

From: The Biology Department,
Brookhaven National Laboratory,
Upton, N. Y., U.S.A.
and
The Department of Periodontology,
Royal Dental College,
Copenhagen, Denmark.

TURNOVER OF THE GINGIVAL EPITHELIUM IN MARMOSETS*

by

MOGENS SKOUGAARD

The majority of reports concerned with the renewal of oral epithelia are based on quantitative studies of mitoses either directly or following colchicine injections. In recent years, however, radioactive labeling of nuclear DNA through injection of tritiated thymidine (H^3 -TDR) is being used extensively for the study of cell population kinetics also in the oral cavity. A review of reports dealing with the renewal of oral epithelia has been published elsewhere (*Skougaard 1965*); the results from these reports are listed in Table 1. In addition to information on methods and experimental animals, the table includes estimates of turnover times for the epithelia; these turnover times are calculated from the findings reported by the investigators.

The turnover time of a tissue is defined as the time taken for the renewal of a number of cells equivalent to the total number of cells in the tissue (*Leblond 1959*). The turnover time (T) can

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be calculated from the mitotic index (number of mitoses per 1000 cells) or from the labeling index (% labeled cells). Provided the cells move through the phases of the mitotic cycle at a constant rate, the following equations will be true:

$$\frac{T_M}{N_M} = \frac{T_S}{N_S} = \frac{T}{N}$$

(T_M = duration of mitosis, N_M = number of mitoses, T_S = duration of DNA synthesis phase (S phase), N_S = number of cells in S phase = number of labeled cells, N = number of cells in the cell population.) From these equations we get:

$$T = \frac{T_M \times 1000}{I_M} = \frac{T_S \times 100}{I_L}$$

(I_M = mitotic index, I_L = labeling index.)

In order to calculate T it is necessary to know the duration of mitosis (T_M) or of S (T_S). For the present calculations it was decided to use a T_M value of one hour in agreement with the results of *Henry et al.* (1952), *Meyer et al.* (1960) and *Fry et al.* (1961). The duration of T_S is, by most investigators, found to be seven to nine hours (*Quastler* 1960, *Quastler & Sherman* 1959, *Koburg & Maurer* 1963, *Skougaard* 1965). A T_S of eight hours was used for the calculation of the turnover times listed in Table 1.

A considerable variation in the turnover times of the oral epithelia, calculated from the mitotic and labeling indices is to be expected. Variations are likely between different species, from one epithelial area to another, and further the different methods used by the various investigators tend to enlarge the variability in the data. However, even if allowance is made for these considerations, the variability in the turnover times listed in Table 1 are extraordinary, with a variation of more than a factor of 100 between the 24 hours found by *Löe* (1961) and the 112 days calculated from the mitotic index reported by *Hayes et al.* (1964).

The present study was undertaken as an attempt to analyze the generative activity of the gingival epithelium under physiological conditions and, hopefully, to elucidate the question of the turnover time for this tissue.

Table 1.
Turnover times for oral epithelia calculated on the basis of the findings of various investigators.

Investigators	Year	Species	Epithelium	Index		Method	Turnover time in days
				Mitotic	Labeling		
<i>Henry et al.</i>	1952	rabbit	buccal	5.1		colchicine	8.7
<i>Mühlemann & Hartl</i>	1955	rat	gingiva	4.0		mitotic counts	11.2
<i>Meyer et al.</i>	1956	man	gingiva	1.56		mitotic counts	28.6
<i>McHugh</i>	1959	rhesus	gingiva	7.1		mitotic counts	6.3
<i>Meyer et al.</i>	1960	mouse	buccal	2.59		mitotic counts	28.6
			floor	2.07			21.5
			palate	1.73			25.8
<i>Messier & Leblond</i>	1960	rat	tongue		5.6	H ³ -TDR	5.2
<i>Löe</i>	1961	dog	gingiva			pocket sealed	1.0
<i>Beagrie & Skougaard</i>	1962	mouse	gingiva		5.5	H ³ -TDR	5.5
<i>Skougaard & Beagrie</i>	1962	marmoset	gingiva		2.8	H ³ -TDR	10.4
<i>Toto & Ohja</i>	1962	mouse	tongue			H ³ -TDR	4.2
<i>Dimassimo</i>	1963	rhesus	gingiva		0.72	H ³ -TDR	40.5
<i>Hayes et al.</i>	1964	man	gingiva	0.4		mitotic counts	111.5

MATERIAL AND METHODS

The majority of cell population kinetics studies based on H³-TDR autoradiography were carried out on mice, whereas most of the experimental studies of the gingival epithelium have been carried out on dogs or monkeys. The size of these animals, however, is of such an order that H³-TDR injection is an expensive procedure.

