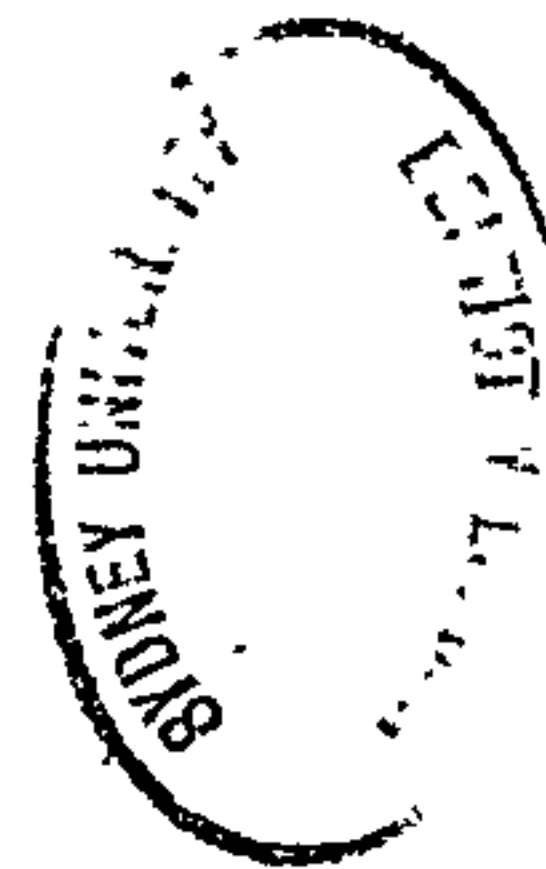




WHITE LESIONS

OF THE

ORAL CAVITY

This critical review of the
literature is submitted in
support of candidature for
the degree of Master of
Dental Surgery.

A. J. Swan.

In studying the subject "White Lesions of the Oral Cavity", the fundamental enquiry must be made as to how the pink oral mucosa becomes white. The answer, as far as the vast majority of white lesions is concerned, is that the stratum corneum, or keratin layer, is thickened and becomes opaque, preventing the vascular bed of the corium from imparting the pink colour to the mucous membrane. Accordingly, full consideration will be given in this review to the literature relating to the keratinization process, as an understanding of the normal is a prerequisite to understanding of the abnormal.

A striking feature in the study of white lesions is the fact that the aetiology of nearly all these conditions is largely unknown. Therefore, the literature is speculative, voluminous, and frustrating. To this reviewer the reports of investigations of normal mucous membrane by microscopy, electron microscopy and histochemical methods are as pertinent to the study of white lesions as the laborious documentation of varying clinical features.

The structure of this review has been founded on the above premise.

Contents.

The Cell.	1
The Normal Anatomy of the Oral Mucous Membrane.	11
Histology and Electron Microscopy of Oral Mucous Membrane.	18
Histochemistry of the Oral Mucosa.	50
Keratinization.	64
Pathologic Processes Involving the Oral Mucosa.	80
Leukoplakia.	100
Leukoedema.	194
Carcinoma.	196
Exfoliative Cytology.	227
Papilloma, Papillomatosis, Verruca Vulgaris.	229
Lichen Planus.	242
Psoriasis.	272
Lupus Erythematosus.	278
Moniliasis.	296
Benign Migratory Glossitis.	315
Hairy Tongue.	320
Fordyce's Granules.	326
Familial White Folded Dysplasia of Mucous Membrane.	328
Keratosis Follicularis.	335
Hereditary Benign Intra-Epithelial Dyskeratosis.	338
Pachyonychia Congenita.	340
Chemical Burns, Thermal Injuries, Cheek, Lip and Tongue Chewing.	342

The Cell.

Introduction.

In reviewing and discussing the subject "White Lesions of the Oral Cavity", one must begin with the fact that the whiteness results from changes in the normally pink covering material of the mouth, namely the oral mucous membrane. Therefore, to be able to understand fully the various changes which produce this whiteness, the gross and minute structure of the mucous membrane must be elucidated.

Many significant developments in the study of white lesions in recent years have stemmed from the use of the electron microscope, and also from histochemical techniques.

The basic component of mucous membrane (and all living matter) is the cell, so that consideration of the exciting recent discoveries by the electron microscope about the cell and its ultrastructure is fundamental to any study of the oral mucous membrane.

The Structure of the Cell.

Where not otherwise acknowledged, the material in this section has been condensed from the work of Zelickson and Hartmann, 1963.

The cell is composed anatomically of a cell membrane and the enclosed protoplasmic substances - cytoplasm, nucleus, and inclusion bodies such as mitochondria, fat globules, etc. The electron microscope, which has increased resolving power from the previous limit of 2000 $\text{\AA}$ with the light microscope, to the present limit of 8 - 10 $\text{\AA}$, permits identification of further minute anatomic units. Some of these are the endoplasmic reticulum, the Golgi Complex, the centrosome and centriole, lysosomes and ribosomes.

Every cell is part of a complex physiologic constellation comprised of many cells, their cell membranes, intracellular substance, and environmental extracellular substance. The stability of this constellation expresses the biologic tendency of cells, organs and organisms to maintain a constancy of environment consistent with their vitality and well-being. This concept of stability or homeostasis is basic to the comprehension of cell and tissue behaviour (Silverman, 1961). To maintain this equilibrium the cell membrane must be in continuous interaction with both the intracellular and extracellular environment.

