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OBSERVATIONS ON THE LIFE CYCLE OF THE GINGIVAL EPITHELIAL CELLS OF MICE AS REVEALED BY AUTORADIOGRAPHY

by

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The purpose of this paper is to present some observations on the life cycle of the epithelial cells in the gingival mucosa of the molar teeth of mice.

It is accepted that in the mucous membrane of the mouth a constant loss of cells occurs from the surface by desquamation and that this loss is compensated for, by new cells forming in the basal layer. According to *Hirt, Hartle & Mühlemann* (1955), the mitotic activity in the epithelial attachment is nearly as great as that found in the rest of the gingivae, whereas *Greulich* (1961) claims that it is greater. *Waerhaug* (1960) poses a question regarding the desquamation of surface cells in the epithelial attachment, but in discussing the biology of this area it is of more importance to determine the complete life cycle of the epithelial cells.

Through the injection of a radioactive isotope which will be incorporated into the new forming and dividing cells only, the fate of the cells can be observed by autoradiography. Several workers (*Reichard & Estborn* [1951], *Mac Donald & Mallory* [1959], *Messier & Leblond* [1960]) have demonstrated a satisfactory method, utilising the fact that prior to mitosis, cells

double their desoxyribonucleic acid (D.N.A.) and take up various D.N.A. precursors. Thymidine is one of these precursors and it is available in a tritiated form (thymidine - H^3). Beta rays are emitted from this isotope with suitably low energy for recording on photographic emulsion (*Mac Donald & Mallory* [1959]).

Messier & Leblond (1960) through calculation of the radioactive index (% radioactive nuclei) have suggested that the cell population of a tissue can be expressed as static, expanding, or renewing. They defined a static cell population as one showing no labelled nuclei and described this in the neurones of the cerebrum, cerebellum and spinal cord. In an expanding cell population a small number of labelled nuclei was present (0.4—1 %). The labelled cells appeared to remain in the tissue indefinitely. They were found in muscle and most connective tissues. Where 3 % or more labelled nuclei were present, with an increase in number during the first twenty-four hours followed by a decrease 3—6 days afterwards, the tissue had a renewing cell population. These workers showed that stratified squamous epithelium of the tongue and oesophagus of rats and mice were of this category.

It is accepted that in stratified squamous epithelium the usual movement of cells is of a passive type, viz. new cells forming in the basal layer pressing older ones towards the surface. In the diapedesis of leucocytes there is an active cell movement. *Messier & Leblond* (1960) have shown that in the gastric mucosa of mice, epithelial cell migration occurred even when cell mitosis was prevented by irradiation. From their findings they claimed that in this epithelium an active as well as a passive movement of cells existed.

The aims of the present experiment are to observe the life cycle of the epithelial cells in the gingivae of mice; to determine the type of cell population through calculating the radioactive index; to investigate the type of cell migration and to compare the epithelial attachment with the rest of the gingival epithelium.

MATERIAL AND METHODS

The material used in this study was taken from 13 male (Q strain) mice aged 8 weeks±1 day from the Animal Genetics Department of the University of Edinburgh. With age, sex, and

