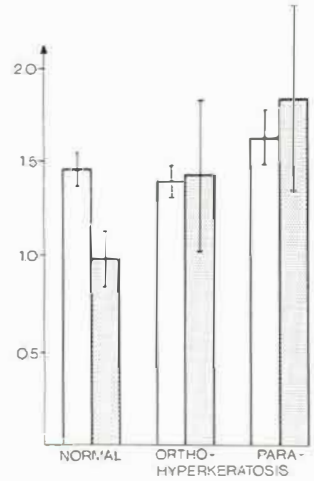

Fig. 2. Measurements on sections from normal oral mucosa and leukoplakia. Unfilled bars indicate basement membrane length relative to given surface length. Filled bars denote the number of mitotic figures per mm surface length. Standard errors of the mean are indicated at each bar.

0.04 to 2.51 ± 0.15 and 1.97 ± 0.11 (means $\pm$ S.E.M.) as normal mucosa was compared with hyperortho- and hyperparakeratotic leukoplakia. These ratios differed significantly from each other ($P < 0.02$–0.001).

The material was also grouped in order to reveal differences in enzymatic activity in labial and buccal mucosa. In the comparison, patient I was excluded, since the material in this case was obtained from the tongue. Hyperorthokeratotic leukoplakia was also excluded due to the small number in one subgroup. Analyses of variance showed significantly higher activities of both enzymes in the labial region, which was evident both in normal and in hyperparakeratotic leukoplakia ($P < 0.01$). LDH and MDH activities were 22% and 27% higher, respectively, in the labial than in the buccal site.

The findings for mitotic and epithelial ridge height indices are shown in Fig. 2. The mitotic index varied considerably in leukoplakias and neither index differed when controls were compared with the leukoplakias or in a comparison of hyperortho- and hyperparakeratinized epithelia of leukoplakias. Regional differences were not discernible in these parameters and a correlation could not be shown between these indices and LDH and MDH activities.

DISCUSSION

At the time of biopsy one patient had oral candidosis and two others had been successfully treated with gentian violet in the immediate past. Enzymatic activities in the candida-infected patient showed the same pattern as in the rest of the material. Since there was no visible discoloration of the mucosa in patients receiving gentian violet treatment, it was assumed that deleterious effects of the dye on epithelial cells were minimal. Consequently, the findings for the above 3 patients were included in the analysis of the material.

In the present study enzymatic activities showed regional differences between labial and buccal mucosa both in the normal epithelium as well in the hyperparakeratinized epithelium in leukoplakia. Since highly significant regional differences were noted, it would seem that the regional pattern of LDH and MDH activities is retained when the epithelium is parakeratinized. In oral mucosa, regional differences in several cellular traits have been reported previously (4, 9, 22, 24, 25, 26). Information on morphological differences between buccal and labial mucosa is, however, lacking. Further studies on human oral mucosa directed towards a correlation of ultramicrochemical findings with morphological fea-

tures may provide an explanation for the regional differences observed in the present study.

Previous studies have shown a great variation in the mitotic index in leukoplakias along with a trend towards a higher mitotic frequency in parakeratinized leukoplakias (8, 23). A similar pattern was found in the present study. The lack of correlation between enzymatic activity and mitotic counts parallels Weber's (35) findings for LDH activity and growth rate in experimental hepatomas.

Alterations in the maturation process in normal oral mucosa evidently occur in order to give rise to the ortho- or parakeratinized layers of leukoplakias. In the two types of keratinized epithelia associated with leukoplakias, LDH levels were increased and MDH decreased (Fig. 1). The psoriatic lesion is another example of altered keratinization. In this pathological state, on the other hand, glycolytic enzymes as well as several enzymes in the citric acid cycle and pentose shunt pathway exhibit increased activities (10, 11).