The Cell Membrane. The cell membrane at the cell surface regulates the interchange of materials between the cell and its environment. Silverman states that there has been considerable controversy as to what extent the cell membrane is an entity having

a characteristic structure, composition and function, and to what extent it merely represents a nonspecific interfacial structure. Zelickson and Hartmann, however, state unequivocally that it is a definite structure which is complete and is linked to the perinuclear space via the system of cytoplasmic membranes, and that it varies in thickness, being usually 50 - 300 Å thick, while Silverman gives its limits as 100 - 200 Å in thickness. Mercer (1961) states that the thickness of the cell membrane is 70 Å (± 10 Å).

Robertson (quoted by Zelickson and Hartmann) has demonstrated the cell membrane to be a 3-layered structure consisting of 2 dense lines approximately 20 Å thick bordering a light central zone. Mercer cites experimental evidence to show that the membrane may be a continuous lipid-like layer covered on each face by a layer of protein. The membrane has numerous infoldings and outpouchings along its surface, and at times is extremely convoluted, or straight. It often is folded into small folds, thus forming the so-called microvilli. The cell most likely accepts foreign materials by pinocytosis or infoldings of its membrane within which is located the foreign matter. The pouch as well as the particles within it are pinched free, and thus assume an intracellular location. Silverman suggests that the cell membrane surface is a sieve-like structure which might permit transit of molecular substances as a simple diffusion process. He also suggests that substances may be transported through the membrane by chemical action.

The membrane often has modifications along its surface called desmosomes (or regions of attachments to other cells, prickles, nodes of Bizzozero, etc.) which allow the cells to adhere to one another. Other cells such as the melanocyte or certain tumour cells lack such attachments and presumably are free to move about more easily (Zelickson and Hartmann).

Nucleus. In thin sections the cell nucleus is usually round, oval or irregular in shape, and is composed of numerous fine, electron-dense granules. Because of the dense concentration of the granules, the nucleus usually appears darker than the rest of the cell. These are the chromatin granules, and the pattern they assume varies with each type of cell.

Nuclear Membrane. The nuclear membrane is a bilamellar structure 200 - 400 Å thick. The inner layer appears thicker than the outer one, probably because of the adherence of clumped chromatin material to it. The outer membrane appears rough, because of the closeness of many ribonucleoprotein (R.N.P) granules to its cytoplasmic surface. The distance between the two membranes is usually constant, though at times the outer membrane bulges some distance into the cytoplasm. The outer membrane is also at times continuous with either the system of cytoplasmic vesicles (endoplasmic reticulum), or with the cell membrane. It is now generally believed that "holes" approximately 500 Å in diameter occur in the nuclear membrane, and that these provide a route of communication between the cytoplasm and nucleoplasm. It is suggested that usually these pores are closed, but may open intermittently. They may vary in number, and it is probable that they allow the passage of rather large particles

between the nucleus and cytoplasm.

Perinuclear Space. The region between the two nuclear membranes is approximately 100 - 600 Å wide, relatively clear and less dense than either nucleus or cytoplasm. Both inner (50 Å) and outer membranes (50 Å) have been referred to as the nuclear envelope. This envelope, when seen in thin sections, has been shown to be a part of the endoplasmic reticulum which encloses the nucleus. The nuclear membrane is thus a part of a complex system of membranes found within all cells. This system of membranes within the cytoplasm has been shown to be continuous with both the cell membrane and the nuclear membrane. This then means that there is a continuous enclosed system of membranes which form a pathway from the perinuclear space to the intercellular space.

The Nucleolus. The nucleolus varies in size, shape and structure, depending on the source and function of the cell. It is usually in the centre of the nucleus and it too is composed of many osmiophilic granules (150 Å). These are darker and slightly larger than those found within the nucleus. The nucleolus does not have a limiting membrane, and may be round, oval or vermiform.

Chromosomes and Mitosis. Most of the chromosomes studied so far are essentially bundles of microfibrils, and they have a basic structure which is similar in all cells. The primary microfibril is of uniform thickness (250 Å) and is coiled. In all organisms it has been found that prophase chromosomes consist of coiled microfibrils approximately 500 Å thick, while chromosomes in

interphase show fibrils about half as thick. During cell division, each fibril doubles in thickness and soon after prophase splits lengthwise into two units each $250 \text{ \AA}$.

The Cytoplasm.

The Endoplasmic Reticulum and Ergastoplasm.

The endoplasmic reticulum consists of a specialised system of cytoplasmic vesicles, more or less flattened, and found in almost all cells. These vesicles are bounded by a membrane approximately $50 \text{ \AA}$ thick. Outside the membrane one finds the cytoplasm, and on the inside or within the vesicles is a fluid or amorphous material. In certain cells the profiles of this system of vesicles almost fill the cytoplasm, while in other cells it may be almost absent. The arrangement of the elements of the endoplasmic reticulum may vary from one moment to the next in the same cell. Some vesicles have granules located on the cytoplasmic surface of their limiting membrane. These granules are electron-dense and vary from $50 - 200 \text{ \AA}$ in diameter. They are often called Palade granules or ribosomes, and may be located either on the membrane of the endoplasmic reticulum (rough surfaced vesicles), or lie free in the cytoplasm. These granules are composed largely of ribonucleoproteins (R.N.P.). The endoplasmic reticulum along with the Palade granules are often referred to as ergastoplasm.

The endoplasmic reticulum may be free of electron-dense granules, and is then referred to as the smooth-surfaced type. The rough-surfaced form is found in abundance in cells which are growing

or actively engaged in protein synthesis, e.g., plasma cells and the malignant melanoma cell.

