Mitotic Activity in the Oral Epithelium of the Albino Rat

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In groups of Wistar rats 3 and 12 months of age, colchicine and radioautography were used to assess mitotic activity in oral epitheliums. Apparent mitotic activity in the epitheliums of the cheek, hard palate, and central, intermediate, and lateral zones of the soft palate depended on the method used and the age of the rat.

Recently, I[1] described three varieties of keratinized, stratified, squamous epithelium in the central, intermediate, and lateral zones of the oral surface of the rat's soft palate. This present study is an investigation into the mitotic activity of these epitheliums in particular to see whether there is a difference in mitotic activity between them; to compare the mitotic activity in the soft palate epithelium with that in the hard palate and cheek; to determine if there is a variation in mitotic activity in these regions in old and young rats; and to compare the mitotic activity obtained with radioautography with that determined with the use of colchicine.

Materials and Methods

Matched groups of 40 Wistar albino rats were used. They consisted of two groups of ten young rats each (75 to 105 gm body wt) and two groups of ten old rats each (320 to 380 gm body wt). These weight ranges corresponded to ages of 3 and 12 months, respectively.

COLCHICINE TECHNIQUE.—Ten young and ten old rats each received an intraperitoneal injection of 0.1% aqueous colchicine[a] in a dosage of 1 mg/kg body weight. The experiment was begun at 6:30 AM and the rats were left undisturbed until 10 AM to ensure the 3.5-hour period recommended by Karring and Löe.[2] The rats were then killed with intraperitoneally administered pentobarbitone; their mandibles and tongues were excised and then fixed in 10% neutral buffered Formol saline. Once fixation was complete, their soft palates were removed as were specimens of the hard palate and cheek. All three specimens were embedded in paraffin wax in a single block. Serial sections were cut at 7 micrometers and every tenth section retained for a total of 100 sections which were mounted on glass slides and stained with Harris' hematoxylin.

RADIOAUTOGRAPHIC METHODS.—Beginning at 7:30 AM aqueous ^3H methyl thymidine[b] was injected intraperitoneally into ten young and ten old rats in a dosage of 1 μCi/mg. All injections were completed five minutes later and the rats were killed at 9:30 AM in an order that ensured a two-hour time interval since the ^3H methyl thymidine administration. Soft palate, cheek, and hard palate specimens were removed, fixed, embedded, and cut as before. Every tenth section for a total of 100 sections was mounted onto glass slides that were coated with a 1% gelatin solution that contained a few granules of $KCr(So_4)_2$ 12 H_2O. They were then dried in an incubator at 37 C. Later, the slides were dewaxed and brought to distilled water before being dipped in a solution of Ilford K5 nuclear emulsion[c] and 6% gelatin at 37 C, using an automatic dipping apparatus set at 240 mm/min. Blank slides and nonradioactive sections were included as controls. All slides were sealed in lightproof boxes and stored at 4 C until development.

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[a] Biochemicals, British Drug House, Poole, Eng.

[b] TRA 120, specific activity 5,000 mCi/mmole, Radiochemical Centre, Amersham, Eng.

[c] Ilford Ltd., Dagenham, Kent, Eng.

TABLE 1

EPITHELIAL MITOTIC INDEX PER SQUARE MILLIMETER OF SURFACE AREA, IN RATS TREATED WITH COLCHICINE, ARRANGED IN DESCENDING ORDER

Age Group	Epithelial Area	N	Mitoses/mm² (Range)	Surface Area (Mean ± *SE*)
12 months old	Lateral soft palate	92	634.9-9,361.7	5,061.4 ± 162.8
	Central soft palate	100	1,481.5-9,436.6	4,943.2 ± 160.0
	Hard palate	100	1,066.7-9,322.0	4,444.7 ± 156.8
	Intermediate soft palate	86	800.0-7,777.8	4,064.8 ± 149.4
	Cheek	90	1,297.7-6,904.8	3,977.8 ± 120.9
3 months old	Cheek	100	666.7-5,270.3	3,174.4 ± 112.5
	Lateral soft palate	90	1,304.3-5,000.0	2,724.0 ± 100.8
	Central soft palate	100	1,132.1-5,774.6	2,692.3 ± 92.0
	Intermediate soft palate	80	952.4-5,000.0	2,047.0 ± 91.8
	Hard palate	89	566.0-4,666.7	1,671.2 ± 86.1

Note: SE, standard error of the mean.

