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Cytological Study of the Migration of the Cells of the Interdental Papillae in the Mouse

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CYTOLOGICAL STUDY OF THE MIGRATION OF THE CELLS OF THE INTERDENTAL PAPILLAE IN THE MOUSE

by

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LIFE

Anjana A. Joglekar was born on May 5, 1937 in Bombay, India.

She attended the primary and secondary schools in Bombay and graduated from the New Era High School in 1954. In June 1956 she finished her pre-dental college education at Jai-Hind College in Bombay. Immediately after, she entered the Nair Hospital Dental College in Bombay City from which she graduated in December 1960 with the degree of Bachelor of Dental Surgery.

In September of 1962 she enrolled in the graduate school of Loyola University of Chicago to pursue a Master of Science degree in the department of Oral Biology.

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INTRODUCTION

A constant loss of cells from the mucous membrane of the mouth is compensated for by new cells forming in the basal cell layer and the prickle cell layer.

The purpose of this study is to observe deoxyribose nucleic acid (DNA) synthesis and the path of cell migration in the epithelium of the interdental papillae of the mouse, through the injection of tritiated thymidine, a radioactive isotope, which will be incorporated into the DNA of the cell preparing for mitosis. The location, path of migration, and fate of such cells can be observed by autoradiography.

Assuming that the newly formed daughter cells would follow the direction determined by the plane of mitotic figures, it is intended to study the direction of the migration of such cells in the epithelium.

The epithelial cells migrate from the basal cell layer of the epithelium, towards the epithelial attachment, or towards the apex of the interdental papillae. This study is concerned with the determination of the path of migration of these cells.

REVIEW OF THE LITERATURE

A. Epithelial Attachment

The subject of the epithelial attachment has been extensively discussed in dental literature. Opinions as to the exact nature of the epithelial attachment fall into two main groups. One claims the existence of an organic connection between enamel and the covering epithelium. The other group objects to this view but believes that the contact of the epithelium with the enamel is maintained through the forces of adhesion between the epithelial cells and the enamel surface.

Black in 1915 described the free gingiva as soft tissue process growing out from the body of the gingiva and covering a portion of the enamel surface of the crowns of the teeth. This part of the tissue has no attachment to the teeth, after passing occlusally of the gingival line, but is simply closely fitted about them. He further stated that a thin blade may readily be passed between the gingiva and the enamel, raising the tissue and exposing the subgingival space between it and the enamel. This tissue has no attachment to the tooth until the gingival line on the tooth is reached; which means that the attachment is to the cementum only.

Early writers of textbooks on dental histology

and pathology (Black, Bodeckar, Broomell) supported the view that the gingival crevice was located at the cemento-enamel junction, even in the teeth that were just erupting.

This concept of the gingival crevice was objected to by Gottlieb in 1927 when he stated that, "After formation of the enamel, the ameloblasts are resorbed and the stratum intermedium and the enamel pulp have atrophied, the outer enamel epithelium is as closely connected organically to the enamel surface as the ameloblasts were before. The eruption of the tooth takes place in such a way that the top of the outer enamel epithelium unites with the mouth epithelium. A hole is formed at the point of union and that part of the enamel which has been disclosed now projects into the mouth. The rest of the entire outer enamel epithelium is still joined to the enamel surface as before." This was supported by Orban and Mueller in 1929, Becks also in 1929, and Kronfeld and Skillen in 1930.

The enamel cuticle, a delicate membrane produced by ameloblasts and which covers the entire crown of the newly erupted tooth, was conceived by Nasmyth, whose name it has borne since its discovery. Gottlieb

believed that this structure was composed of two membranes which he has designated as the primary and secondary cuticles. The primary cuticle is closely attached to the enamel, and closely attached to it is the secondary cuticle which is produced by reduced enamel epithelium covering the crown.

Orban in 1929 stated that the average crevice is about 0.8 mm deep and that the gingival crevice is not located at the cemento-enamel junction, but high on the surface of the crown and that between this point and the cemento-enamel junction, the epithelium is attached to the enamel.

Gottlieb in 1927 claimed that "the outer enamel epithelium detaches itself pari passu from the enamel surface." Further, the outer enamel epithelial layer is capable of forming a secondary cuticle before becoming detached from the surface of the enamel. When, in the course of further eruption, the bottom of the crevice approaches the cemento-enamel junction, a "downward growth" of the epithelium always sets in.

Skillen in 1930 agreed with Beck's idea of 1929 in the concept of the degeneration of the entire epithelial attachment. They believed that the degeneration of the epithelial attachment was followed by

the proliferation of the oral epithelium downwards behind the epithelial attachment as if to replace the epithelial attachment as well as to compensate for the apical migration of the epithelial attachment and gingiva.

To support Gottlieb's view, Bodecker and Applebaum in 1939 showed the gingival attachment to the enamel in ground sections of the teeth of animals and man. Turner in 1927 and Kronfeld in 1930 observed the adherence of epithelium to extracted teeth. Toller (40) utilizing frozen sections of teeth and their surrounding tissues in young dogs, pulled away the free margin of the gingiva from the teeth. It was shown that after a certain depth at which the epithelium peeled off clearly from the enamel surface, the epithelial attachment separated and left part of the epithelial attachment on the enamel surface, thus demonstrating the strong attachment of the epithelium to the enamel.

Macapanpau in 1956 confirmed this view by his findings on rat molars and on human teeth. He suggested that tears and splits in histologic sections were evidence that "the union between enamel and epithelium was stronger than the union between enamel and dentine." However, the fallacy in this reasoning

was exposed by Zander in 1955. He stated that they were artifacts and not tears or splits.

In a study of the human enamel cuticle, Ussing in 1955 was able to demonstrate by means of the electron microscope, fibrils passing into the homogenous layer of the enamel cuticle from the ameloblast side as well as from the enamel side. In unerupted molars of mice, these latter fibrils were seen to be intimately connected with similar fibrils from the enamel matrix and are assumed to be an essential part of the epithelial attachment.

Though during amelogenesis an organic connection is known to exist between enamel epithelium and enamel matrix, the presence and the fate of this attachment at later stages has been a controversial subject.

On the basis of his findings by the phase contrast microscopy on the nature of the epithelial attachment, Baume (1952, 53, 54) states that the epithelial attachment is a fibrillar structure. Tonofibrils are present in oral epithelium as well as in reduced enamel epithelium, but in a different way. In oral epithelium they are arranged in bundles of 15-25 units which pass parallel to the side of the nucleus from one cell into the other. The fusiform extracellular portions are

identical with the intercellular bridges observed with conventional histologic methods. It was further mentioned by Baume that the tonofibrils of the reduced enamel epithelial cells and the ameloblasts in particular are coarse and run more as individuals rather than compounds from one cell into the other and eventually in the long axis of the ameloblasts. The continuity of the fibrils is maintained during the physiologic replacement of the ameloblasts by young proliferating prickle cells and the fusion of the latter with the basal layer of oral epithelium.

Baume further states that the existence of the tonofibrils depends upon the vitality of the epithelial cells; tonofibrils disintegrate when the cell dies.