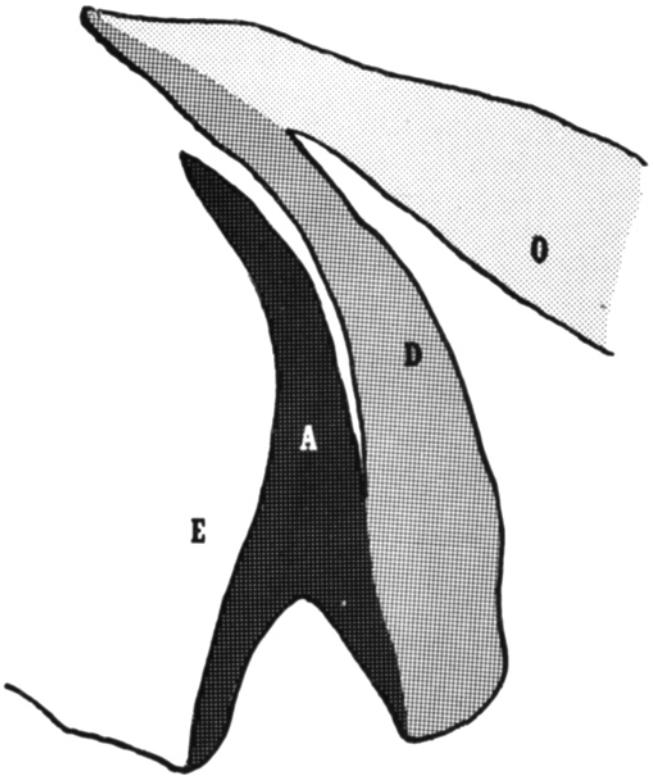

Fig. 1. For the purpose of description the diagrammatic division of the gingival papilla. O — oral epithelium; D — downgrowing oral epithelium; A — epithelial attachment; E — enamel space.

genetic background similar, it was expected that more stable results would be given from the experiment. Only the gingivae of the molar teeth were observed. *Mahn* (1890) has shown that the last molar tooth of the mouse erupts approximately twenty-eight days after birth. Our observations were thus related to the gingivae of fully erupted teeth. The average weight of the mice was 28 grams. They were injected subcutaneously with 1 micro curie tritiated thymidine per gram bodyweight. All injections were given within one hour so that diurnal variations would not influence the result. The animals were sacrificed at intervals of 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 36 hours, 2 days, 3 days, 5 days, 7 days, 10 days, 12 days and 15 days. The vascular system

of the animal was perfused via the left ventricle of the heart with 10 % neutral buffered formalin after which the heads were removed, fixed in the same solution, decalcified in formic acid and double embedded in celloidin and paraffin wax. Sections were cut bucco-lingually and mesio-distally at 6—8 microns and covered with autoradiographic stripping film (Kodak AR 10) as described by *Pelc* (1947). The film-covered sections were placed in light-tight boxes and stored at a constant temperature of 4°C for 21 days. The films were then developed and fixed, after which the sections were stained in haematoxylin.

Hirt, Hartle & Mühlemann (1955) investigating the distribution of mitosis in rat gingival epithelium described the tissue around the tooth as composed of two parts — the downward growing oral epithelium and the epithelial attachment. The mouse has a similar type of gingival epithelium and for descriptive purposes it was decided to use the same nomenclature and division of the tissue (Fig. 1).

RESULTS*

All sections examined showed clear labelling of the cells throughout the periodontium. Some of the sections showed a diffuse scatter of spots on the film but these were considered to be "background". The background problem in autoradiography

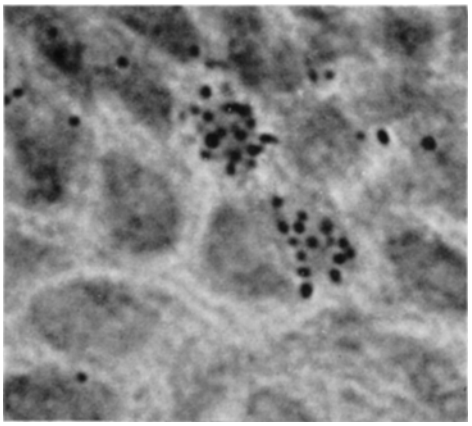

Fig. 2. Typically labelled epithelial cells.

* Only sections showing the main pattern of the experiment are reported.