TABLE 2

EPITHELIAL MITOTIC INDEX PER SQUARE MILLIMETER OF SURFACE AREA, IN RATS TREATED WITH ^3H METHYL THYMIDINE, ARRANGED IN DESCENDING ORDER

			Labeled Cells/mm²	
Age Group	Epithelial Area	N	Range	Mean ± *SE*
12 months old	Hard palate	90	1,413.0-1,1451.6	4.551.8 ± 209.3
	Cheek	90	952.4-1,1754.4	4,286.7 ± 177.3
	Lateral soft palate	90	666.7- 7,666.7	3,106.6 ± 136.3
	Central soft palate	90	882.4- 7,555.6	2,713.9 ± 137.0
	Intermediate soft palate	90	588.2- 4,000.0	1,955.5 ± 82.8
3 months old	Cheek	88	909.1-10,000.0	4,459.2 ± 249.5
	Lateral soft palate	88	952.4- 9,285.7	4,364.9 ± 258.1
	Hard palate	90	1,320.8-10,333.3	4,104.5 ± 199.3
	Intermediate soft palate	90	882.4- 9,230.8	3,620.5 ± 183.6
	Central soft palate	90	666.7-11,875.0	3,408.2 ± 192.8

Note: SE, standard error of the mean.

TABLE 3

VARIOUS EPITHELIUMS EXAMINED ARRANGED IN DESCENDING ORDER OF MITOTIC ACTIVITY TO SHOW PATTERN OF MITOTIC ACTIVITY IN 12-MONTH-OLD RATS

Colchicine Technique	^3H Methyl Thymidine Technique
Within the soft palate	
* [Lateral, Central] *NS*; [Central, Intermediate] *	* [Lateral, Central] *NS*; [Central, Intermediate] *
Soft palate as a whole compared to the hard palate and cheek	
* [Soft palate, Hard palate] *NS*; [Hard palate, Cheek] *NS*	* [Hard palate, Cheek] *NS*; [Cheek, Soft palate] *

Note: NS, not significant.
* $P < 0.001$.

After 21 days, the emulsion was found to be sufficiently exposed and after development in an amidol mixture, the sections were lightly stained with Mayer's hematoxylin.

ASSESSMENT OF MITOTIC ACTIVITY.—The mitotic index per square millimeter of surface area devised by Karring and Löe was used. Specimens were examined in a Wild M12 binocular[d] microscope fitted with a drawing tube, and an area of each epithelium on each section was selected for its easily identifiable landmarks. Its surface was then traced onto paper at a linear magnification of ×116 and the length of the epithelial surface on the tracing was measured with an opisometer. From this and the section thickness setting, the surface area was calculated. Statistical analysis of the results was performed using an analysis of variance, Student's *t* test, and Scheffe's test.[4] The number of mitotic figures in the colchicine groups, and the number of nuclei having more than five silver grains overlying them in the radioautography groups were counted. This enabled the mitotic index per square millimeter of the surface to be derived.

Results

The absolute mitotic indexes per square millimeter of surface area are given in Tables 1 and 2. Extremely variable results were obtained. The order of mitotic activity differed between the young and old rats within the colchicine and ^3H methyl thymidine groups and also differed within the same age group when the results obtained with both techniques were compared.

In Tables 3 and 4, the results of the statistical evaluation of the findings are given. Here the variation seen in the absolute values is confirmed. Within the soft palate, the only consistent results were seen in the 12-month-old rats where the order of activity was the same using both the colchicine and ^3H methyl thymidine techniques.

The mitotic activities in the 12- and 3-month-old rats were also compared statistically. When the colchicine technique was then used for each epithelial specimen except that from the cheek, there was a significantly greater activity in the older rats ($P < 0.001$). In the cheek, the mitotic activities were similar in both age groups. In the instance of the ^3H methyl thymidine technique, the amounts of radioactive labeling seen in the cheek, hard palate, and central soft palate were similar in the two age groups. However, the intermediate and lateral zones of the soft palate showed significantly more labeling in the young rats ($P < 0.001$), a reversal of the findings in the colchicine group.

[d] Wild Ltd., Heerbrugg, Switz.

Discussion

In quantitative studies of mitotic activity in stratified squamous epithelium, various forms of index have been used. These include the ratio of mitoses to the total number of cells present,[5] and the number of dividing cells relative to a unit length of the basement membrane.[8] Recent work[7,8] suggested that the number of mitoses should rather be related to a length of the epithelial surface. This reference unit was defined by Karring and Löe[3] as 1 mm^2 of the outer surface of the epithelium and was shown by them to be the most reliable method for stratified squamous epithelium. Thus, this index was used in the present study.