There exist monkeys that combine a small size (200–400 g bodyweight) with the characteristics of primates. One of these monkeys, the marmoset, has proved useful for autoradiographic tracer work (*Skougaard & Beagrie 1962*). The animals used in the present experiment are cotton topped marmosets (*oedipomidas oedipus*).

The marmosets were not born and reared in the animal house of the laboratory, their age was therefore not known. They were all assumed to belong to the category: young adults, based on:

- 1) the color of the fur, which tends to become lighter and more grey with age, and

- 2) the condition of the periodontal tissues. Inflamed gingiva and occasionally more progressive periodontal disease are common findings in older marmosets.

None of the marmosets used in the present experiment showed clinical signs of periodontal disturbances.

Three male marmosets were injected intraperitoneally with 2 μ Ci H³-TDR per g of bodyweight and sacrificed one hour later. The jaws were dissected out, fixed in neutral buffered formalin for 48 hours and decalcified in EDTA in a 36° C incubator for four days. The jaws were cut in blocks, each containing two to three teeth, imbedded in paraplast and serially sectioned at 4 μ bucco-lingually or mesio-distally. Autoradiographs were made following standard procedures for dipping emulsions (*Joftes & Warren 1955*). The autoradiographs were analyzed by recording the unlabeled cells on a hand tally counter and scoring the labeled cells on an adding machine with the number of grains over the nuclei, including those showing only one, two or three grains. The epithelial attachment as well as the epithelium of the attached gingiva were examined from 10 different gingival crevices taken from three marmosets. A minimum of 2×10^3 epithelial

cells were scanned from each area. The total number of cells scanned was 5×10^4 . The raw data were analyzed by computer, for each set of grain counts the computer produced a histogram, mean grain count and labeling index. For background, correction autoradiographs of sections from a biopsy taken from an uninjected animal were made and treated identically to the rest of the material.

An additional four marmosets were injected intraperitoneally with $2 \mu\text{Ci H}^3\text{-TDR}$ per g of bodyweight. Two of the animals were sacrificed 24 hours after injection, the remaining after 48 and 72 hours. Autoradiographs were made from sections of teeth and surrounding tissues as previously described.

RESULTS

Position of labeled cells

One hour after the injection of H³-TDR the labeled cells were located in the basal as well as the spinal cell layers of all samples observed. In the attached gingiva 62 % of the labeling occurred in the basal layer and 38 % in the spinal cell layers. Fig. 1 demonstrates an autoradiograph from the attached gingival epithelium.

In the epithelial attachment only 30 % of the labeling was found in the basal layer and 70 % in the spinal cell layers. In this area labeled cells occasionally were found at the surface of the epithelium in contact with the enamel. This was only observed in the apical third and only where the epithelial attachment consisted of few cell layers (Fig. 2).

Labeled cells were located in the marginal part of the epithelial attachment as well as near the cemento-enamel junction. There appeared to be no systematic difference in the frequency of the mitotic activity between the apical and marginal parts of the area. In some cases the highest number of labeled cells were located in the apical half of the area (Fig. 3) but other samples showed the opposite distribution.

After 24—72 hours the number of labeled cells had increased and the cells had moved towards the surface in the attached gingiva as well as in the epithelial attachment. In the latter the