He concluded that the cohesion of the cells of the gingival epithelium among themselves and with the neighboring tissues is secured by tonofibrils. The epithelial attachment to the tooth has a fibrillar structure which is in continuity with the primary enamel cuticle. This has been supported by Ussing in 1955 and McHugh in 1961.

Stern in 1962 working on rat incisors reported that there is an organic union between the ameloblasts and the enamel. This connection consists of hemidesmosomes, a membrane-like structure and fine filaments.

Fine filaments course to the hemi-desmosomes which are attached to the membrane-like structure (the cuticular membrane) which in turn, appears to have an organic union with the enamel by means of fine filaments.

The controversy of the epithelial attachment was aroused again by Waerhang in 1953. He denied the attachment of the epithelium to the tooth and returned to the concept of a potential space, a pocket, extending from the gingival margin to the cemento-enamel junction, from the time the tooth had erupted into the oral cavity. He based his findings on the observations that a thin and narrow steel blade could be inserted into this space without any pressure. On sections of the tooth and its supporting structures he claimed that there was no difference between the epithelium in the intact areas and in the areas of the insertion of the blade. He also claimed that after a gingival flap had been peeled away from the surface of the enamel and the flap repositioned, no difference could be seen between the operated and non-operated areas. However, Orban and his collaborators in 1956 after the repetition of these procedures have opposed his observations. When a blade or strip of plastic was inserted into the alleged space between epithelium and tooth, or when a flap is pulled away

from the surface of the tooth and then replaced, the signs of such a procedure were always seen in the microscopic sections and with the most absolute clarity. They stated that the space created by the blade or strip, and the tear created by the flap operation was between the layers of the attached epithelium which proved that the attachment of the epithelium to the tooth was stronger than the cohesion of the cells to each other.

Henning and Zander in 1963, described a method for the study of the gingival crevice with regard to the relationship of the soft and hard tissues. The method makes use of the principle of insufflation which is defined as the act of blowing powder, vapor, or gas into an orifice.

Carbon penetration was limited to an area between the enamel epithelium and the oral epithelium. They found that in incisor regions on the labial surface, there was presence of carbon particles between oral and enamel epithelium with pooling in connective tissue. The path of entry of the carbon particles seemed narrow and subsequently widened, once the connective tissue is reached. On the lingual surface, carbon was seen between epithelium and connective tissue. In the molars,

mesiodistal sections showed carbon particles lodged between oral epithelium and the tooth to the level of epithelial attachment.

During the study on the development of gingival epithelium in monkeys, McHugh in 1961 and 1963, stated that the pattern of development observed in these animals indicates that during and after the eruption of a tooth, its enamel epithelium is gradually replaced by an epithelial cuff. The outer layers of enamel epithelium proliferate and join the downgrowing oral epithelium to form this cuff, while the inner layers degenerate.

McHugh also found that the epithelium over cementum has approximately half the mitotic activity of oral epithelium from the attached gingiva of the same specimens. According to him, some of this activity may be directed towards further downgrowth over cementum. But since the downgrowth over the cementum is slow, this activity is believed to be concerned with the turnover of existing cells.

Schultz-Haudt and others in 1963 have discussed on the nature of contact between the gingival epithelium and the tooth enamel surface. This is based mainly on their observations pertinent to epithelial stickiness. It was noted by Berwick and Coman (1962) that the

squamous epithelial cells of the human buccal mucosa exhibit sticky properties. The sticky properties of the cells depended upon the elaboration of a surface material, a mucpoly saccharide protein complex. Cellular stickiness became reduced by the actions of acid and alkaline phosphatases. No other specific evidence is available with regard to the chemical nature of an extraneous coating of the crevicular epithelial cells.

There is, however, some suggestive data. Thonard and Scherp (1961) by histochemical methods obtained indications to the extent that intercellular material of the gingival epithelium contained acid mucopoly saccharides. Similar studies by Schultz-Haudt and others in 1963 have indicated the presence of glycoproteins between the epithelial cells of the gingival crevice.

The existence of crevicular fluid passing from the connective tissue into the gingival crevice is established by Brill and Krasse in 1955. The composition of this fluid has also been reported to influence epithelial stickiness to the enamel surface.

Based on these observations, Schultz-Haudt and others assumed that the crevicular epithelial cells do

possess a sticky property which would contribute to maintain epithelial contact with the enamel.

B. Mitosis in Epithelium

Introduction of Earlier Investigators

The method of replacement or regeneration of epithelia has held the attention of investigators since the formulation of the cell doctrine. The greatest difference of opinion encountered is in regards to the localization of the particular portion of the stratum germinativum which continues actively in the reproduction of new cells.

Minot in 1908, in quantitatively analyzing the mitotic activity by studying the epidermis of rabbit embryos at varying age ranges found different mitotic indices in various tissues. He described the mitotic index as "the number of cells to be found at any given moment in the active process of division out of a total of 1,000 cells."

Thuringer in 1924 noticed that new cells are not only reproduced in the lower layers of stratum germinativum, but throughout the entire stratum spinosm. The proportion of mitotic figures in fact is greater in the middle and outer one third than in the deeper layers.

Thuringer established the earliest quantitative

counts of cell division. He noticed the difference in the mitotic indices in different regions. He counted one mitosis for 2,414 cells on the scalp; for 378,325 cells on the leg; and for 268,275 cells on the ear of a human subject.

In 1928, Thuringer, working on the prepuce of younger (17 days) and older (3 years) children, found regeneration was faster in some regions than in others. It was greater in younger than in older children. The greatest proliferation centers in prepuce were found in lower 1/3 of stratum spinosm. In this respect it differs from the scalp.

It is generally recognized that a periodic mitotic rhythm exists in the regeneration of plants and animals. Many investigators have shown the existence of a daily rhythm in both animals and man. As yet however, there is no agreement as to the timing of peak mitotic activity.

Henry found an average mitotic index of 5.1. At different hours of the day and night it reached ranges from 3.8 to 7.2 in the oral mucosa of a 3½ month old rabbit.

Halberg and associates in 1952, reported a low at night and a high in the morning mitoses in the retro-molar epithelium and periodontal ligament in 5 month

old black male rats. No day and night difference was detected in the interdental papilla.

Distribution of Mitoses

The site of regenerative activity in the epithelium is the stratum germinativum.

Thuringer in 1924 found 12% of mitoses in the basal layer of cat epidermis, and 88% in the spinous layer.

Henry found 60% in the basal layer and 40% in the spinous layer of rabbit buccal mucosa. Marwah in 1956 reported 23% of mitoses in the basal layer and 77% in the inner spinous layer of human attached gingiva. Thuringer and Cooper working on human epidermis noted increased mitoses in older individuals; 50% of mitoses were encountered in the basal layer and 50% in the spinous layer.

Cowdry and Thompson in 1944, working on the epidermis of the hind foot pads of the buffalo mouse, were able to show that the site of maximum mitotic frequency in the untreated animals was in the spinous cells of the proximal 1/3 of the suprabasal epithelium, while in all of the animals treated with .25 cc of 1:10000 aq. colchicine subcutaneously, maximum mitoses was located in those of the middle 1/3. The possibility is suggested that the level of maximum mitoses in the epidermis is not

fixed, but subjected to changes in different physiological and pathological conditions.