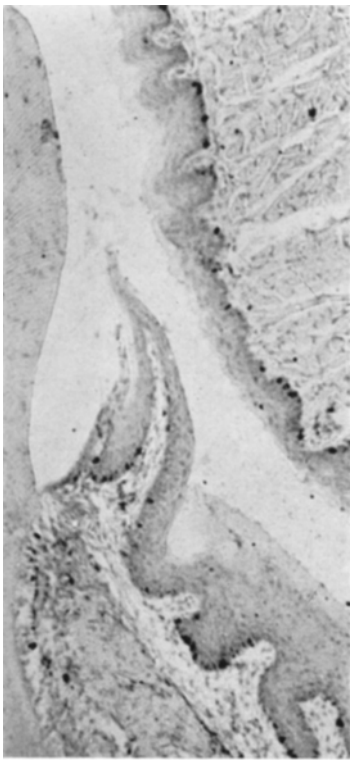

Fig. 3. A lightly stained section showing the distribution of labelled cells in the basal layers of the epithelium one hour after injection of thymidine -H^3. Some labelling is also present in the periodontal membrane.

has been discussed by several workers notably *Doniach & Pelc* (1950) and *Mac Donald & Mallory* (1959). In our investigation a cell had to have labelling, of the type illustrated in Fig. 2, to be accepted as satisfactory. All sections were examined under oil immersion to determine the individual identity of the cells. In this way, it was possible to avoid counting a labelled leucocyte which might have migrated through the epithelium.

The *one hour* specimen showed labelling throughout the basal layers of all epithelium examined (Fig. 3). The largest number of labelled cells appeared to be in the downgrowing oral epithelium. In the epithelial attachment some sections showed a few labelled cells on the surface layer, but this was only observed in close proximity to the cemento-enamel junction.

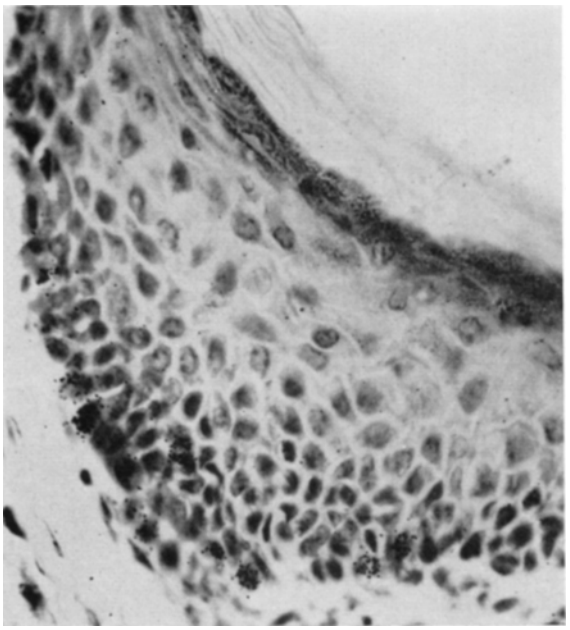

Fig. 4. Labelled cells in the basal layer of the oral epithelium 3 hours after injection.

The observations on *three hour* specimens are exemplified in Figs. 4 and 5. An increased number of labelled cells, some of which were paired, was present in the basal layer of all three epithelia. In the epithelial attachment near the cemento-enamel junction labelled cells were also found in the middle and superficial cell layers.

After *24 hours* the coronal part of the epithelial attachment was significantly devoid of labelled cells, in contrast to the remainder in which an even distribution of labelling was found (Fig. 6). The other epithelia now showed marked cells in the spinous layer as well as in the basal epithelium.

After *three days* the only part of the epithelial attachment

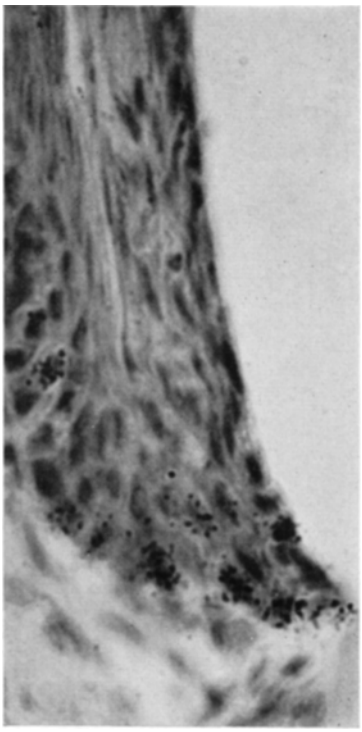

Fig. 5. A 3 hour section of the epithelial attachment next the cemento-enamel junction showing the distribution of labelled cells from the basal to the surface layer.

showing fully labelled cells was the coronal tip (Fig. 7). This observation was also made in a few of the five day sections. Throughout the rest of the epithelial attachment only lightly labelled cells, with a few exposed silver grains, were present. The oral and downgrowing oral epithelium showed labelling mainly in the spinous layer (Fig. 8).