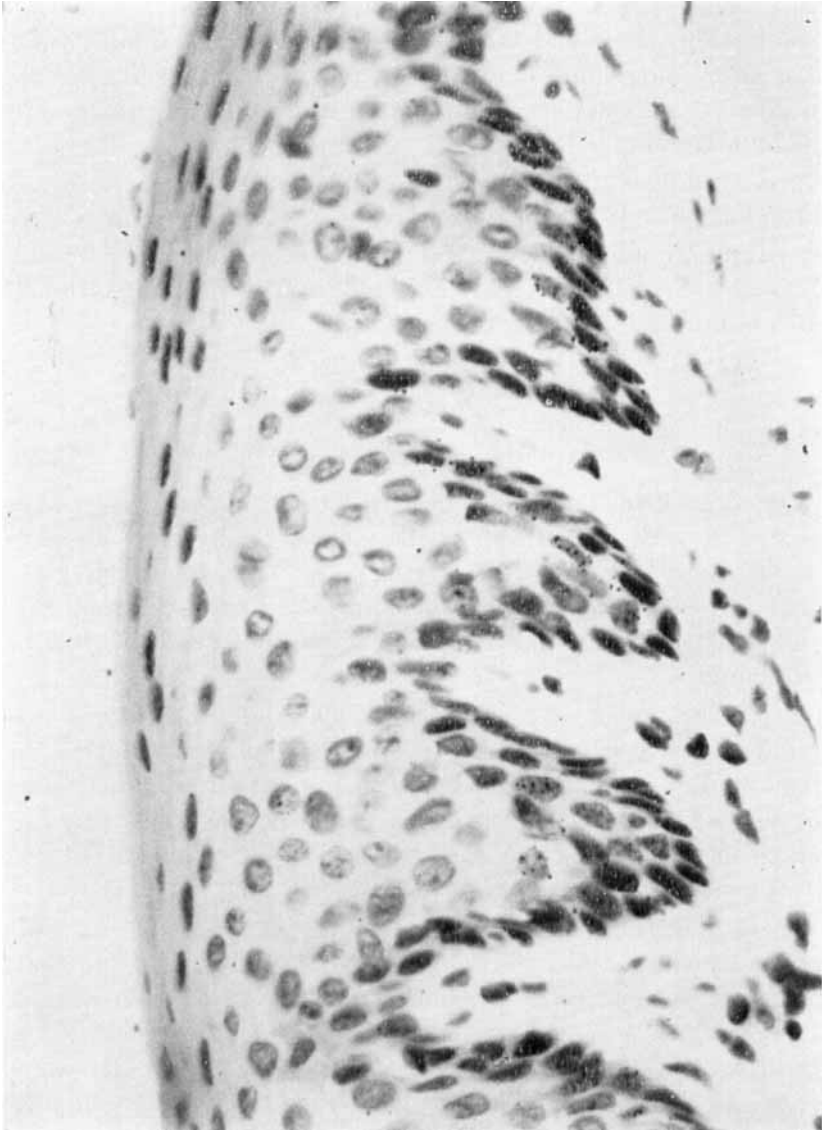


Fig. 1. Autoradiograph from the attached gingival epithelium 1 hour after ^3H -TDR injection.



Fig. 2. Epithelial attachment from marmoset sacrificed 1 hour after H^3 -TDR injection. The high magnification shows a labeled cell in contact with the enamel.



Fig. 3 a. Apical half of the epithelial attachment of marmoset sacrificed 1 hour after H^3 -TDR injection. Note the many labeled cells present in this area.



Fig. 3 b. Marginal part of the epithelial attachment shown in Fig. 3 a.
Only few labeled cells are seen here.



Fig. 4. Epithelial attachment from marmoset sacrificed 72 hours after injection of H^3 -TDR.

number of labeled cells in contact with the enamel had increased compared to the one hour samples (Fig. 4).

Labeling index

The labeling indices from the one hour specimens are listed in Table 2. These data are compiled from the scanning of a total of 5×10^4 cells, and cells carrying more than three grains over the nuclei were scored as labeled. The average labeling index for the epithelial attachment was 7.2 and for the attached gingiva 4.2. The standard errors were calculated at 0.6 and 0.5, respectively, indicating that the difference found between the labeling indices in the two epithelial areas of the marmoset gingiva was significant ($P < 0.01$).

A careful examination of the epithelial attachment areas showed that signs of inflammation were present in practically all cases. No histologically detectable pathological changes were observed in the connective tissue. The findings were confined to the epithelium, and consisted of hyperplasia of the epithelium, an uneven demarcation between the connective tissue and the epithelium and occasionally the presence of a few leucocytes. No case of epithelial downgrowth along the cementum was observed.