Variations in Mitotic Activity

Marwah in 1956 reported the age difference in the mitotic index of the human attached gingival epithelium. The older group had 50% more mitotic activity than the younger group.

Bullough working on age and mitotic activity in the male mouse stated that "during immature age the animals are still growing and their mitotic rate is generally greater. During mature age the mitotic rate is lowered. During middle age the mitotic rate increases, but in senility it is again reduced.

Bullough reported that mitotic activity increased with the increase in the glycogen content of ear epidermis of the mouse and decreased with the decrease in blood sugar level induced by insulin.

Muhlmann in 1954, noted higher mitotic rates in the periodontal ligament adjacent to the alveolar bone as compared with the rest of the periodontal ligament and an accumulation of mitosis was found in certain areas around the molar roots.

Hirt, et al, in 1955 working on the distribution of mitoses in the epithelium of the interdental papillae

of rat molars, found mitotic figures all over the papillary epithelium in the basal and prickle cell layers. They were most abundant in the downgrowing oral epithelium. There was considerably less mitoses in the zone where the downward proliferating oral epithelium joined with the epithelial attachment. Cell division in the epithelial attachment increased steadily in frequency from the occlusal to the apical. Mitotic figures were also located near enamel and root cementum. It is more near the cementum.

Schoenheider in 1960 observed that 8% of basal cells on the dorsum of the tongue synthesized DNA within one hour, while on the ventral surface it was 65%. The basal cells which synthesized DNA disappeared from the basal layer in slightly more than 50 hours.

Gargiulo in 1961, observed that after single exposure of 30% hydrogen peroxide for a thirty day period, an apparent prolongation of the mitotic period occurred in normal human oral epithelium. This seemed to be more specific in prophase. This apparently gave evidence that the mitotic process is retarded by hydrogen peroxide.

McHugh in 1961 stated that the epithelium in contact with cementum does not keratinize. An appreciable proportion i.e., 13% of the total number of mitotic figures in

this epithelium were in cells actually in contact with cementum.

Greulich in 1961 stated that mitotic activity in the region of the gingival margin and attached epithelial cuff was considerably greater than that of adjacent oral mucosal epithelium. In another study in 1962 he found mitoses in the cell layer immediately adjoining the enamel space. At later time intervals, labeled cells were more numerous in this layer and were distributed apically towards the gingival surface. These were presumed to be migrating daughter cells arising from mitotic division of initially labeled stem cells.

Beagrie and Skougaard in 1962, observed the gingival epithelial cells using tritiated thymidine and found that the greatest amount of labeling was in the oral epithelium, which was growing down between the epithelial attachment and connective tissue. One hour after injection, 5.5% of the epithelial attachment cells, 7.9% of the downgrowing oral epithelium cells, and 7.0% of the surface oral epithelium were labelled. These percentages increased for 24 hours and decreased thereafter. They considered that the cells of the epithelial attachment near the cemento-enamel junction were renewed in 24 hours, and the cells then took 3 to 5 days to migrate up to

the tip of the gingival crest.

Dimassimo in 1963, working on gingival epithelium in monkeys found labeled cells in the basal layer in all specimens from 0 to 9 hours. In the apical part of the epithelial cuff about 70% of the labeled cells were in the basal layer and the rest were anywhere, even against the surface of the cementum and enamel. By 6 days 14% of the labeled cells were still in basal layers. In other parts of the epithelial cuff, they had reached the surface of the epithelium. By 14 days, occassional labeled cells were found in the basal layer. Other cells were either gone, or were still against the tooth surface.

Autoradiography

According to Gross, et al, (1951) autoradiography is a method for detecting radioisotopes based on their ability to affect the silver bromide crystals of photographic emulsion. Such crystals act as micro-detectors of radiation and are, therefore, useful in visualizing locations of radio-elements in the microscopic structure of the body. In the autoradiograph, an increased density of silver granules occurs in the emulsion over the site of radioactivity.

One of the advantages of autoradiographic technique

to the biologist is its ability to localize a radioactive element or compound to a particular organ, histologic unit, or to a distinct cell group in an organism. Many labeled compounds and elements have been traced to specific zones of concentration in animal and human organs. The ultimate goal of autoradiography is to identify the site of the radioactive element, or compound, in terms of intracellular structure such as the nucleus.

A theoretical study was carried out by Gross and his co-workers in 1951 to determine the conditions giving the most satisfactory radiographic images. 1) The resolution of the image is best when the thickness of section and emulsion, and especially the space between the two, are reduced to a minimum. 2) The preparation should be underexposed to obtain densities as low as may be conveniently identified. 3) The emulsion used should contain a high concentration of small grains of fairly uniform size.

An autoradiograph is obtained by placing a tissue section in contact with photographic emulsion, allowing sufficient time for exposure, and then developing as in ordinary photography. The resultant autoradiogram consists of accumulations of black-silver granules

overlying those areas in the tissue section which contain the radioactive material.

As with the method involving the use of radioisotopes, it is assumed that the chemical behavior of a labeled substance is identical with that of its stable counterpart. This will also be true of the biological behavior provided; 1) the amount of radioactivity administered be small enough not to have significant radiochemical effect, and 2) the amount or weight of the material injected by sufficiently small not to produce a significant increase in the amount of substance in the circulation. The labeled substance will then truly act as a tracer of normal metabolism.

Taux in 1959 describes in detail the etymology of autoradiography. Many names have been applied since the first autoradiographic technique in 1896. Among them are autoradiography, radioautography, autophotography, and histoautoradiography.

Becquerel in 1896, after Roentgen's discovery of x-rays, wrapped a photographic plate in black paper, placed a crystal on top and exposed the combination to sunlight. After developing the plate, a faint outline of the crystal was observed, the first autoradiogram. Although he discovered the facts of radioactivity, he failed to understand them.

London in 1904 was first to use autoradiography for locating radioactivity in minerals. He exposed a frog to radium emanation, and after its death, placed it on a photographic plate, resulting in an autoradiogram.

Bouchard, P. Curie, et al, in 1902 were using the microscopic autoradiographic method to study the distribution of inhaled radium emanation in tissue sections of a guinea pig.

Kotzareff in 1922 reported autoradiograms showing the concentration of radium in the cortex of the kidney of a guinea pig. He also injected radium into a tumor of a human and produced an autoradiogram.

Chanie in 1927 found that solutions with tracer amounts of polonium, radium, and thorium contain radioactive aggregates called radiocolloids. Autoradiography can show the radiocolloids in the tissue and can be better interpreted.

Lomholt in 1930, apposed 20 micro tissue sections to a plate and later removed them for staining. In this manner the same section making the autoradiogram could be studied histologically.

Bulliard and his associates in 1938 were the first to produce a biological autoradiogram with induced radioactivity, when they demonstrated phosphorous-32 in

the adrenal gland.

Belanger and Leblond in 1946 developed a technique for locating radioactive elements in the tissue by placing liquid emulsion in direct contact with the sections. This technique obviated 2 main pitfalls of the previous methods since an intimate contact between tissue and emulsion was obtained and autoradiography was no longer a problem.