Two other dilemmas exist in mitotic studies, namely, at what time should mitotic activity be studied? and, which technique should be used to indicate the mitotic activity?

There is a diurnal variation in mitotic activity in oral epithelium, which in rodents is highest in the early morning and lowest in the evening.[6,9–15] Usually, investigators have allowed for this variation either by examining the mitoses at regular intervals during a 24-hour period or by doing experiments at the same time of the day. If this latter technique is used, Karring and Löe[2] suggested that it should be at the time of maximum mitotic activity. In rodents, this has been reported to lie between 8:45 AM and 10 AM[2,13,14]; hence, the choice of timing in the present investigation.

Regarding a technique to indicate mitotic activity, two methods are commonly used, colchicine which arrests cell division in metaphase and radioactive labeling of nuclei with tritiated thymidine. Both have their supporters and critics. Colchicine has been shown to be a valid technique by some authors,[2,16–19] whereas others have thought that radioactive labeling is better.[14,20] Both techniques were used in the present study to produce, as far as is possible, a clear picture of mitotic activity in the tissues studied. The

TABLE 4

VARIOUS EPITHELIUMS EXAMINED ARRANGED IN DESCENDING ORDER OF MITOTIC ACTIVITY TO SHOW PATTERN OF MITOTIC ACTIVITY IN 3-MONTH-OLD RATS

Colchicine Technique	^3H Methyl Thymidine Technique
Within the soft palate	
* [Lateral, Central] *; [Central, Intermediate] NS	NS [Lateral, Intermediate] NS; [Intermediate, Central] NS
Soft palate as a whole compared to the hard palate and cheek	
† [Cheek, Soft palate] *; [Soft palate, Hard palate] *	NS [Cheek, Hard Palate] NS; [Hard Palate, Soft palate] NS

Note: NS, not significant.
* $P < 0.001$.
† $P < 0.01$.

methods used in each technique were based on the work of other authors[2,18,21,22] and were standardized as far as was possible.

The mitotic activity of the three types of epithelium within the soft palate has not been previously reported and may be due to different functional demands in the different regions. However, the activity in the soft palate as a whole has been reported elsewhere.[2,13,14] Using the colchicine technique in the 12-month-old rats, the activity in the soft palate was greater than in the hard palate, which was followed by the cheek. This order of activity is similar to that seen by England and Burke[14] who used tritiated thymidine assessed by liquid scintillation counting, and Trott and Gorenstein[13] who used colchicine. Karring and Löe[2] who used colchicine found mitotic activity to be greatest in the hard palate. In the 3-month-old rats, the order was changed to cheek, soft palate, and hard palate. In the study by Meyer, Medak, and Weinmann,[23] the activity in the cheek was greater than in the palate.

The ^3H methyl thymidine technique produced results different from those obtained with colchicine. In 12-month-old rats, the order of activity was hard palate, cheek, and soft palate which was the same as that seen by England and Burke[14] using the same technique but in 3-month-old rats. It was also similar to the findings of Karring and Löe[3] and Trott and Gorenstein[13] in colchicinized rats.

The colchicine and deoxyribonucleic acid labeling techniques are supposed to yield data of equal validity as far as turnover time is concerned.[24] However, as regards patterns of activity, the present study has shown considerable variation in the different epitheliums in the two age groups.

This variation in mitotic activity in various oral epitheliums depended on the method used and the age of the rat. The inconsistent and contradictory results put in doubt the validity of one or both of the methods used and must lead to the posing of the question: Which, if any, of the methods are to be selected in mitotic studies of oral epithelium?

Conclusions

In the colchicine-treated and ^3H methyl thymidine-treated material, there was inconsistent variation in mitotic activity both within the various epitheliums and between the groups studied, suggesting that the methods used should, perhaps, be reevaluated.

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References

1. CLEATON-JONES, P.: Histological Observations in the Soft Palate of the Albino Rat, *J Anat* 110: 39-47, 1971.
2. KARRING, T., and LÖE, H.: The Use of

Colchicine for the Assessment of Mitotic Activity in Rat Oral Epithelium, *Scand J Dent Res* 80: 474-485, 1972.