The smooth or agranular form has a tubular rather than a cisternal shape. This form is common in cells that have a form to maintain, and is also common in cells which are actively synthesizing steroids. It is possible to show a connection sometimes between the smooth and rough surfaced endoplasmic reticulum in the same cell.

In summary, the function of the endoplasmic reticulum involves the synthesis of protein, and probably offers a means whereby proteins escape from the cell as well as move intracellularly. There is also strong evidence that the endoplasmic reticulum plays a part in glycogen storage and release as well as in the manufacture of steroids for secretion. It has long been known that cells connected with protein synthesis contain large amounts of ribonucleic acid, and it has now been shown with the electron microscope that this mechanism functions at the site of these R.N.P. granules. It has also been suggested that the combination of reticulum and R.N.P. granules functions with the granules acting as building blocks which are able to synthesize different proteins, depending on the type of cell. The secretion then accumulates in the vacuoles of the endoplasmic reticulum, and from here it probably passes to the Golgi Complex to be compacted and modified into its final form. The secretory product is then able to pass from this region to the exterior of the cell.

In addition, the endoplasmic reticulum apparently regulates the osmotic pressure of the cell, for if a cell is placed in a hypotonic medium, the endoplasmic reticulum, as well as the perinuclear

space, swells to a larger volume.

Mitochondria. All have the same form in all cells. These are limited by a bilamellar membrane (each 30 - 60 $\bar{A}$ thick, enclosing a 40 - 70 $\bar{A}$ space). The outer membrane is smooth-surfaced and encloses the mitochondrion. Pores and holes have not been seen in this outer membrane. The inner membrane, also smooth and osmiophilic, folds in upon itself to form numerous shelves or crests (each 150 - 250 $\bar{A}$ wide). The shelves are of uniform thickness but their number varies greatly, and because they extend only part way across the mitochondrion, the space inside these organelles is a continuum. The latter contains mitochondria sap, consisting of a fine, granular material, which is usually not very dense, but the density may vary.

The shelves provide a tremendous internal surface area, and the general consensus is that the numerous oxidative enzymes present within the mitochondria are located in an orderly array upon these shelves. The shapes of the mitochondria are from round or oval to filamentous. They are usually about 1 micron in diameter and are rich in the aerobic enzymes, especially those of importance in the Krebs cycle.

The Golgi Complex. The Golgi Complex has a specific structure essentially similar in all cells. It consists of groups of large, flat, saucer-shaped, smooth-walled profiles. These in turn are surrounded by numerous small, smooth-walled vesicles, which are derived by a pinching off of the ends of the larger,

flattened sacs. In thin sections the Golgi Complex usually lies to one side of the nucleus and surrounds the centrosome of the cell. The vesicles of the Golgi Complex differ from those of the endoplasmic reticulum by being smaller and agranular, and the vesicles and sacs are closely grouped.

As far as function is concerned, it is likely that the Golgi Complex is related to the storage and organisation of certain substances, in particular the lipids.

Centrosome and Centriole. The centrosome occupies the centre of the cell. It is relatively clear and is often surrounded by the profiles of the Golgi Complex. In the centre of the centrosome are found the centrioles. They are small cylinders about $1500 \text{ \AA}$ in diameter, and $5000 \text{ \AA}$ in length. The sides of the cylinders are composed of 9 groups of 3 small tubules, each about $200 \text{ \AA}$ in diameter. 9 spherules surround each centriole. There is one spherule for each group of three tubules, and each spherule is connected by a bridge to each of the three tubules. The centrioles lie perpendicular to each other.

The centriole plays a part in mitosis. Prior to the splitting of the chromosomes, the centrioles diverge and travel to opposite poles of the cell, thus forming the asters which play an important role in mitosis. Thus centrioles are often seen in cells which are rapidly proliferating, especially so in many malignant tumours.

Lysosomes. The lysosomes, or dense bodies, are often seen in cells. Their exact definition is not as yet complete. They are identified mainly by exclusion, and are limited by a smooth-walled, single membrane. They may contain electron-dense granules, and often have a whorling pattern, along with an internal cavity. They have been associated with the acid phosphatase activity of the cell, and it is suggested that their function is to break down the larger molecules within the cell, and thus allow the smaller units to enter the mitochondria where they will undergo aerobic metabolism. They may also play a part in pinocytosis and phagocytosis. The lysosome has been related to the Golgi Complex, and to mitochondria formation, as well as to taking part in the storage of materials and to ageing.

The Ground Substance of the Cytoplasm. The ultra-structure of the cytoplasm varies from cell to cell and differs in regions of the same cell. There is often a fine vesicular or reticular structure, along with some fine filamentous elements. Inclusions are also seen. They may be lipid granules which are amorphous, electron-dense particles with no definite shape or size and no limiting membrane. Glycogen is also present and resembles the lipid masses, but is not as dense. Filaments are present and organised in certain cells, and may at times fill the cytoplasm.

The geometrical arrangement of these fibrils and their attachment to desmosomes on the cell membranes suggests a mechanical role in maintaining cell shape and rigidity. They stain as basic proteins, and occur in enhanced amounts in precisely the situations where support is demanded (Mercer).

The Normal Anatomy of the Oral Mucous Membrane.

In framing this section, the following authorities have been consulted: Orban and Wentz, 1960; Silverman, 1961; Sicher, 1962, and McCarthy and Shklar, 1964. No individual references will be included here.