Boyd and Williams in 1948 used stripping film techniques by which back scattering, emulsion staining, chemical fogging, and photographic developer damage to the tissue can be eliminated.

It is known that mitoses is preceded by a synthesis of deoxyribonucleic acid (DNA). If a radioactive DNA precursor is administered at that time, the nucleus becomes radioactive and may be recognized by autoradiography even before mitoses takes place. In the past, Leblond, et al, 1948 used phosphate p^{32}, and Pelc in 1957 used adenine C^{14} to label newly formed DNA and investigate cell population. The use of these substances raised serious problems. The doses required for adequate autoradiography were high enough to cause radiation damage. The detection of labeled DNA required the extraction of other labeled compounds from the sections,

which was a difficult and often incomplete procedure. Finally, the autoradiographic resolution is poor with p^{32} and only fair with the C^{14}.

Thymidine was tritiated at Brookhaven in 1955 by Huges and independently in Belgium. Although thymidine is apparently not a normal precursor of DNA, it can enter the synthetic chain and label DNA at the time of DNA doubling prior to mitoses. Thus, if the label is sufficiently intense and permanent, one can by autoradiography follow cells from the time of DNA synthesis to ultimate death.

Thymidine labeled with tritium H^3 makes it possible to overcome the difficulties encountered with the other DNA precursors. The danger of radiation damage seems to be reduced. Sections need not be subjected to chemical extraction, since DNA is the only labeled substance present in significant amount in the sections following tritiated thymidine administration and DNAse treatment eliminates all autoradiographic reactions (Amano, 1958). Due to the low beta-ray energy of tritium, the photographic grains produced by tritium containing structures are found within 2 or 3 microns, most of them within 1 micron from the source as measured within NTB^3 emulsion.

According to Firket and Vesly, 1957, thymidine is

a specific precursor of DNA. The very low energy of tritium electrons permits precise localization on autoradiographs of the atom that disintegrates.

Lajtha, Phil, and Oliver in 1959 confirmed thymidine as a specific component of DNA, important because it labels only DNA. The range of beta particles has a maximum of 8 microns and an average of 1.5 microns.

It has been concluded that tritiated thymidine is an adequate tool for the autoradiographic detection of newly formed sites of cell formation.

MATERIALS AND METHODS

Materials:

Thirty-three C57 white mice, 60 days old, weighing an average of 32 grams, being fed a diet of Wayne Lab Blox for mice or rats, were injected intraperitoneally with 1 microcurie of tritiated thymidine, specific activity 1.9 curie per milli molecule,* per gram of body weight. They were sacrificed at intervals of ½ hour, 1 hour, 2 hours, and at an interval of every 2 hours up to 50 hours, 2½, 3, 3½, 4, 4½, and 5 days following the injection.

The mandibles with the gingiva were dissected out for autoradiographic preparation as follows---

Method:

The mandibles with the gingival tissue were fixed in 10% formalin for 24 hours. They were then subjected to decalcification in the solution of 90% Formic Acid with Sodium Citrate solution prepared as follows:

Solution A - 50 grams of Sodium Citrate and 250 cc of distilled water.

Solution B - 125 cc of 90% Formic Acid and 125 cc of distilled water.

*Schwarz Bioresearch; Mountain View Avenue; Orangeburg, N.Y.

Equal parts of solutions A and B. The solution was changed every day for 3 to 4 days. Roentgenographs were taken to detect the decalcification of the tissue.

Tissues were then dehydrated in 75% alcohol, followed by 95% and finally by absolute alcohol.

The tissues were then embedded in paraffin (tissue mat, Fisher Company). The embedding was done in Vacuo, at a melting point of 56.5° C, under a pressure of 15 pounds per square inch for a period of 15 minutes.

Serial sections were then cut at a thickness of 3 microns. Each sixth section was selected for autoradiographs.

The mounted sections were then deparaffinized in the following manner: Immersion for five minutes each in two changes of xylol, then for five minutes in absolute alcohol, followed by three minutes in 95% alcohol and subsequently in 75% alcohol for three minutes. Finally, the sections were washed in distilled water for five minutes.

The tissues were then subjected to autoradiography.

Autoradiography (Strip Film Technique):

1. Under dark room conditions a Wratten #1 red Safelite 10 watt bulb was used. A humidity of 70% and below is recommended.

2. Kodak AR 10 fine grain emulsion was placed on a cutting jig, and cut into squares with a scalpel.

3. Using a scalpel, a corner of the square was picked up, stripped from the glass plate, and floated, emulsion side down, in a glass dish containing distilled water four inches deep.

4. The histologic slide (section side up) is inserted into water. The slide was brought up under the floating emulsion and engaged.

5. The slides were then air dried for 10 minutes.

6. Then slides were placed in a black, light-proof exposure box, section side up. Lithium chloride was placed in the box for maintaining a low humidity, and black masking tape was used to seal the box.

7. The box was exposed for 30 days at low humidity and temperature. During the exposure time, the box was maintained in a position which kept the sections upright.

Developing:

1. The slides were placed in a staining rack and developed for 5 minutes at 60° F (18° C in Kodak D196 developer).

2. The slides were rinsed in distilled water for 30 seconds.

3. Then placed in acid fixer for 10 minutes.

4. Then washed in running tap water for 30 minutes.

5. Slides were covered (to prevent dust from settling on them) and allowed to dry in a stream of air.

6. When the slides were dried, they were dipped in water fro 30 seconds and the excess emulsion was trimmed from the slides.

Slides were then taken and stained with Hematoxylin, Eosin method. They were then dehydrated with 95% alcohol followed by absolute alcohol (at least two changes). Removal of alcohol was done by Xylene (2 changes).

The coverslips were finally mounted with Canada balsam.

Sections were also stained by the PAS staining technique to study the keratinization.

For each specimen, labeled cells were counted in 10 sections and an average reading was taken as final count. All the epithelial cells were counted in inter-dental papillae of each of the slides and an average was taken to have a per cent of labeled cells in each papilla.

FINDINGS

Morphologically, the interdental papilla is roughly triangular in shape. It is that part of the gingiva which fills the space between two adjoining teeth and being limited at its base by an imaginary line connecting the margin of the gingiva from the center of one tooth to the center of the next. The interdental papilla is composed of the free gingiva and the attached gingiva. The morphology of the papillae depends largely upon the relation of the neighboring teeth to each other.

Histologically, a mesio-distal section of the interdental papilla consists of stratified squamous epithelium with a dense fibrous connective tissue core. The curvature of the epithelial surface follows the curvature of the enamel surface. The surface layer of the epithelium is non-keratinized but consists of flattened cells with pyknotic nuclei.

The prickle cell layer varied in its thickness. For the purpose of description the epithelium is divided into three zones based on the thickness of prickle cell layers. That portion of the epithelium which is considered as the terminal end of the epithelial attachment on the tooth was almost always at the cemento-enamel junction.

It is about 2 to 3 cell layers thick, and is designated as zone I.

The intermediate zone, or zone II, is that portion of epithelium which extends from the coronal end of zone I to approximately the bottom of the gingival sulcus. It is 4 to 5 layers thick.