Table 2

Labeling indices from the epithelial attachment and the epithelium of the attached gingiva from 10 gingival crevices taken from 3 marmosets 1 hour after injection of H^3 -TDR

Marmoset No.	1				2					3				Overall	
Location	P ₃ Sup L	P ₃ Sup R	P ₁ Inf L	Mean	P ₃ Sup R	C Sup L	P ₁ Inf L	P ₃ Sup L	Mean	P ₁ Inf R	P ₃ Sup L	C Sup L	Mean	Mean	S.E.
Epithelial attachment	5.4	3.7	9.6	6.7	7.6	7.4	6.4	7.6	7.3	7.2	7.3	9.8	8.1	7.2	0.6
Attached gingiva	3.2	2.1	6.4	3.9	2.3	4.5	5.9	4.1	4.2	3.1	4.6	5.8	4.5	4.2	0.5

Ratio between mitotic figures and labeled cells

The number of mitotic figures observed in the sections was surprisingly small compared with the number of labeled cells. The ratio between mitotic figures and labeled cells to be expected is of the order of 1:8 in accordance with the ratio between duration of mitosis and S-phase. In the present material, however, only one mitotic figure was found for every 75 labeled cells.

Turnover time

The turnover time was computed from the labeling index and the duration of S-phase as previously described. These parameters for the epithelial attachment and the attached gingiva are listed in Table 3.

The turnover time for the attached gingival epithelium is seen to be almost twice as long as that of the epithelial attachment.

Table 3

*Duration of S-phase (T_S), labeling index (I_L), turnover time (T) and the corresponding standard deviations**

	T_S hours	σ_{T_S} hours	I_L	σ_{I_L}	T hours	σ_T hours	T days	σ_T days
Epithelial attachment	8.0	1.0	7.2	1.7	111	30	4.5	1.2
Attached gingiva	8.0	1.0	3.7	1.4	214	82	8.9	3.4

* T is calculated from:

$$T = \frac{T_S \times 100}{I_L} \text{ and } \sigma_T \text{ from the equation}$$

$$\sigma_T = \sqrt{\left(\sigma_S \times \frac{100}{I_L}\right)^2 + \left(\sigma_{I_L} \times \frac{T_S \times 100}{I_L^2}\right)^2}$$

The standard deviations for the turnover times were calculated as indicated in the table and demonstrate that particularly for the attached gingival epithelium the scatter was considerable.

DISCUSSION

Type of cell population

The present study confirmed that mitotic activity takes place in all parts of the gingival epithelium of marmosets. The labeling indices determined for the epithelial attachment clearly classify this area as a renewing cell population according to *Messier & Leblond* (1960). The labeling index for the attached gingival epithelium was in some cases below 3 % (Table 2). Although *Messier & Leblond* (1960) suggested 3 % as a minimum for renewing cell populations, the epithelium of the attached gingiva in the marmosets, however, undoubtedly undergo a continuous renewal. This was demonstrated by the increase in the number of labeled cells between 1 and 24 hours after injection of H^3 -TDR and the corresponding dilution in the labeling intensity in the same time interval. It seems questionable whether a 3 % labeling index is necessary to qualify a tissue as a renewing cell population.

Position of labeled cells

The mitotic activity revealed by the labeled cells took place in the generative layers of the epithelium. The distribution of the labeled cells between the basal and the spinous layers was largely in agreement with the observations by *Meyer et al.* (1956) and *Hayes et al.* (1964) of mitotic figures in the gingival epithelium of man.

The position of the labeled cells after division indicated that part of the divided cells stayed in the generative layers for further division while others migrated towards the surface and subsequently were exfoliated. The fact that mitotic activity occurred in the basal as well as in the spinous cell layers of the epithelia made an estimate of the ratio of migrating cells versus cells staying in the generative cycle impossible. The steady state condition indicates that the ratio is 1:1. This ratio would occur if in all cases one of the two daughter cells migrated towards the surface and the other remained in the generative layers as suggested by *Rolshoven* (1951). A 1:1 ratio would also occur, however, if in some cases both daughter cells migrated and a similar number of cell pairs remained in the generative compartment.