The morphologic structure of the mucous membrane varies in the different areas of the oral cavity in correlation with the function of specific zones and the mechanical influences that bear upon them. For example, the mucous membrane around the gingiva of the teeth is subjected to the mechanical forces of mastication of coarse food, whereas that in the floor of the mouth, which is protected by the tongue, is subjected generally only to liquid or softened foods. Hence, the epithelium of the gingiva is heavily keratinized or toughened to resist abrasion, whereas the epithelium of the floor of the mouth is less keratinized.

The mucous membrane is attached to the underlying structures by a layer of connective tissue, the submucosa, which itself varies in different areas, depending upon whether the mucosa is firmly or loosely attached to the bony structure, and whether there is muscle tissue between itself and the underlying bone.

The oral mucosa is composed of two layers - the surface epithelium and the lamina propria. A basement membrane separates the two layers.

The following is a classification of the different areas

of the mucous membrane:-

A. Masticatory Mucosa.

- | | | |
|--|---|-------------------------|
| 1. Gingiva, | } | Keratinizing
Mucosa. |
| 2. Mucous Membrane of the Hard Palate, | | |

B. Lining Mucosa.

1. Lining of the lips and cheeks,
2. Inferior surface of the tongue,
3. Floor of mouth,
4. Mucous membrane of the soft palate,

C. Specialised Mucosa (Tongue).

1. Anterior Papillary Zone,
2. Posterior Lymphatic Zone.

D. Transition between Skin and Mucous Membrane.

A. Masticatory Mucosa.

1. Gingiva. The gingiva or gums refers to the tissue surrounding the teeth and covering the alveolar bone. The gingival tissue is tightly matted down to the periosteum of the alveolar bone. That portion of the gingiva extending beyond the alveolar bone and attaching to the teeth is referred to as the marginal gingiva or free gingival margin. The surface of the marginal gingiva is smooth; that of the attached gingiva is stippled. The gingival tissue appears a light pink colour normally. Beyond the gingiva and separated from the gingiva by a fairly clear demarcation (the mucogingival junction) is the alveolar mucosa. The latter is a loose,

delicate tissue not matted down to underlying bone and containing numerous small blood vessels which can be seen through this delicate mucosa. The alveolar mucosa on the outer or buccal aspect is continuous with the mucous membrane of the lip and cheek, and a vestibule or trough is formed where they blend into one another - the mucobuccal fold or vestibule of the mouth.

2. The Mucous Membrane of the Hard Palate. The mucosa of the hard palate covers the palatal osseous structure and is firmly attached to the underlying connective tissue, although some displacement of this mucosal tissue is possible. The submucosal tissue is absent only in the peripheral zone, where the palatine tissue is identical with the gingiva, and in a narrow zone along the midline, starting in front with the palatine or incisive papilla, and continuing as the palatine raphe over the entire length of the hard palate. The attachment of the mucosa to the periosteum of the maxillary and palatine bones is accomplished by dense bands and trabeculae of fibrous connective tissue that join the lamina propria to the periosteum. The submucous space is then subdivided into irregular intercommunicating compartments of various sizes. These are filled with adipose tissue in the anterior part and with glands in the posterior part of the hard palate. The ducts of the glands appear on the palatal surface as large numbers of small pores. Anteriorly the hard palate presents numerous folds of firm tissue - the palatine rugae.

B. Lining Mucosa.

1. The Lining of the Lips and Cheeks. The mucosa is light pink in colour and smooth in texture. Where it is adjacent to or covering muscles, it is immovably fixed to the fascial covering of the respective muscles, and it is highly elastic. These two factors protect the mucosa during function and thus prevent cheek biting.

In the vestibular fornix, where the mucosa of the lips and cheeks reflects to become the mucosa of the alveolar ridge, it is connected by a loosely textured submucosa which permits the movements necessary.

There is usually a ridge or fold of tissue along the occlusal line of the teeth, representing a tissue response to constant stimulation by the teeth during mastication. This fold may be grey-white in colour, and is occasionally confused with white lesions of the buccal mucous membrane. Above the occlusal line and in the posterior part of the buccal mucosa opposite the maxillary molar teeth is the papilla of the parotid (Stenson's) duct.

2. Inferior Surface of the Tongue. The mucosa of the ventral surface of the tongue is smooth and delicate, no papillary structures being present. It is firmly attached to the epimysium fascia of the tongue musculature. The submucosa is not identified as a layer.

3. The Floor of the Mouth. In the floor of the mouth the lining mucosa is very thin and very loosely attached to the underlying structures, permitting free and extensive mobility of the tongue. The submucosa contains adipose tissue and the sublingual glands.

Anterior to the attachment of the lingual frenum are two diagonal ridges meeting in the midline to form a V configuration pointing anteriorly. Along these ridges or sublingual caruncula open the numerous ducts of the sublingual glands.

4. The Soft Palate. The lining mucosa of the soft palate represents a transition between the firmly adhering mucosa of the lips and the loosely adhering mucosa of the vestibular sulcus. It is highly vascular and reddish compared to the pale colour of the hard palate. The lamina propria has a distinct layer of elastic fibres separating it from the submucosa, which is rather loose and contains a continuous layer of mucous glands. Thus, the lining mucosa of the soft palate is free to move over the muscles, but it is kept firm in contour and adaptation to the musculature by the elastic layer in the lamina propria.