Zone III starts at the coronal end of zone II and terminates at the tip of the papilla. This is 8 to 10 layers thick. (See figures #1 and 2)

The basal layer shows short and broad ridge-like structures, the epithelial ridges.

The lamina propria of the interdental papilla consists of bundles of collegenous fibres with fibroblasts scattered in between. There was little inflammation in the connective tissue of the lamina propria with a few polymorphonuclear leucoeytes.

Labeled cells were observed in all the interdental papillae taken from the mice at different intervals, ranging from $\frac{1}{2}$ hour to 5 days (120 hours) after the administration of tritiated thymidine. (See table #1)

Basal Cell Layer:

An average of eight labeled cells per section were present in the basal cell layer of all the three zones

at ½ hour, and they gradually increased to a maximum of 15 cells per section at 10 hours. The maximum number of labeled cells in the basal cell layer continued to the 18 hour interval. The number of labeled cells in the basal cell layer then decreased gradually and at 48 hours, most of the labeled cells had moved out of the basal cell layer into the prickle cell layer. In the 5 day specimen no labeled cell was seen in the basal cell layer of the 3 zones. (See table #1)

Prickle Cell Layer:

In the early specimens (2 to 6 hours) the number of labeled cells in the prickle cell layer showed a gradual increase from two cells per section. They were seen to increase to a maximum of 10 cells per section at 10 hours. After this period there was a gradual decrease in the number of the labeled cells in the basal cell layer and for the corresponding periods the number of labeled cells in the prickle cell layer were increased. (See table #1)

Surface Cell Layer:

At ½ hour a labeled cell was observed in the surface layer in zone I. However, for the first 6 hours labeled cells were only seen in zone I. From 6 hours to 18 hours

labeled cells were also seen in zone II. At 18 hours they were seen in all three zones. After 42 hours most of the cells had moved out from zone I, only occassionally was a cell seen in zone I.

The labeled cells in the surface layer showed a gradual increase in number from 0 at ½ hour to a maximum of 6 at 26 hours. The surface cell number started decreasing at 32 hours and continued to a 5 day period at which time no labeled cell was seen in the surface layer.

The percentage distribution of labeled cells rises to a maximum at 10 hours. A plateau is formed from 10 to 22 hours, and then there is a decline. This may be seen in table #1.

There was a decrease in the number of grains in the labeled cells at the 10 hour interval.

DISCUSSION

The observation of labeled cells in the basal cell layer and the prickle cell layer of the epithelium shows that mitotic activity occurs in these layers. The mitotic potential of the basal cell layer is greater than the prickle cell layer, as the basal cell layer contains four times more labeled cells at ½ hour.

The number of labeled cells in the basal cell layer and the prickle cell layer underwent mitotic division and reached their maximum at 10 hours. There was an increase of about 100% in the number of labeled cells in the basal cell layer between 30 minutes and 10 hours. This finding supports those of Toto and Ojha (1960). The increase in the number of labeled cells in the prickle cell layer was greater than that of the basal cell layers. This level of labeled cells remained fairly constant in the basal and prickle cell layers until the 22 hour period. Increase in the number of labeled cells in the prickle cell layer shows not only the mitotic activity in both layers, but also the migration of the divided cells from the basal cell layer into the prickle cell layer.

The decrease in the number of grains in the labeled cells at the 10 hour interval indicates that the radio-

active material is divided into 2 daughter cells, thus diminishing the amount available for each new daughter cell.

There was a gradual decrease in the percentage of labeled cells after the 22 hour period. If the labeled cells proceed into division, there should be an increase in the number of labeled cells at later periods. A probable explanation for the failure to observe this is that the number of cells undergoing mitosis is not large and the rate of migration of these cells is fast. The decrease in the number of cells is also likely to be due to the dilution of the isotopes after mitosis or some of the cells might have died because of the lethal action of the radioactive element.

The gradual decrease in the number of labeled cells in basal cell layer may in part be attributed to the migration of these cells into prickle cell layer.

In zone I, where the epithelium is only 2-3 cell layers thick, one labeled cell was observed in the layer adjacent to the enamel of the tooth at 30 minutes intervals. It is possible that either the cell has undergone synthesis and is acting as a prickle cell or it might have migrated from the basal cell layer. It is also possible that the basal cell layer in zone I has

curved inwards, so that it lies against the tooth surface and the labeled cell may be a part of the basal cell layer.

The cells in zone II next to the enamel space may be regarded as cells of the prickle cell layer.

The cells in the basal cell layer adjacent to the tooth surface in zone I can migrate in three directions. They can migrate either towards the tooth or in the direction of the adjacent basal cell layer or upwards against the tooth surface. The cells cannot move towards the tooth as it acts as a barrier. This epithelial layer in zone I is only 2 to 3 cell layers thick and it is not likely that they move towards the basal layer, so the possible path of migration is that against the tooth surface until they reach the gingival sulcus where they are exfoliated.

Cells of the basal layer in zone II can migrate in either of two ways; towards the tooth surface or towards the prickle cell layer of zone III.

Cells from the basal cell layer of zone III can migrate wither towards the tip of the interdental papilla from where they are exfoliated, or towards the tooth surface to be exfoliated in gingival sulcus. It took longer for these cells in zone III than in zones I or II

to move towards the surface layer, as there is a greater number of cells in this zone.

One-half hour to eighteen hours following the administration of tritiated thymidine more labeled cells were found in the basal cell layer than in the prickle cell layer. This is due to a greater number of cells in DNA synthesis in the basal cell layer. In specimens from 22 hours to 44 hours more cells were found in the prickle cell layer than in the basal cell layer. In the superficial layers of the epithelium, maximum number of cells were observed in the 22 to 32 hour specimens. This gradual increase in the number of cells in the prickle cell layer and the superficial layer of the epithelium demonstrates a movement of the labeled cells from the basal cell layer towards the prickle cell layer and the superficial layer.

Normally the entire oral mucosa of the mouse is keratinized except in the region of the gingival sulcus. The possible explanation could be that these cells are the cells of the prickle cell layer and they migrate fast to be exfoliated either towards the gingival sulcus or towards the tip of the interdental papilla.

Dimassimo in 1963 showed that there was mitotic activity in cells of the epithelial attachment including

those cells against the tooth surface, demonstrating their viability. This epithelium, therefore, constitutes a germinative layer.

McHugh in 1963 demonstrated the presence of dividing cells in the epithelial attachment against cementum. He suggests that the cells do not mature and move from the basal cell layer towards the tooth, but they themselves undergo mitosis. He suggested that it seemed likely that these cells move towards the gingival crevice to be finally desquamated into the mouth. Our findings are in general agreement with those of Beagrie and Skougaard (1962), Greulich (1962), Dimassimo (1963), and McHugh (1963) for proliferation and migration of cells. The fate of epithelial cells upon reaching the tooth surface is still uncertain.

These observations are recorded in young mice; it is possible that they could be different in older animals.

The layers of the gingival epithelium are taking part in the physiologic renewal which is seen by their mitotic potential and the proliferated cells migrate towards the superficial layers from the basal cell layer, which is seen by the reduction of labeled cells in the basal layer of later specimens.