In the *five day* sections labelled cells were similarly distributed but were nearer the granular and keratinous layers.

After *twelve days* very light labelling was present in all epithelium examined with an occasional heavily labelled cell showing near the surface of the oral epithelium. This was in contrast to the periodontal membrane in which cells were labelled as heavily as in the earlier sections (Fig. 9).

Fig. 6. A section of the gingival papilla 24 hours after injection of thymidine - H^3. One or two labelled cells are seen in the spinous layer of the oral and downgrowing oral epithelium. In the epithelial attachment some cells are seen on the surface; the coronal tip is devoid of labelled cells.

Radioactive Index

The radioactive index (% labelled cells) was calculated for the 1 hour, 3 hour, 6 hour, 12 hour, 24 hour and 3 day specimens. These time intervals are the same as those suggested by *Messier & Leblond* (1960). The average percentages are shown in Table 1. These were calculated by examining 10 random sections from each specimen, and the results were compiled from counting 1000 cells in each epithelial area.

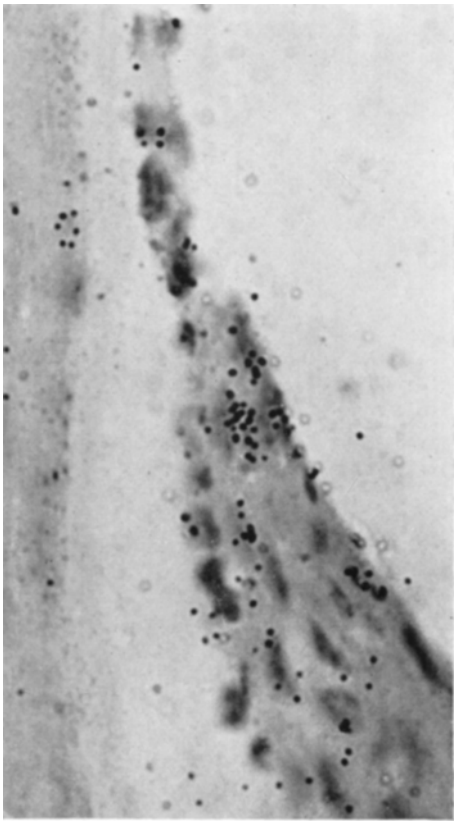

Fig. 7. The tip of the epithelial attachment showing a heavily labelled cell still present after 3 days. The remainder of the attachment shows only light labelling.

Table 1.

Radioactive Index (% Labelled Cells)
at different time intervals after thymidine - H^3 injection.

	1 hour	3 hours	6 hours	12 hours	24 hours	mean	3 days
Epithelial Attachment	5.5	8.2	10.3	9.3	12.7	9.2	2.4
Downgrowing Oral Epithelium	7.9	10.0	9.9	9.5	10.5	9.5	1.6
Oral Epithelium	7.0	7.6	8.6	7.5	10.3	8.2	3.2

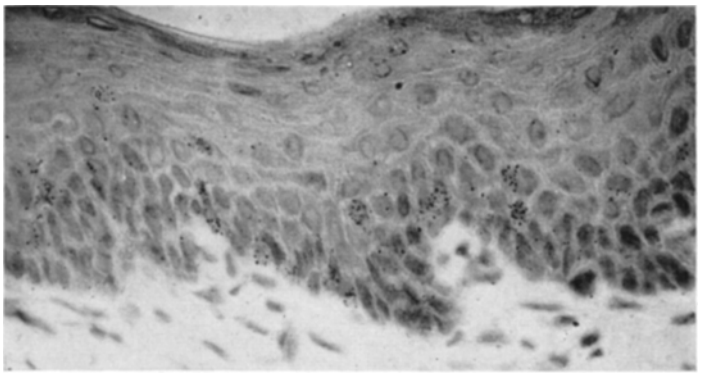

Fig. 8. A 3 day section of oral epithelium with typical distribution of cells in the spinous layer.