C. Specialised Mucosa (Tongue).

1. Anterior Papillary Zone. The lingual mucous membrane covers a highly complex muscular system and must be adapted to continuous irritational stimuli of varying nature. The mucosa covering the dorsal surface of the tongue is rough, and composed of large numbers of minute papillae. The major group of lingual papillae are the filiform papillae which are small and punctate in appearance, giving the tongue a file-like texture. Scattered among these are the larger fungiform papillae which appear somewhat more red in colour. At the posterior border of the lingual dorsum are the large circumvallate papillae arranged in a V-shaped

configuration, with the point facing the oropharynx. There are approximately 8 to 10 circumvallate papillae arranged in two diagonal rows. They do not protrude above the surface, but are bounded by a deep circular furrow that seems to cut them out of the substance of the tongue. Their free surface shows numerous secondary papillae that are covered by a smooth, thin epithelium. On the lateral surface of the circumvallate papillae, and occasionally on the walls surrounding them, the epithelium contains numerous taste buds. Into the trough open the ducts of small serous glands, von Ebner's glands, which serve to wash out the soluble elements of food that stimulate the tastebuds from the deep circular groove.

At the angle of the V-shaped terminal groove on the tongue is located the foramen caecum, the remnant of the thyroglossal duct.

2. The Posterior Lymphatic Zone. Posterior to the circumvallate papillae, the surface of the tongue is irregularly studded with round or oval prominences, the lingual follicles. Each of these shows one or more lymph nodules, sometimes containing a germinal centre. Most of these prominences have a small pit at the centre, the lingual crypt, which is lined with stratified squamous epithelium. Ducts of the small posterior lingual mucous glands open into the crypts. Together the lingual follicles form the lingual tonsil.

On the lateral border of the posterior parts of the tongue, sharp parallel clefts of varying length can often be observed. They

bound narrow folds of the mucous membrane and are the vestige of the large foliate papillae found in many mammals. They contain taste buds.

D. Transition Between Skin and Mucous Membrane.

This is the red zone, or vermilion border of the lip. This region is characterised by numerous densely arranged long papillae of the lamina propria, reaching deep into the epithelium and carrying large capillary loops close to the surface. Hence blood is visible through the thin parts of the translucent epithelium covering the papillae, resulting in the red colour of the lips.

The boundary between the red zone of the lip and mucous membrane is found where keratinization of the transitional zone ends. The epithelium of the mucous membrane of the lip is not keratinized.

HISTOLOGY AND ELECTRON MICROSCOPY OF ORAL MUCOUS MEMBRANE.

Oral mucous membrane is essentially similar to skin but lacks the secondary structures or skin appendages. The oral mucosa is composed of a surface stratified squamous epithelium and an underlying connective tissue corium or lamina propria.

The normal stratified squamous epithelium is usually keratinized only in the gingiva and the hard palate. This view is not unanimously supported by all writers, as will be seen later in this section.

The following headings will be used:-

A. Stratified Squamous Epithelium;

1. Stratum Germinativum in (a) Keratinizing Epithelium,
(b) Non-keratinizing Epithelium.
2. Stratum Spinosum in (a) Keratinizing Epithelium,
(b) Non-keratinizing Epithelium.
3. Stratum Granulosum in (a) Keratinizing Epithelium,
(b) Non-keratinizing Epithelium.
4. Stratum Lucidum and Stratum Corneum.
5. The melanocytes.
6. Nerve supply and nutrition of the Epithelium.
7. The basement membrane.
8. Differentiation in the epithelium.
9. The life cycle of the epithelium.

B. The Lamina Propria and Submucosa.

Authorities consulted in preparing this section are Orban and Wentz (1960), Sicher (1962), Silverman (1961), Mercer (1961), McCarthy and Shklar (1964) and Zelickson and Hartmann (1963). Some other references will be quoted.

A. Stratified Squamous Epithelium.

1. The Stratum Germinativum.

The stratum germinativum or basal layer is immediately adjacent to the connective tissue, and represents the germinal layer of the epithelium. The basal cells are cuboidal or columnar with large deeply staining nuclei and appear as a uniform row one or two cells thick.

- (a) In Keratinizing Epithelium. The nucleus of the basal cell is oval, has two membranes, an inner one about $300 \text{ \AA}$ thick, bounded on its inner surface by many fine particles. Numerous fine particles cover the cytoplasmic surface of the outer nuclear membrane, which is separated from the inner one by a clear area $600 \text{ \AA}$ wide. In places, the outer membrane diverges from the inner zone, at times appearing to form part of the endoplasmic reticulum. Pores are seen in the outer membrane, and may be present in the inner membrane, though none have been recorded. The nucleoplasm is made up of small particles ($200 \text{ \AA}$). The nucleolus appears to be a condensation of particles into a worm-like structure without a limiting membrane.

The Cytoplasm of the basal cells contains multiple organelles, vesicles and filaments. The many mitochondria which are present throughout the cytoplasm average $7300 \text{ \AA}$ in length and $2400 \text{ \AA}$ in width, and follow the usual form of mitochondria formerly described.

The endoplasmic reticulum is present but not well developed. Some vesicles have smooth surfaces while others appear rough, due to the presence of numerous adherent ribonucleoprotein granules (R.N.P.). A number of smooth-walled vesicles is often noted in irregular cytoplasmic pegs, which project into the corium. Numerous fine, electron-dense particles (200 - 300 Å) are seen throughout the cytoplasm, and these make up the background of the cytoplasm of all epidermal cells.

Tonofilaments, either arranged in bundles or lying in random fashion, are noted throughout the cytoplasm. In the basal cell, the tonofilaments appear long and pronounced, lie perpendicular to the surface of the epidermis, and attach to the inner surface of the desmosomes. It appears that the actual quantity of filaments in the basal and succeeding layers of the same skin specimens is identical, and that only the degree of organisation of these filaments into fibrils differs between layers.