In malignant tumours there is a change in cellular metabolism towards an increased rate of glycolysis and lactate production (1, 35). Based on the reports published by Bär et al. (3) and Shonk et al. (31, 32), LDH/MDH ratios were calculated for twelve different normal tissues and their malignant counterparts. The ratios were significantly increased, from 0.89 ± 0.09 to 1.30 ± 0.15 (means $\pm$S.E.M.). In most cases this change was due to an increase in LDH and a decrease in MDH activity, a pattern that was also evident in our leukoplakia material.

Each of the five electrophoretically separable LDH isoenzymes contain four sub-units composed of proteins designated M and or H (17). When the M component predominates, LDH can reduce larger amounts to pyruvate and thus increase lactate production (17). It is known that in several malignant tumours, in psoriasis, and in homogeneous leukoplakias, a shift occurs in the LDH isoenzyme pattern such that there is a higher proportion of LDH molecules with predominantly M sub-units (2, 3, 7, 14, 15, 21). However, on the basis of the LDH isoenzyme pattern and our present findings, it would be premature to suggest increased glycolytic capacity in homogeneous leukoplakias. The present study stresses the need to investigate other enzymes, especially those designated as rate limiting in aerobic and anaerobic pathways. Further studies should perhaps also include the category of speckled leukoplakias. The clinical finding of a higher incidence of malignant change in the speckled than in homogeneous lesions (28) might be reflected in a comparison of their enzymatic patterns.

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REFERENCES

1. Aisenberg, A. C.: The Glycolysis and Respiration of Tumors. Academic Press, New York and London, 1961.
2. Bohn, L. & Nissen, N. J.: Mælkesyredehydrogenase isoenzymmønster i serum ved haematologiske og neoplastiske sygdomme. Nord Med *79:* 275, 1968.
3. Bär, U., Schmidt, E. & Schmidt, F. W.: Enzyme-Muster und Isoenzyme menschlicher Tumoren. Klin Wschr *41:* 977, 1963.
4. Cutright, D. E. & Bauer, H.: Cell renewal in the oral mucosa and skin of the rat. I. Turnover time. Oral Surg *23:* 249, 1967.
5. Dabelsteen, E. & Fulling, H. J.: A preliminary study of blood group substances A and B in oral epithelium exhibiting atypia. Scand J Dent Res *79:* 387, 1971.
6. Einhorn, J. & Wersäll, J.: Incidence of oral carcinoma in patients with leukoplakia of the oral mucosa. Cancer *20:* 2189, 1967.
7. Elhilali, M., Oliver, J., Sherwin, A. & Mackinnon, K.: Lactate dehydrogenase isoenzymes in hyperplasia and carcinoma of the prostate: a clinical study. J Urol *98:* 686, 1967.
8. El-Labban, N., Lucas, R. B. & Kramer, I. R. H.: The mitotic values for the epithelium in oral keratosis and lichen planus. Brit J Cancer *25:* 411, 1971.
9. Gerson, S. J., Meyer, J. & Mattenheimer, H.: Quantitative histochemistry in rat oral mucosa: Lactate and malate dehydrogenase. J Invest Derm *47:* 526, 1966.
10. Halprin, K. M. & Ohkawara, A.: Carbohydrate metabolism in psoriasis. An enzymatic study. J Invest Derm *46:* 51, 1966.
11. Hammar, H.: β-hydroxyacyl-CoA dehydrogenase and glucose-6-phosphate dehydrogenase in normal human skin and in some papulosquamous diseases of the skin. Acta Dermatovener (Stockholm) *51:* 93, 1971.
12. Kramer, I. R. H., Lucas, R. B., El-Labban, N. & Lister, L.: A computer-aided study of the tissue changes in oral keratoses and lichen planus and an analysis of case groupings by subjective and objective criteria. Brit J Cancer *24:* 407, 1970.