SUMMARY AND CONCLUSIONS

The proliferation and migration of the epithelial cells in the interdental papillae of the mouse has been studied using tritiated thymidine to label the cells in DNA synthesis.

Material was obtained from sixty day old C57 white mice weighing an average of 32 grams. They were injected with one microcurie of tritiated thymidine per gram of body weight. They were sacrificed at an interval of ½ hour, 1 hour, 2 hours, and at intervals of every 2 hours up to 50 hours, 2½, 3, 3½, 4, 4½, and 5 days.

The mandibles with gingiva were dissected out, fixed in 10% formalin, decalcified, dehydrated, and embedded in paraffin.

Serial sections were taken at a thickness of 3 microns. Each sixth section was then selected to make an autoradiograph using NTB_3 emulsion. The sections were then stained by hematoxylin and eosin method.

The interdental papilla was divided into 3 zones based on the thickness of the prickle cell layer. (Figure #1.) The movement of the labeled cells has been observed.

Our observations led us to the following conclusions:

1. All the zones of the epithelium are taking part in

the physiologic renewal of the epithelium.

2. The cells migrate from the basal cell layer to the prickle cell layer and from the prickle cell layer to the surface cell layer.

3. The cells from zone I, zone II, and some of the cells from zone III migrate towards the gingival sulcus to be desquamated. The rest of the cells from zone III migrate towards the tip of the interdental papilla where they are desquamated.

TABLE NUMBER 1

The distribution of labeled cells in different cell layers of epithelium at different time intervals.

TIME	BASAL CELL LAYER	PRICKLE CELL LAYER	SURFACE LAYER	TOTAL NUMBER OF CELLS
½ hour	8	2	1	11
1 hour	6	4		10
2 hours	9	4	2	15
4 hours	10	9		19
6 hours	8	6	2	16
8 hours	5	9	3	17
10 hours	15	10	2	27
12 hours	6		1	7
14 hours	14	8	3	25
18 hours	14	9	3	26
20 hours	8	6	3	17
22 hours	9	13	4	26
24 hours	6	9	1	16
26 hours	8	4	6	18
32 hours	7	4	6	17
34 hours	5	9	2	16
36 hours	3	4	2	9
42 hours	2	5	3	10
44 hours	2	5	3	10

TABLE NUMBER 1 (CONTINUED)

TIME	BASAL CELL LAYER	PRICKLE CELL LAYER	SURFACE LAYER	TOTAL NUMBER OF CELLS
46 hours	3	4	2	9
48 hours	1	6	2	9
50 hours	2	6	1	9
2½ days	4	7	3	14
3 days	2	4	1	7
3½ days	2	3	1	6
4 days	3	7	4	14
4½ days	2	3		5
5 days		3		3

TABLE NUMBER 2

Percentage of labeled cells in the epithelium at different time intervals.

TIME	PERCENTAGE OF LABELED CELLS
½ hour	6%
1 hour	6%
2 hours	9%
4 hours	10.5%
6 hours	9%
8 hours	10%
10 hours	15%
12 hours	4%
14 hours	14.5%
18 hours	15%
20 hours	10%
22 hours	15%
24 hours	9%
26 hours	10.4%
32 hours	10%
34 hours	9%
36 hours	5%
42 hours	6%
44 hours	6%

TABLE NUMBER 2 (CONTINUED)

TIME	PERCENTAGE OF LABELED CELLS
46 hours	5%
48 hours	5%
50 hours	5%
2½ days	8%
3 days	4%
3½ days	3.5%
4 days	8%
4½ days	3%
5 days	1.7%

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Figure 1: Diagram of the interdental papilla showing division of epithelium into three zones.

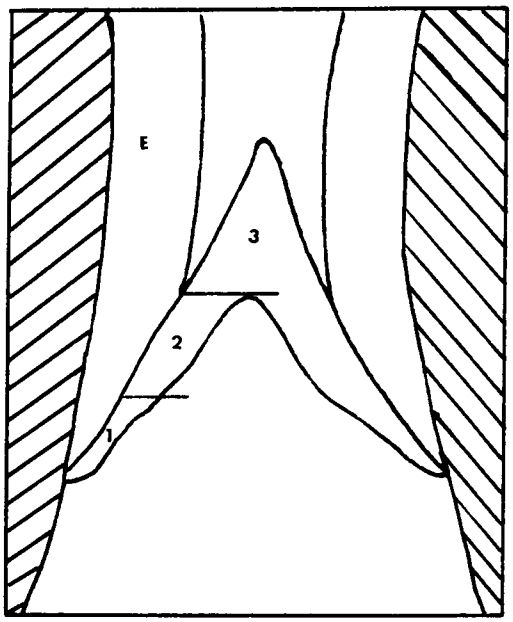
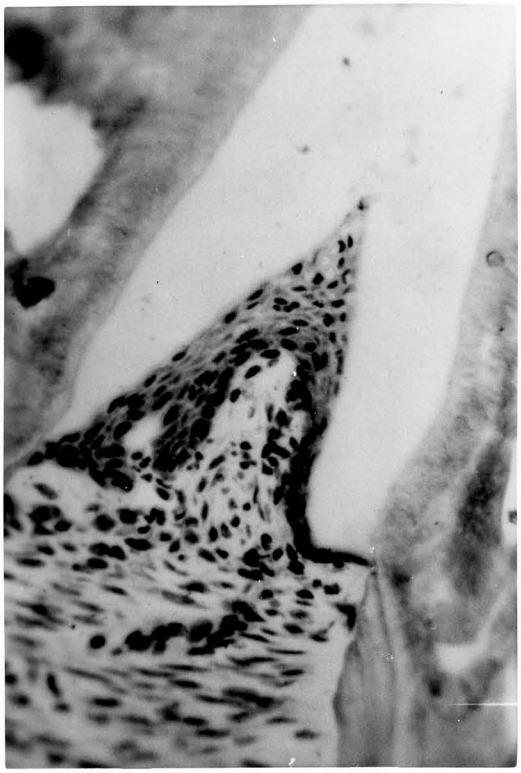

E
3
2
1

Figure 2: Photomicrograph of the interdental papilla of the mouse showing division of the epithelium into three zones. X 400.