This question has been examined by *Leblond, Greulich & Pereira* (1964) for oesophageal and plantar epidermis epithelium. These workers found that the migration of cells from the generative layers occur at random mainly due to pressure from dividing cells. The present material was not examined systematically with regard to this question. However, examination of serial sections demonstrated the frequent presence of apparently paired labeled cells 48 hours after H³-TDR injection, which is in agreement with the findings of *Leblond et al.* (1964).

Varitability in labeling

It was evident from the present findings that a considerable variation exists in the frequency of labeling observed in the epithelial attachment as well as in the epithelium of the attached gingiva.

The labeling index was significantly higher in the epithelial attachment than in the epithelium of the attached gingiva. This coincided with inflammatory changes in the epithelial attachment, and it may be that the mitotic activity was increased as a result of the inflammation. This is in agreement with the findings of *Marwah, Weinmann & Meyer* (1960) and *Schultz-Haudt & From* (1961).

The accelerated mitotic activity of the epithelial attachment was undoubtedly primarily concerned with renewal rather than with expansion of the epithelium. This could be seen from the fact that in the many gingival crevices examined, the size and shape of the epithelial attachments from animals of varying age showed only comparatively small differences, although new cells were formed at a rate rapid enough to duplicate the number of cells within five days.

Comparison between turnover times calculated for gingival epithelia from various investigations

In Table 4, turnover times for gingival epithelium are calculated from the mitotic and labeling indices reported by the respective investigators.

Table 4

Turnover times for gingival epithelium based on the findings of various investigators*

Investigator	Year	Species	Epithelium	Turnover time (days)
<i>Mühlemann & Hartl</i>	1955	rat	intramolar	11.2—16.5
<i>Beagrie & Skougaard</i>	1962	mouse	a.g.	4.3
			e.a.	5.5
<i>Løe</i>	1961	dog	e.a.	1
<i>McHugh</i>	1959	rhesus	a.g.	6.3
			e.a.	10.9
<i>Skougaard & Beagrie</i>	1962	marmoset	a.g.	10.4
			e.a.	5.8
Present invest.		marmoset	a.g.	8.9
			e.a.	4.6
<i>Dimassimo</i>	1963	rhesus	a.g.	40.5
			e.a. (1)	10.0
			e.a. (2)	40.5
<i>Meyer et al.</i>	1955	man	a.g.	28.6—45.5
<i>Hayes et al.</i>	1964	man	a.g.	112

* a.g. = attached gingival epithelium; e.a. = epithelial attachment.

The reports by *McHugh* (1959), *Beagrie & Skougaard* (1962), *Skougaard & Beagrie* (1962), *Dimassimo* (1963) and the present study give information on the epithelial attachment as well as on the epithelium of the attached gingiva. In mice (*Beagrie & Skougaard* 1962) and in rhesus monkeys (*McHugh* 1959) the mitotic activity was found to be higher in the oral epithelium than in the epithelial attachment. The findings of *Dimassimo* (1964) in rhesus monkeys and those of the present study indicate, however, that the mitotic activity is highest in the epithelial attachment. In the present study the assumption was made that the higher activity in the epithelial attachment is related to inflammatory changes. The rhesus monkeys used in *McHugh's* experiments appeared to be very young animals and no signs of inflammation of the epithelial attachment area were seen in the illustrations. The same was true for the mouse crevices examined by *Beagrie & Skougaard* (1962). The apparent discrepancy in the

results thus seems to strengthen the assumption that inflammatory changes lead to an increased mitotic activity of the gingival epithelium.

The duplication time for the epithelial attachment of dogs reported by *Löe* (1961) was extremely short and indicated a high mitotic activity rate. The 24 hour turnover time corresponds to a labeling index of the order of 40 for the epithelial attachment of dogs. Such index is close to the indices found in intestinal crypt cells in mice (*Quastler & Sherman* 1959) which is the highest rate of cell proliferation so far reported. It seems likely that the sealing of the gingival crevices of the dogs in *Löe's* experiment has caused an acceleration of the mitotic activity beyond a physiological level. The high number of leukocytes observed in the sealed pockets suggests that the changes were inflammatory.