DISCUSSION

The observations on the gingival epithelium are in agreement with those described by *Messier & Leblond* (1960) for oesophageal and tongue epithelium, viz. initial labelling in the basal layer followed by migration towards the surface. In some of the one hour sections, particularly in the epithelial attachment, labelled cells were apparently present in the middle of the spinous layer (Fig. 10). Examination of serial sections, however, showed that in reality these were basal cells so placed, because of an invagination of the connective tissue (Fig. 6).

As mentioned earlier radioactive thymidine is incorporated into a cell just prior to mitosis. A mature labelled cell on division distributes its radioactivity in equal quantity between its two daughter cells. Some of these new cells migrate towards the surface, while others are retained for further division and a proportional decrease of radioactivity. Migrating cells do not undergo further division (*Messier & Leblond,* 1960) and when these are marked and observed an expression of the time taken for tissue renewal is given. The tissue renewal time is that taken for the last fully labelled cell to be lost from the surface layer.

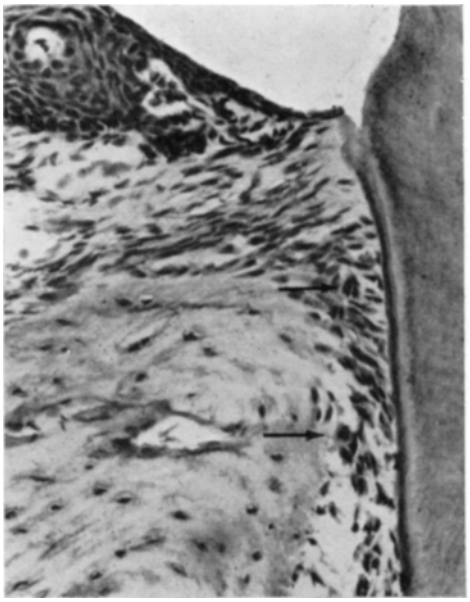

Fig. 9.

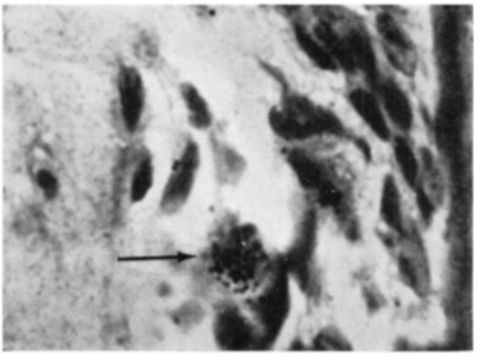

Fig. 9 A.

Fig. 9. 12 day autoradiograph. A few lightly labelled cells are present in the epithelial attachment in contrast to the heavier labelled cells of the periodontal membrane. Higher magnifications are shown in Figs. 9 A and 9 B (labelled cells arrowed).

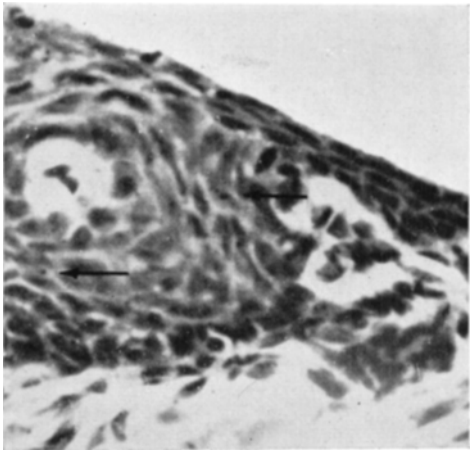

Fig 9 B.