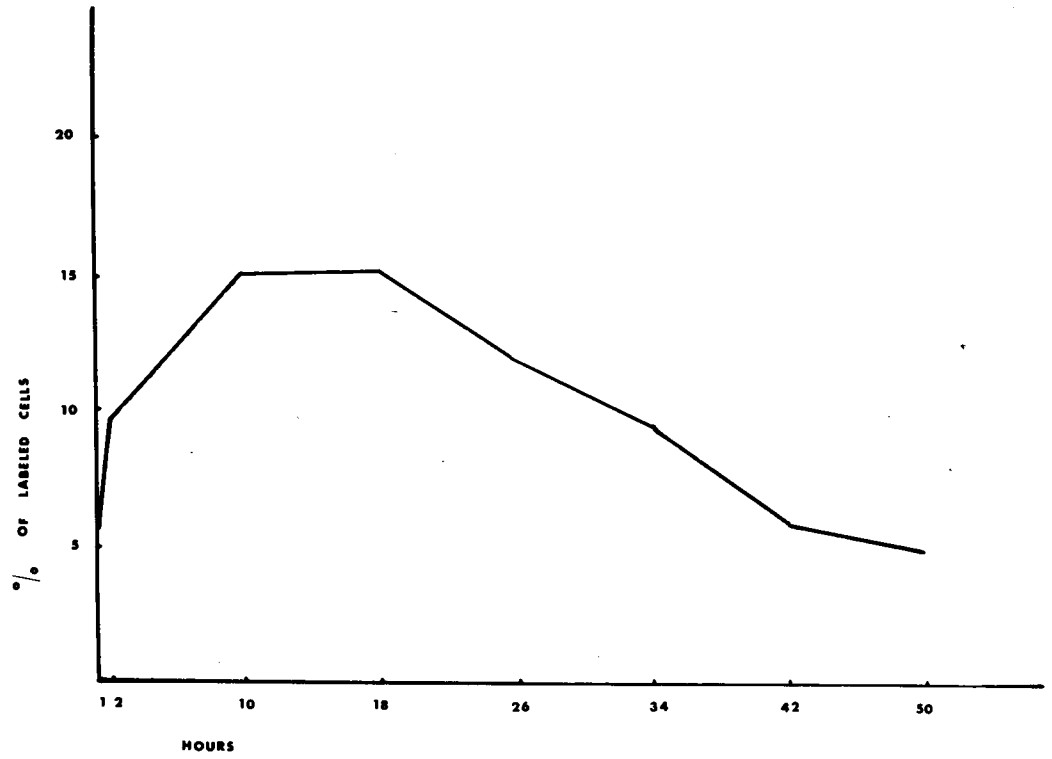


Fig. 3 % Of Labeled Cells At Different Time Intervals.

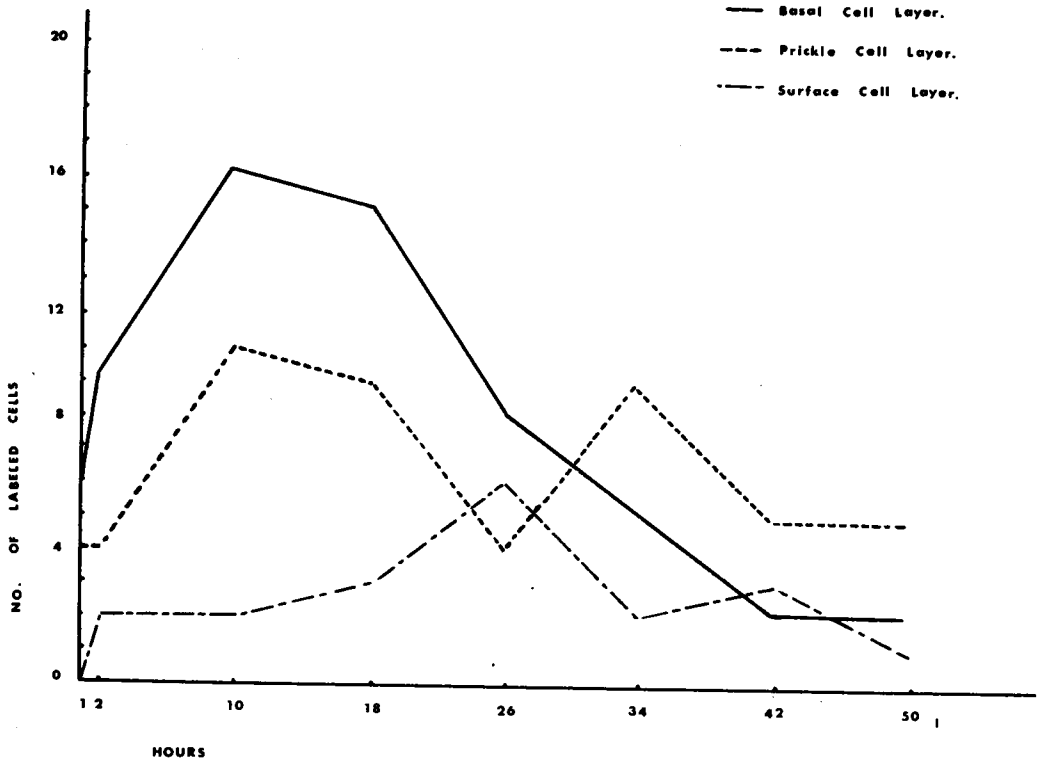


FIG. 4 The Number Of Labeled Cells In Different Cell Layers At Different Time Intervals.

Figure 5: Photomicrograph of the interdental papilla at 30 minutes interval showing a labeled cell in the surface layer in Zone I. X 400.

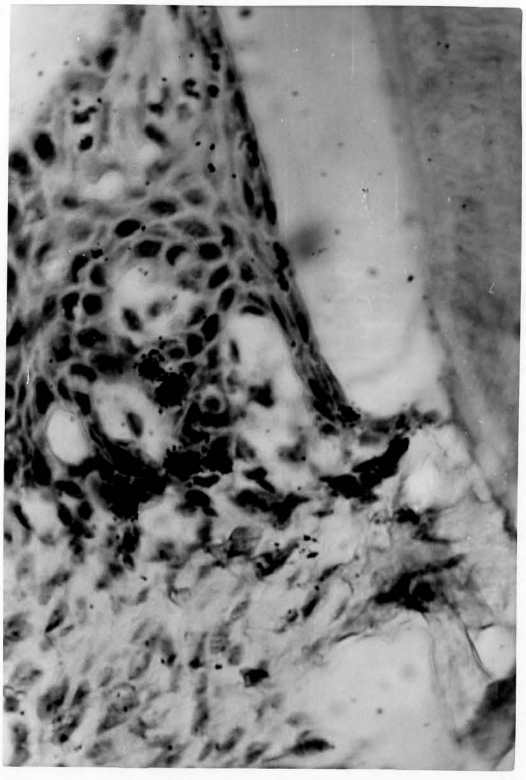


Figure 6: Photomicrograph of the interdental papilla at 2 hour interval showing distribution of labeled cells in Zone I and Zone 2. X 400.