The number of mitotic figures reported by *Meyer et al.* (1956) and by *Hayes et al.* (1964) from human material was extremely low and of the same order of magnitude as the frequency of mitosis found in the present material from the marmosets. Assuming a duration of mitosis of 1 hour, these figures lead to turnover times for the gingival epithelium about 10 times as long as the values estimated from other investigations. It is of course possible that the turnover time of the gingival epithelium in man is considerably longer than that of rodents and monkeys. The data in Table 4 seem to indicate an increase in the turnover time of the gingival epithelium with the size of the animals. This is exemplified by the turnover times for the gingival epithelium of mice and marmosets estimated at 4.2—5.3 days and 5.8—10.4 days, respectively (*Beagrie & Skougaard* 1962 and *Skougaard & Beagrie* 1962). It seems unlikely, however, that the turnover time for the gingival epithelium of young human beings is almost 50 times longer than that of young rhesus monkeys (*Hayes et al.* 1964, *McHugh* 1959). I was felt pertinent to make an attempt to explain this discrepancy in results.

The mitotic cell division is controlled by a highly complex mechanism of mitotic inhibitors. According to *Bullough & Laurence* (1961, 1964) and *Bullough* (1962) adrenalin and noradrenalin are the most effective of the hormonal inhibitors and responsible for the diurnal variations in the mitotic activity. These

workers have further demonstrated a depression of the number of cells in mitosis during stress and related this to an increased adrenalin concentration (*Bullough & Laurence 1961*).

The antimitotic stress effect opens a possibility for explaining the low mitotic index found in the marmoset biopsies. Although precautions were taken to secure peaceful surroundings, attempts to domesticate the marmosets failed completely. It was thus not possible to catch the animals without considerable excitement, probably resulting in an increased adrenalin blood concentration. Such increased adrenalin concentration would not affect the number of cells synthesizing DNA, i.e., the number of labeled cells. However, the 1 hour time interval between H³-TDR injection and biopsy leaves the cells already in mitosis time to conclude the division, whereas no new cells will be allowed to pass early prophase. A possible adrenalin blockade would therefore result in a marked reduction in the number of mitotic figures at the time of biopsy.

The very low mitotic index reported by *Hayes et al.* (1964) was found in gingival biopsies taken from children prior to tooth extractions. It seems possible that the adrenalin concentration of the tissue may have been well above physiological level assuming conventional local anaesthetics containing adrenalin (nor-adrenalin) were used.

The present study was not undertaken for the purpose of participating in the discussion on the epithelial attachment. Some information of interest for this discussion has, however, been obtained. In the fully erupted teeth of the marmosets the epithelial attachment was found to be renewed at a higher rate than the attached gingiva. The rate of DNA synthesis as well as the duration of the S-phase appeared to be identical in the two gingival epithelia examined. Since the accelerated cell turnover in the epithelial attachment may be explained by incipient inflammatory changes, it seems reasonable to conclude that as far as DNA synthesis and cell turnover are concerned, no distinction can be made between the cell populations of the two epithelia.

The present material does, however, not offer any information as to the kinetics of these cells in unerupted or erupting teeth. This problem requires further study.

SUMMARY AND CONCLUSIONS

The present investigation was concerned with the generative activity in the gingival epithelium of adult marmosets as revealed by autoradiography after injection of H^3 -TDR.

Seven marmosets were injected with H^3 -TDR and sacrificed 1 hour to 72 hours after the injections. Autoradiographs were made from sections of teeth and surrounding gingiva and the labeling which appeared in the epithelial attachment and the epithelium of the attached gingiva was recorded.

In the epithelial attachment the labeling index was significantly higher than in the epithelium of the attached gingiva, viz., 7.2 and 3.7, respectively. It is suggested that this was related to inflammatory changes in the epithelial attachment.

For the epithelial attachment the turnover time was 4.6 days \pm 1.2 days and for the attached gingival epithelium 8.9 days \pm 3.4 days.

Turnover times for gingival epithelia found by various investigators were compared with the present findings. It is suggested that the extremely rapid duplication of the cells in the epithelial attachment of sealed gingival crevices from dogs is related to unphysiological conditions. It is finally suggested that the very low mitotic activity observed in gingival biopsies from children might be the result of a blockade of mitoses caused by adrenalin (or noradrenalin) from local anaesthesia.

RÉSUMÉ ET CONCLUSIONS

RENOUVELLEMENT DE L'ÉPITHÉLIUM GINGIVAL CHEZ LE OUISTITI

Le travail ci-dessus concerne l'activité proliférative de l'épithélium gingival du ouistiti adulte étudiée par la méthode autoradiographique après injection de H^3 -TDR.