The desmosomes, dense oval thickenings called attachment plaques, are located in opposed areas of adjacent cell membranes and the tonofibrils terminate at the internal face of the "attachment plaque", and thus do not transverse the 300 - 600 Å distance between opposed plaques. A node of Bizzozero consists of a pair

of "attachment plaques", one from each of two adjacent cells, and 7 intervening lamellae. Each plaque is in its 3-dimensional form, an oval plate of more than 100 $\bar{A}$ in thickness and about 3000 - 7000 $\bar{A}$ in diameter.

Kurahashi and Takuma (1963) reported that the desmosomes found in the basal and prickly cell layers consisted of 3 intercellular lamellae and thickened attachment plates in the membranes of the adjacent cells; also that tonofibrils were inserted into the cytoplasm side of the plates.

The dermal side of the basal cell is made up of irregular prolongations of the cell in the dermis, each containing, besides the vesicles mentioned, an occasional thickening of its membrane ("attachment plaque") but without a similar structure opposing it. This latter formation has also been referred to as a half-desmosome. Tonofibrils are attached to the cytoplasmic surface of these plaques.

About 300 $\bar{A}$ below the cell membrane, another fairly definite broader membrane runs parallel with the dermal border of the basal cell - the basement membrane. Several dense lamellae are also noted between this membrane and the "attachment plaques" or thickenings of the basal cell membrane. Kurahashi and Takuma (1963) showed that the basement membrane of the gingival epithelium is a filamentous structure 300 - 700 $\bar{A}$ wide.

Melanin granules are sometimes present in basal cells. While pigmentation is a normal occurrence in Negroes, it is found too, in the Caucasian race, especially in people with a dark complexion. The granules are often arranged as a cap or group at the superior pole of the nucleus, or may be dispersed throughout the cytoplasm. They are of the mature type and the internal striations of the immature granule are rarely seen. The granules are round to oval, many have a limiting membrane and are composed of groups of particles. Though stored by the basal cells, the pigment is elaborated by specific cells, the melanoblasts.

On rare occasions centrioles, which are usually seen in the stratum germinativum, appear in the plane of section. They lie in a clear zone (centrosome) at the superior pole, just outside the outer nuclear membrane.

A Golgi Complex is often seen in this same region. It usually consists of several smooth-walled elongated vesicles (900 Å) surrounded by numerous smaller, smooth-walled, round vesicles (500 Å).

The cell membrane of adjacent basal cells is for the most part straight and perpendicular to the mucosal surface. The contour, however, is interrupted by villous foldings of the cell membranes which are compressed between the two cells. In its attachment to the spinous cell, the basal cell is less regular in contour, and has numerous larger foldings and villi.

(b) In Non-keratinizing Epithelium.

The basal cells are oval with their longest diameter perpendicular to the surface. They have numerous and irregular elongations which project into the corium. Desmosomes are present, but are few, thus the basal cells are only loosely held together and are separated from their neighbours by large intercellular clefts which extend from the corium toward the surface for a distance corresponding to the length of 2 or 3 cells. Cytoplasmic projections resembling microvilli project into these intercellular clefts. The desmosomes consist of thickenings of opposed cell membranes with 2 or 3 dense lamellae distinguishable between them, and are similar morphologically to the description in the previous section. Short intracellular filaments terminate at the desmosomes, and do not cross the cell membrane. Regular thickenings of the basal cell wall also occur on that surface which faces the basement membrane. These half-desmosomes are approximately 700 Å in length.

The nucleus is usually round to oval and contains an irregular nucleolus. Sognnaes et al (1958) found that in the deeper cell regions of the mucous membrane the nucleoli are distinct, the nuclei are rounded, and the cell membranes are relatively smooth, that is, devoid of the marked indentations which occur in the more superficial layers. The nucleus has two membranes, the outer of which is

sometimes close to the cell membrane. Nuclear pores are present.

Mitochondria are abundant and are localized around the nucleus. They range in length from 2500 - 10000 Å and often contain small, dense granules. In section they are round to oval, and have shelves which extend about half way across the interior of the structure.

The Golgi Complex is especially well developed, there being large groups of small, smooth-walled vesicles situated at the opposite poles of the nucleus. Also there are numerous vesicles arranged in the pattern typical of the Golgi Complex. These are elongated, flattened, and smooth-walled.

The endoplasmic reticulum is present, but poorly developed. It is mainly of the rough-surfaced type with numerous R.N.P. granules adhering to the cytoplasmic surface of the cisternae.

Tonofilaments are present, but few in number. They are short and attach to the cytoplasmic surface of the desmosome, but do not cross the cell membrane.

The basement membrane is separated from the epithelium by an almost constant distance of 200 - 300 Å.

2. The Stratum Spinosum.

The stratum spinosum, or prickly-cell layer, is a relatively wide zone of polyhedral cells with large nuclei. The nuclei stain less intensely than those of the basal layer, and for

this reason the basal layer is usually well demarcated from the overlying prickle-cell layer. The individual cells of the stratum spinosum are clearly outlined by cell walls, and the cells are slightly separated and appear to be joined by fine protoplasmic processes, referred to as intercellular bridges. These processes, or intercellular fibrils are responsible for the prickle-cell connotation. The cells of the stratum spinosum tend to flatten as they approach the surface layers (McCarthy and Shklar).

From the ensuing description of the ultrastructure of the stratum spinosum, it will be obvious that Zelickson and Hartmann do not describe any fibrils as crossing from one cell to another - they terminate at the desmosomes, which form the only attachments between cells. The "prickles" are actually the villous and irregular folds of the cell membranes.