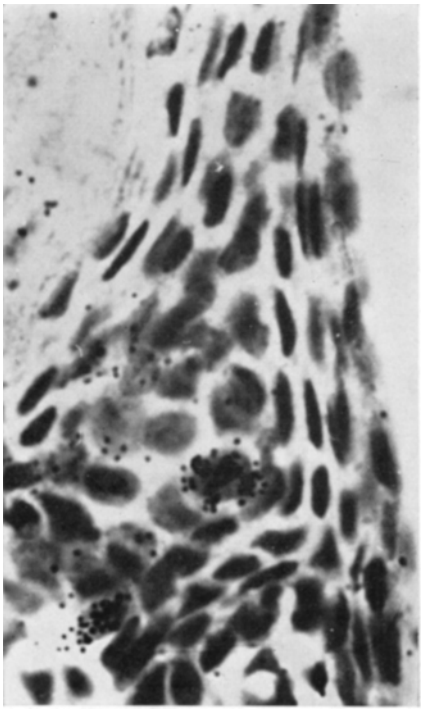

Fig. 10. 1 hour section showing typical labelled cells in the basal layer of the epithelial attachment. The cell in the centre of the epithelium was so placed through an invagination of the connective tissue. This was shown from examination of serial sections.

The criteria for a renewing cell population are:

1: a radioactive index > 3 %

2: an increase in the number of labelled cells in the first 24 hours, followed by

3: a decrease in the 3—6 day period.

According to this definition the results shown in Table 1 indicate that the three epithelial areas examined have a renewing cell population.

Cell migration in the oral epithelium was easily followed to the surface layer (Figs. 4, 6, and 8). It appeared to be of a passive type, with the fully labelled cells disappearing in the granulous layer between ten and twelve days.

In the epithelial attachment cell migration seemed to be horizontal in the apical part and more vertical or oblique in the remainder. The downgrowing oral epithelium also seemed to demonstrate this combined migration to some extent. It is probable that only a passive type of cell migration occurs, but with the complicated direction of movement in these two areas, the possibility of active cell migration cannot be ruled out.

It would appear from our observations that in the epithelial attachment nearest to the cemento-enamel junction, a complete renewal of cells occurs in 24 hours, but that labelled cells take 3—5 days to reach the coronal tip.

Hirt, Hartle & Mühlemann (1955) found in rats, no mitotic figures at the junction between the epithelial attachment and the downgrowing oral epithelium, and claim this as an area of degeneration. Our observations in mice have failed to demonstrate in this part a basal layer of cells where mitosis could take place. Instead, we found that some cells at the tip of the epithelial attachment and the downgrowing oral epithelium terminated their life cycle here.

Our findings do not give support to the contention that the epithelial attachment is a degenerating tissue (*Skillen,* 1930). It is shown to have a renewing cell population. The cells which migrate against the enamel have an age range of 24 hours to 5 days. For surface cells, they are thus younger than their counterparts of the neighbouring oral and downgrowing oral epithelium. The nucleus of a cell in the oral epithelium breaks up before the cell passes into the keratinous layer. Against the enamel there is no keratinous layer and heavy labelling from the nucleus is maintained until the cell is lost. The environment of a cell detached from this surface will be little changed if the transudate described by *Brill & Krasse* (1958) has the same composition as blood plasma (*Brill & Brönnestam,* 1960). Consequently, such a cell might well maintain its vitality until it is lost to the oral cavity.

Recently *Löe* (1961) contended that in the dog a complete turnover of cells in the epithelial attachment was to be expected in 24 hours. This is not in agreement with our findings, but an accurate comparison cannot be made between the gingival epithelium of such different experimental animals.

SUMMARY AND CONCLUSIONS

The life cycle of the cells of the gingival epithelium of mice has been investigated using tritiated thymidine to label the new forming and dividing cells.

The downgrowing oral epithelium and the epithelial attachment has been compared to the oral epithelium. The type of cell population has been determined by means of the radioactive index and the three epithelia were considered to have a renewing cell population.