Sept ouistitis recurent une injection de H^3 -TDR et furent sacrifiés de 1 à 72 heures après l'injection. Des autoradiographies de coupes de dents et de l'épithélium environnant furent pratiquées et le marquage fut étudié dans l'attache épithéliale et dans l'épithélium de la gencive adjacente.

Dans l'attache épithéliale l'index de marquage était, de manière significative, plus élevé que dans la gencive adjacente, re-

spectivement 7.2 et 2.3. Il est suggéré que ceci est du aux modifications inflammatoires dans l'attache épithéliale.

Pour l'attache épithéliale le temps de "turnover" était de 4.6 jours \pm 1.2 et pour l'épithélium de la gencive adjacente 8.9 jours \pm 3.4.

Les temps de turnover de l'épithélium gingival, trouvés par divers investigateurs ont été comparés aux résultats ci-dessus. Il est suggéré que le dédoublement extrêmement rapide des cellules dans l'attache épithéliale dans la poche gingivale scellée chez le chien était lié aux conditions non physiologiques.

Finalement il est suggéré que l'activité mitotique extrêmement basse trouvée dans les biopsies gingivales d'engants pourrait être le résultat du blocage des mitoses causé par l'adrénaline (ou la noradrénaline) de l'anesthésie locale.

ZUSAMMENFASSUNG UND SCHLUSSFOLGERUNGEN

DIE ERNEUERUNG DES ZAHNFLEISCHEPITHEL BEI MARMOSETS

Die mitotische Aktivität im Zahnfleischepithel erwachsener Marmosets wurde autoradiographisch nach Injektion von H^3 -TDR untersucht.

Sieben Marmosets wurden 1 bis 72 Stunden nach der Injektion von H^3 -TDR getötet. Autoradiographien von Schnitten der Zähne und des umgebenden Zahnfleisches wurden genommen, auf denen die Menge markierter Substanz im Epithelansatz und im Epithel des anschliessenden Zahnfleisches bestimmt wurde.

Im Epithelansatz war der markierte Anteil deutlich höher als im anschliessenden Zahnfleisch (7.2 : 3.7). Es wird angenommen, dass dieser Befund auf entzündliche Veränderungen im Epithelansatz zurückzuführen ist.

Für den Epithelansatz wurde als Umsatzzeit 4.6 ± 1.2 Tage, für das umgebende Zahnfleisch 8.9 ± 3.4 Tage ermittelt.

Von verschiedenen Autoren gefundene Umsatzzeiten für Zahnfleischepithel wurden mit den vorliegenden Ergebnissen verglichen. Die extrem schnelle Zellvermehrung im Epithelansatz abgeschlossener Zahnfleischtaschen bei Hunden ist auf unphysiologische Bedingungen zurückzuführen.

Abschliessend wird festgestellt, dass die nach Zahnfleischbiopsien bei Kindern auftretende, sehr geringe Aktivität bei der Zell-

teilung wahrscheinlich die Folge einer Blockade der Mitose durch bei der Lokalanästhesie verwendetes Adrenalin oder Noradrenalin ist.