- (a) In Keratinizing Epithelium. The round nuclei are bounded by two membranes. The nucleoli may be vermiform in shape, lacking a limiting membrane, and made up of many fine particles. Nuclear pores are present in the membrane. The outer nuclear membrane at times bulges away from the nucleus, and often approaches the cell membrane. The inner membrane appears wider than the outer, which is covered on its cytoplasmic side by R.N.P. granules, giving it a rough appearance.

Mitochondria are numerous throughout the cytoplasm, at times encircling and indenting the nucleus.

Throughout the cytoplasm of the spinous cells are vesicles

of many sizes and shapes; some are smooth and others are coated with ribosomes, giving them a rough-walled appearance. Some smooth, double-walled vesicles are seen close to the cell membrane. These probably represent cross-sections of invaginating villi from adjacent cells.

The cell membrane of the spinous layer is quite villous, with each cell held close to its neighbour by the resistant desmosomes. Between the desmosomes the membrane is thrown into irregular folds and the cell is separated from its neighbour by an intercellular space, which is perhaps more apparent than real.

The ultrastructure of the desmosomes is the same as in the basal layer.

A Golgi apparatus and centrioli have not been noted in the spinous layer.

Tonofilaments are relatively abundant, being arranged in bundles at their attachment to the cytoplasmic side of the desmosome, and lying in a random manner in the rest of the cell. The filaments have about the same diameter (50 - 100 Å), but are of varying length, probably due to the sectioning technique. Charles and Smiddy (reported by Zelikson) suggested that both ends of the tonofibril or tonofibrillar bundle may be attached to the cell wall at the desmosome

or half-desmosome (in basal cell). The result of such an arrangement would be to link the whole of the tonofibrillar network of the epithelium into an elastic system suitable for absorbing the distortional stresses to which it is subjected.

As the cell approaches the stratum granulosum, it undergoes definite flattening, with its longest diameter becoming oriented parallel to the surface. The desmosomes are still clearly visible, and retain their characteristic internal structure. The tonofibrils, although appearing less distinct at this level, are arranged in a fairly regular fashion, lying horizontal rather than perpendicular to the surface.

In the higher regions of the stratum spinosum, elements of the endoplasmic reticulum or mitochondria are observed. In place of the cell organelles, various workers have found numerous round, smooth-surfaced, thick-walled granules or vesicles, which are also found in the stratum granulosum. It has been suggested that these could be virus particles, but the consensus of opinion is that they are attenuated mitochondria.

- (b) In Non-keratinizing Epithelium. The cells of the stratum spinosum become much more elongated and are orientated parallel to the surface. An increasing number of plications occur in the cell membrane. The cell boundaries are here more closely apposed, with an

absence of the clefts of the basal layer.

Desmosomes are present, but few. In the more elongated cells, the mitochondria are spread laterally from the poles of the nucleus. Filaments are few, short, and not arranged in bundles, and the cytoplasm is more granular than in the deeper levels. The perinuclear vesicles and other elements of the Golgi Complex are still present. These structures, along with the mitochondria, are constantly found together forming a perinuclear complex which is observed in most cells throughout the mucosa.

At a slightly higher level, numerous smooth, thick-walled granules tend to localise near the cell membrane, and are present in cells from which the mitochondria are disappearing. It appears nearly certain that these granules are, in fact, degenerating mitochondria.

3. The Stratum Granulosum.

The stratum granulosum or granular layer lies above the stratum spinosum and is composed of several layers of flattened cells with large numbers of deeply staining granules in the cytoplasm. These have been referred to as keratohyalin granules. They are not as well marked in non-keratinizing areas of the mucosa.

- (a) In Keratinizing Epithelium. The process of differentiation continues. Present, but in smaller numbers, are mitochondria, vesicles of the endoplasmic reticulum, and the previously mentioned smooth, thick-walled granules. Most characteristic, however, are the electron-dense,

irregularly shaped keratohyalin granules. Charles (quoted by Zelickson) suggested that keratohyalin is a substance which has been deposited on the tonofibrils, the tonofibrils having the appearance of being ensheathed by the dense keratohyalin. He thought they are not significant in cornification, being merely the first precipitation from the cytoplasm. Selby (quoted by Zelickson) found the keratohyalin granules to be non-crystalline and sufficiently dense and irregular in shape and contour to be considered cellular debris. Zelickson thinks that since the keratohyalin granules are observed in cells with normal nuclei, their identity as nuclear remnants does not seem likely.

Nuclei may still be present in the granular layer, and when seen are irregular in outline. If the nucleus is present, the inner membrane is still definite, but the outer zone is difficult to resolve. An occasional nucleolus is observed. Mitochondria, if present, are often perinuclear, but their membranes and crystal appear shrivelled. The endoplasmic reticulum is not prominent.

The cell membrane has definite foldings and desmosomes, which appear to be closer and more numerous than in the deeper layers. This may be due to the new, somewhat crenated shape the cell has assumed, with the same surface membrane now encompassing a lesser volume.

As the cell continues to flatten, the keratohyalin material seems to be closely associated with the tonofilaments.

- (b) In Non-keratinizing Epithelium. The cell membranes are even more folded than in the deeper layers, and interdigitate with those of neighbouring cells, but here fewer desmosomes are seen. The cytoplasm at this level is clear, as most of the cytoplasmic organelles have disappeared. The cells, which are elongated and narrow, but not flat, are shed in this state, according to Zelickson.

4. The Stratum Lucidum and the Stratum Corneum.

In skin there is often a stratum lucidum or thin clear zone above the stratum granulosum. However, this zone is normally absent in oral mucosa.