13. Langvad, E. & Roed-Petersen, B.: Studies in oral leukoplakias. XVII. Lactate dehydrogenase isoenzyme patterns in oral leukoplakia. Acta Path Microbiol Scand *75:* 193, 1969.
14. — Lactate dehydrogenase isoenzyme patterns in oral leukoplakias and in clinically uninvolved oral mucosa of the same persons. Acta Path Microbiol Scand (A) *78:* 505, 1970.
15. Latner, A. L., Turner, D. M. & Way, S. A.: Enzyme and isoenzyme studies in preinvasive carcinoma of the cervix. Lancet *II:* 814, 1966.
16. Lehner, T.: Immunopathology of oral leukoplakia. Brit J Cancer *24:* 442, 1970.
17. Lehninger, A. L.: Biochemistry, p. 411. Worth, New York, 1970.
18. Lowry, O. H.: The quantitative histochemistry of the brain. Histological sampling. J Histochem Cytochem *1:* 420, 1953.
19. Lowry, O. H., Roberts, N. R. & Chang, M. W.: The analysis of single cells. J Biol Chem *222:* 97, 1956.
20. Lowry, O. H., Roberts, N. R. & Kapphahn, J. I.: The fluorometric measurement of pyridine nucleotides. J Biol Chem *224:* 1047, 1957.
21. Marghescu, S.: Über das Lactate Dehydrogenase-Zymogram in Hornschicht, Epidermis und Blutserum unter normalen Bedingungen und bei Psoriasis. Arch Klin Exp Derm *225:* 163, 1966.
22. Meyer, J., Alvares, O. & Barrington, E. P.: Volume and dry weight of cells in the epithelium of rat cheek and palate. Growth *34:* 57, 1970.
23. Meyer, J., Daftary, D. K. & Pindborg, J. J.: Studies in oral leukoplakias. XI. Histopathology of leukoplakias in Indians chewing "pan" with tobacco. Acta Odont Scand *25:* 397, 1967.
24. Meyer, J., Medak, H. & Weinmann, J. P.: Mitotic activity and rates of growth in regions of oral epithelium differing in width. Growth *24:* 29, 1960.
25. Osmanski, C. P.: Disintegrative process in regions of the oral epithelium. *In* Current Concepts of the Histology of Oral Mucosa (ed. C. A. Squier & J. Meyer), p. 129. C. Thomas, Springfield, Ill., 1971.
26. Osmanski, C. & Meyer, J.: Differences in the fine structure of the mucosa of mouse cheek and palate. J Invest Derm *48:* 309, 1967.
27. Pindborg, J.: Oral leukoplakia as a precancerous condition. Acta Otolaryng (Stockholm) Suppl. *224:* 15, 1967.
28. Pindborg, J. J., Jølst, O., Renstrup, G. & Roed-Petersen, B.: Studies in oral leukoplakia: A preliminary report on the period prevalence of malignant transformation in leukoplakia based on a follow-up study of 248 patients. J Amer Dent Ass *76:* 767, 1968.
29. Pindborg, J. J., Renstrup, G., Poulsen, H. E. & Silverman, S.: Studies in oral leukoplakias. V. Clinical and histologic signs of malignancy. Acta Odont Scand *21:* 407, 1963.
30. Seeger, P.: Variance Analysis of Complete Designs, pp. 67, 80. Almqvist & Wiksell, Stockholm, 1966.
31. Shonk, C. E., Arison, R. N., Koven, B. J., Majima, H. & Boxer, G. E.: Enzyme patterns in human tissues. III. Glycolytic enzymes in normal and malignant tissues of the colon and rectum. Cancer Res *25:* 206, 1965.
32. Shonk, C. E., Majima, H., Koven, B. J. & Boxer, G. E.: Enzyme patterns in human tissues. IV. Comparison of glycolytic enzymes in surgical biopsies and autopsy specimens. Cancer Res *26:* 607, 1966.
33. Smith, C. & Pindborg, J. J.: Histologic Grading of Oral Epithelial Atypia by the Use of Photographic Standards. C. Hamburger, Copenhagen, 1969.
34. Snedecor, G. W. & Cochran, W. G.: Statistical Methods, 6th ed., Iowa State University Press, Ames, 1967.
35. Weber, G.: Carbohydrate metabolism in cancer cells and the molecular correlation concept. Naturwissenschaften *55:* 418, 1968.

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