3. KARRING, T., and LÖE, H.: The Reliability of Various Mitotic Index Systems in Assessing Mitotic Activity in Stratified Squamous Epithelium, *J Periodont Res* 7: 271-282, 1972.
4. SCHEFFE, H.: The Analysis of Variance, New York: John Wiley & Sons, 1959, pp 66-72.
5. MINOT, S.: *The Problem of Age, Growth and Death: A Study of Cytomorphosis Based on Lectures at the Lowell Institute, March 1907*. London: John Murray, 1908, pp 218-250.
6. MÜHLEMANN, H.R.; EBNETER, M.; and RUPF, W.: Diurnal Variation in Mitotic Activity of Oral and Corneal Epithelium in Colchicinized Albino Rats, *Helv Odontol Acta* 3: 30-34, 1959.
7. LÖE, H., and KARRING, T.: A Quantitative Analysis of the Epithelium—Connective Tissue Interface in Relation to Assessments of the Mitotic Index, *J Dent Res* 48: 634-640, 1969.
8. MACDONALD, D.G.: Cell Renewal in Oral Epithelium, in SQUIER, C.A., and MEYER, J. (eds) : *Current Concepts of the Histology of Oral Mucosa,* Springfield, Ill: Charles C Thomas, 1971, pp 61-79.
9. CARLETON, A.: A Rhythmical Periodicity in the Mitotic Division of Animal Cells, *J Anat* 68: 251-263, 1934.
10. BULLOUGH, W.S.: Mitotic Activity in the Adult Male Mouse, *Mus musculus L*: The Diurnal Cycles and Their Relation to Waking and Sleeping, *Proc Roy Soc Lond (Biol)* 135: 212-233, 1948.
11. BULLOUGH, W.S.: Age and Mitotic Activity in the Male Mouse, *Mus musculus L, J Exp Biol* 26: 261-286, 1949.
12. MUHLEMANN, H.R.; MARTHALER, T.M.; and LOUSTALOT, P.: Daily Variations in Mitotic Activity of Adrenal Cortex, Thyroid and Oral Epithelium of the Rat, *Proc Soc Exp Biol* 90: 467-470, 1955.
13. TROTT, J.R., and GORENSTEIN, S.L.: Mitotic Rates in the Oral and Gingival Epithelium of the Rat, *Arch Oral Biol* 8: 425-434, 1963.
14. ENGLAND, C., and BURKE, W.: Diurnal Variation in Deoxyribonucleic Acid and Synthesis in Selected Epithelial Tissue of Male Rats, *J Dent Res* 47: 478-481, 1969.
15. KARRING, T., and LÖE, H.: The Effect of Age on Mitotic Activity in Rat Oral Epithelium, *J Periodont Res* 8: 164-170, 1973.
16. BULLOUGH, W.S.: The Action of Colchicine in Arresting Epidermal Mitosis, *J Exp Biol* 26: 287-291, 1949.
17. BERTALANFFY, F.D., and LEBLOND, C.P.: The Continuous Renewal of the Two Types of Alveolar Cells in the Lung of the Rat, *Anat Rec* 115: 515-541, 1953.
18. HOOPER, C.E.S.: Use of Colchicine for the Measurement of Mitotic Rate in the Intestinal Epithelium, *Am J Anat* 108: 231-244, 1961.
19. TANNOCK, I.F.: A Comparison of the Relative Efficiencies of Various Metaphase Arrest Agents, *Exp Cell Res* 47: 345-356, 1967.
20. MESSLER, B., and LEBLOND, C.P.: Cell Proliferation and Migration as Revealed by Autoradiography After Injection of Thymidine-H^3 into Male Rats and Mice, *Am J Anat* 106: 247-285, 1960.
21. AMANO, M.; MESSIER, B.; and LEBLOND, C.P.: Specificity of Labelled Thymidine as a Deoxyribonucleic Acid Precursor in Radio-Autography, *J Histochem Cytochem* 7: 153-155, 1959.
22. BLENKINSOPP, W.K.: Cell Proliferation in Stratified Squamous Epithelium in Mice, *Exp Cell Res* 50: 265-276, 1968.
23. MEYER, J.; MEDAK, H.; and WEINMANN, J.P.: Mitotic Activity and Rates of Growth in Regions of Oral Epithelium Differing in Width, *Growth* 24: 29-46, 1960.
24. BERTALANFFY, F.D.: Tritiated Thymidine Versus Colchicine Technique in the Study of Cell Population Cytodynamics, *Lab Invest* 13: 871-886, 1964.