The movement of labelled cells has been observed. In all areas of the gingivae labelled cells were first seen in the basal layer from which they migrated to the surface. This movement was complete in oral epithelium by 10—12 days; in downgrowing oral epithelium 10—12 days, and in the epithelial attachment from 24 hours to 5 days, depending on the distance between the basal and the surface layer.

The epithelial attachment does not appear to be undergoing degeneration.

Cell migration in the oral and downgrowing epithelium was considered to be of a passive type. It is likely that in the epithelial attachment a similar type of cell migration occurs, but with the complicated movement of cells in this area further investigation is necessary to draw a definite conclusion.

Acknowledgment

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RÉSUMÉ ET CONCLUSIONS

OBSERVATIONS SUR LE CYCLE VITAL DES CELLULES DE L'ÉPITHÉLIUM GINGIVAL DE SOURIS MIS EN ÉVIDENCE PAR AUTORADIOGRAPHIES

Le cycle vital des cellules de l'épithélium gingival de souris a été étudié en utilisant la thymidine au tritium pour marquer les nouvelles cellules en voie de formation et en voie de division.

L'épithélium buccal se developpant vers le bas et l'attachement épithelial ont été compares à l'épithélium buccal. Le type de population cellulaire a été déterminé au moyen de l'index radioactif et on a considéré que les trois épithéliums avaient une population cellulaire se renouvelant.

Le mouvement des cellules marquées a été observé. Dans toutes les régions de la gencive, les cellules marquées ont d'abord été vues dans la couche basale d'où elles se deplaçaient vers la surface. Ce mouvement etait achevé dans l'épithélium buccal en 10 à 12 jours, dans l'épithélium buccal se developpant vers le bas en 10 à 12 jours, et dans l'attachement épithélial en 24 heures à 5 jours, suivant la distance entre la couche basale et la couche superficielle.

L'attachement épithélial ne semble pas subir de dégénération.

La migration cellulaire dans l'épithélium buccal et dans l'épithélium descendant a été considérée comme étant d'un type passif. Il est vraisemblable que dans l'attachement épithélial, un type similaire de migration cellulaire se produit, mais, étant donné le mouvement complexe des cellules dans cette zone, des recherches ultérieures sont necessaires pour pouvoir tirer des conclusions definitives.

ZUSAMMENFASSUNG UND KONKLUSIONEN

UNTERSUCHUNGEN ÜBER DEN LEBENSLAUF DES GINGIVALEPITHELS VON MÄUSEN MITTELS AUTORADIOGRAPHIE

Der Lebenslauf der Zellen des Gingivalepithels von Mäusen ist unter Verwendung von H^3 - thymidin, welches die neuen Zellen und die eben vor Teilung stehenden Zellen markieren, untersucht worden.

Das niederwachsende Mundepithel und der Epithelansatz ist mit dem Mundepithel verglichen worden. Die Art der Zellen ist durch den radioaktiven Index bestimmt worden, und es stellte sich heraus, dass die drei Epithelien aus "renewing cell population" bestehen.

Die Wanderungen der markierten Zellen wurden beobachtet. In allen Teilen der Gingiva wurden markierte Zellen zuerst in der Basalschicht wahrgenommen, wovon sie zur Oberfläche übersiedelten. Im Mundepithel vollzog sich diese Wanderung in 10—12 Tagen, im niederwachsenden Mundepithel in 10—12 Tagen und im Epithelansatz in 24 Stunden bis 5 Tagen, vom Abstand der Basalschicht zur Oberflächeschicht abhängig.

Der Epithelansatz scheint keiner Degeneration unterworfen zu sein.

Es wurde gefunden, dass im oralen und niederwachsenden Epithel die Zellenwanderung passiver Art ist. Wahrscheinlich gehen im Epithelansatz in ähnlicher Weise Zellenwanderungen vor sich, aber wegen der verwickelten Bewegungen der Zellen in diesem Gebiet sind weitere Untersuchungen benötigt um eine endgültige Konklusion zu formulieren.

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