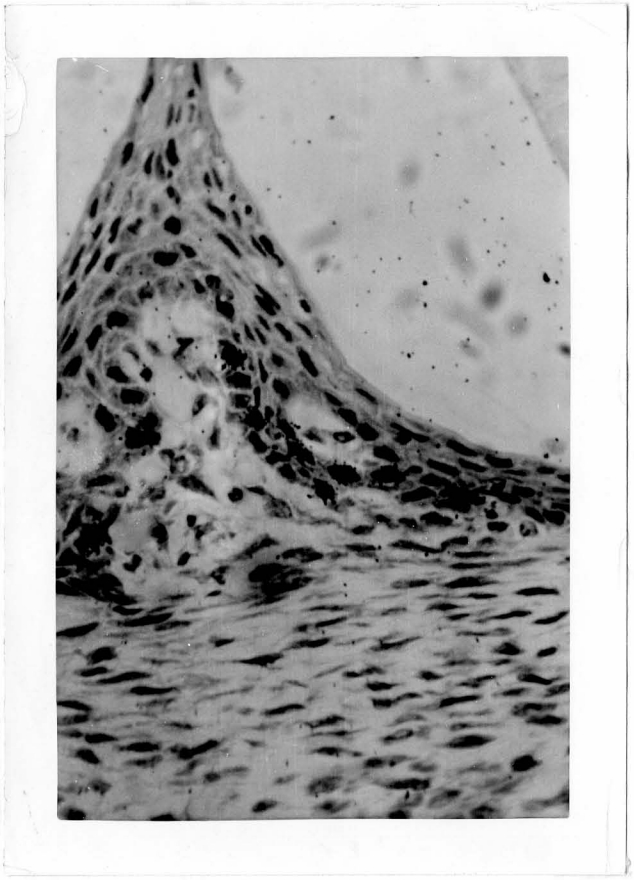


Figure 7: Photomicrograph of the interdental papilla at 10 hour interval showing maximum number of labeled cells in Zone I, 2 and 3. X 400.

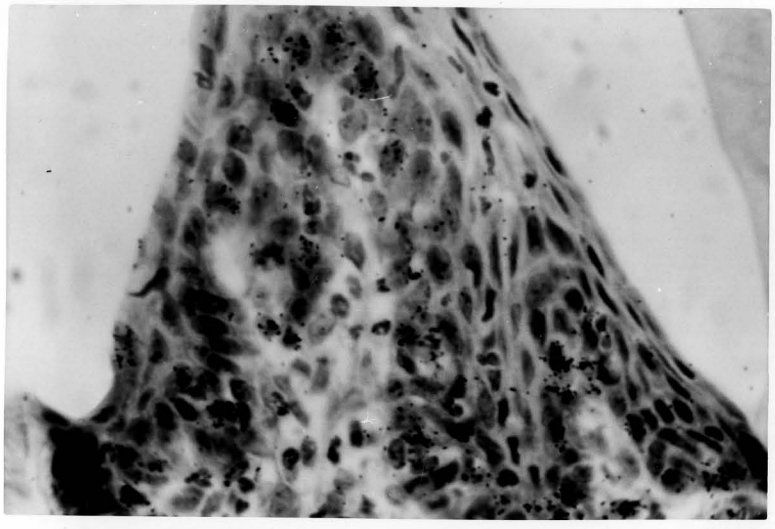


Figure 8: Photomicrograph of the interdental papilla at 26 hour interval showing maximum number of labeled cells in surface layer of the epithelium. X 400.

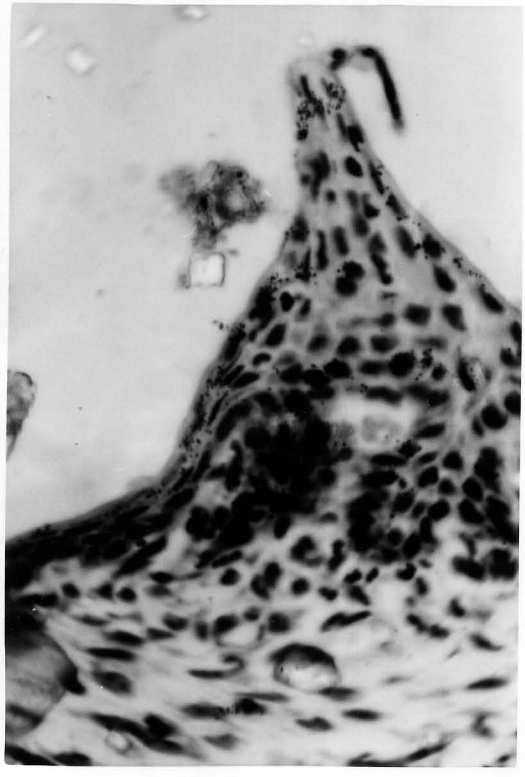


Figure 9: Photomicrograph of the interdental papilla at 50 hours showing general decrease in the number of labeled cells, with the maximum number of labelled cells in Zone 3. X 400.

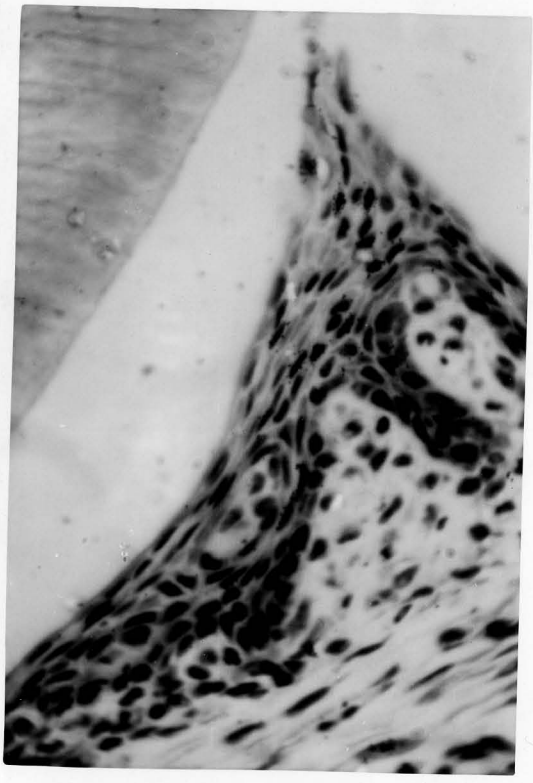
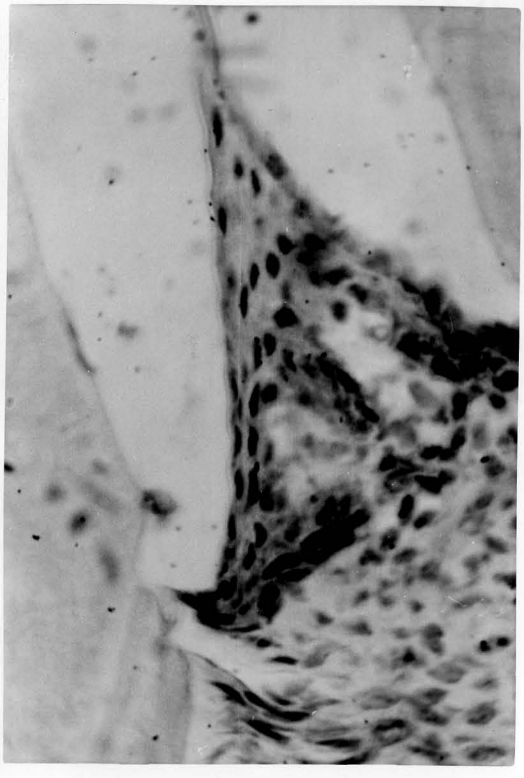


Figure 10: Photomicrograph of the interdental papilla at 5 day interval showing the presence of only 7 cells in the epithelium. X 400.



APPROVAL SHEET

The thesis submitted by Anjana A. Joglekar has been read and approved by four members of the Department of Oral Biology.

The final copies have been examined by the Director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 14 1964
DATE

SIGNATURE OF ADVISOR