REFERENCES

- Beagrie, G. S. & M. R. Skougaard*, 1962: Observations on the life cycle of the gingival epithelial cells of mice as revealed by autoradiography. *Acta odont. scand.* 20: 15.
- Bullough, W. S.*, 1962: The control of mitotic activity in adult mammalian tissues. *Biol. Rev.* 37: 307.
- Bullough, W. S. & E. B. Laurence*, 1960: The control of mitotic activity in mouse skin. *Dermis and hypodermis. Exp. Cell Res.* 21: 394.
- 1961: Stress and adrenaline in relation to the diurnal cycle of epidermal mitotic activity in adult male mice. *Proc. roy. Soc. B* 154: 540.
- 1964: Mitotic control by internal secretion: The role of the chalone-adrenalin complex. *Exp. Cell Res.* 33: 176.
- Dimassimo, C.*, 1963: *Proliferation and migration of cells in the gingival epithelium*. Thesis, Univ. of Rochester, New York.
- Fry, R. J. M., S. Lesher & H. I. Kohn*, 1961: A method for determining mitotic time. *Exp. Cell Res.* 25: 469.
- Hayes, R. L., M. Silberkweit, N. N. Soni & T. H. Simpson*, 1964: Pattern of mitotic activity in oral epithelium of rabbits. *Arch. Path.* 54: 281.
- Henry, J. L., J. Meyer, J. P. Weinmann & I. Schour*, 1962: Pattern of mitotic activity in oral epithelium of rabbits. *Arch. Path.* 54: 281.
- Joftes, D. L. & S. Warren*, 1955: Simplified liquid emulsion radioautography. *J. biol. photogr. Ass.* 23: 145.
- Koburg, E. & W. Maurer*, 1962: Autoradiographische Untersuchung mit (³H) Thymidin über die Dauer der deoxyribonukleinsäure Synthese und ihren zeitlichen Verlauf bei den Darmepithelien und anderen Zelltypen der Maus. *Biochim. biophys. Acta* 61: 229.
- Leblond, C. P.*, 1959: Classical technics for the study of the kinetics of cellular proliferation. p. 31 in *F. Stohlman (Ed.): The Kinetics of Cellular Proliferation*. Grune and Stratton, New York.
- Leblond, C. P., R. C. Greulich & J. P. M. Pereira*, 1964: Relationship of cell formation and cell migration in the renewal of stratified squamous epithelia. p. 39 in *W. Montagna & R. E. Billingham (Eds.): Advances in Biology of Skin. Vol. V*. Pergamon Press, Oxford.
- Löe, H.*, 1961: Physiological aspects of the gingival pocket. An experimental study. *Acta odont. scand.* 19: 386.
- Marwah, A., J. Meyer & J. P. Weinmann*, 1956: Mitotic rate of gingival epithelium in two age groups. *J. Periodont.* 27: 313.
- Marwah, A., J. P. Weinmann & J. Meyer*, 1960: Effect of chronic inflammation on the epithelial turnover of the human gingiva. *Arch. Path.* 69: 147.
- McHugh, W. D.*, 1959: *The development and structure of the gingival epithelium*. Thesis, Univ. of St. Andrews, Scotland.

- Messier, B. & C. P. Leblond*, 1960: Cell proliferation and migration as revealed by radioautography after injection of thymidine- H^3 into male rats and mice. *Amer. J. Anat.* 106: 247.
- Meyer, J., A. S. Marwah & J. P. Weinmann*, 1956: Mitotic rate of gingival epithelium in two age groups. *J. Invest. Dermat.* 27: 237.
- Meyer, J., J. Medak & J. P. Weinmann*, 1960: Mitotic activity and rates of growth in regions of oral epithelium differing in width. *Growth* 24: 29.
- Mühlemann, H. R. & S. Hartl*, 1955: Daily variations of mitotic rate and inflammatory cell migration in the epithelium of the intermolar rat papilla. *Bull. Schweiz. Akad. med. Wiss.* 11: 379.
- Quastler, H.*, 1960: Cell population kinetics. *Ann. N. Y. Acad. Sci.* 90: 580.
- Quastler, H., & F. G. Sherman*, 1959: Cell population kinetics in the intestinal epithelium of the mouse. *Exp. Cell. Res.* 17: 420.
- Rolshoven, E.*, 1951: Über die Reifungsteilungen bei der Spermatogenese mit einer Kritik des bisherigen Begriffes der Zellteilungen. *Verh. d. Anat. Ges.* 49: 189.
- Schultz-Hautdt, S. D. & S. From*, 1961: Dynamics of periodontal tissues, I. The epithelium. *Odont. T.* 69: 430.
- Skougaard, M.*, 1965: *Cell Population Kinetics of the Gingival Epithelium*. Rhodos Sci. Publications, Copenhagen.
- 1965: Duration of DNA synthesis in the gingival epithelial cells of mice and marmosets. *Acta odont. scand.* 23: 615.
- Skougaard, M. & G. S. Beagrie*, 1962: The renewal of gingival epithelium in marmosets (*Callithrix Jacchus*) as determined through autoradiography with thymidine- H^3 . *Acta odont. scand.* 20: 467.
- Toto, P. D. & G. Ojha*, 1962: Generation cycle of oral epithelium in mice. *J. dent. Res.* 41: 388.