The surface zone is the keratinized or cornified layer referred to as the stratum corneum. The stratum corneum is composed of varying amounts of keratin, usually structureless fine layers of eosinophilic material with occasional cell nuclei present, or clear spaces representing degenerated nuclei. The width of the stratum corneum varies considerably in oral mucosa, depending upon the nature of the tissue. Areas of the mouth receiving little stimulation or traumatic influences either are non-keratinized or present a very thin stratum corneum. The floor of the mouth, ventral surface of the tongue, and soft palate, fall into this category. Generally, however, according to McCarthy and Shklar,

the oral mucosa presents a definite stratum corneum, although the width is considerably less than that of skin. In this respect there seems to be some conflict between the various authorities; Orban and Wentz, Silverman, and Sicher all either state directly or imply that the only areas where there is keratin, i.e., a stratum corneum, are the "masticatory mucosa" areas of the gingiva and hard palate. McCarthy and Shklar state also that "the mucosa in these areas (living mucosa) is relatively slightly keratinized".

It has been shown that stimulation of the oral mucosa results in increased keratinization. Where the stratum corneum is thin, the stratum granulosum is indistinct and may appear to be absent. As the width of the stratum corneum is increased, the stratum granulosum becomes wider and more obvious.

The electron microscope shows the characteristic homogeneous appearance of the stratum corneum cells. A nucleus is usually no longer seen. The cytoplasmic structures - melanin, mitochondria, endoplasmic reticulum, Golgi Complex, and smooth, thick-walled vesicles, are usually absent. The cell is long and flat, and oriented parallel to the surface. Still present are the desmosomes attaching each cell to its neighbour. The internal structure of the desmosome is altered, and appears to have taken part in the process of keratinization. The outer cells desquamate only when the desmosomes break, this being the last step in the progression of the cell.

According to Sicher, in the gingiva, the formation of

true keratin, orthokeratosis, is, in a majority of individuals, replaced by parakeratosis. Sometimes the epithelium is non-keratinized, though the gingiva has to be regarded as normal. According to the behaviour of the surface layer, four types of gingival epithelium can be distinguished:-

- (1.) In fully keratinized epithelium, layers consist of flat, tightly packed, horny scales - the transformed surface cells. Nuclei are absent.
- (2.) In parakeratosis, the surface cells seem to consist of keratin, but have retained pyknotic nuclei.
- (3.) In incomplete parakeratosis, specific stains, e.g., Mallory's stain, show the surface layer divided into two layers. The deeper layer stains like keratin, but this stain is lost in the superficial layer, probably by the influence of oral fluids on the incompletely differentiated keratin of the nuclei-containing cells.
- (4.) Where keratinization is lacking, the flat surface cells retain their nuclei.

The most frequent type is parakeratosis, about 50%.

Next is incomplete parakeratosis, 25%; then full keratinization, 15%; and non-keratinization, 10% (according to Sicher).

5. The Melanocytes.

The melanocytes, which elaborate melanin pigment, are situated in the basal layer of the epithelium. The melanin is not retained in the melanocytes, but stored by the basal cells. The melanocytes have long processes, and are also termed dendritic cells.

In the usual haematoxylin-eosin specimen, these cells have a clear cytoplasm and are also known as clear cells.

The electron microscope shows that the dendritic processes of the melanocytes extend between neighbouring epithelial cells. The dendrites squeeze between the cells and tend to spread, but not break down the resistant desmosomes. The melanocyte has a few filaments but lacks desmosomes. The cytoplasm contains numerous mitochondria spread throughout the cell, and a well-developed endoplasmic reticulum. An easily recognizable Golgi Complex is located at one side of the round nucleus. Few if any mature melanin granules are present within the melanocyte, but pre-melanin granules are found in the perikaryon and in the dendritic processes of the melanocyte.

6. Nerve Supply and Nutrition of the Epithelium.

What may be peripheral nerve endings are occasionally seen in the mucosa. These are round to oval in sections, and lie in close proximity with, but not attached to, the membranes of adjacent cells. They contain numerous neurovesicles and mitochondria. The neurovesicles are round, small, and their contents are of a greater density than the surrounding cytoplasm. Their mitochondria are of the filamentous type. These nerve endings closely resemble sectioned melanocyte processes, but because of the above features, a differentiation is possible.

If it were not for the basement membrane, there would be no apparent separation between the mucosal epithelium and the underlying corium. This close relation between the epithelium and the

corium is suggested by the large intercellular clefts with blood cells infiltrating them, and also by the irregular projections of the basal cells into the corium, making for a closer continuity between these elements.

Blood vessels are plentiful in the upper corium. The cytoplasm of the endothelial cells of the capillaries contains numerous mitochondria and a well-developed endoplasmic reticulum along with numerous pinocytic vesicles bordering the lumen. One noteworthy feature is the absence of a capillary basement membrane, and the existence of clear passages between the endothelial cells, placing the lumen in direct communication with the corium.

These channels between the capillary endothelial cells quite possibly allow free passage of material from the vascular tree through the corium to the epithelium.

Again, the complex plication of the epithelial cell membrane probably enhances the transfer processes across cell membranes as a consequence of increased surface area.

7. The Basement Membrane.

Sognnaes and Albright (1958) stated that the basal cells are not bordered from the underlying connective tissue by anything that can be characterized as a structural unit referred to as a basement membrane in optical microscopy.

However, Zelickson (1963) reports that about 300 Å below the cell membrane of the basal cells, another fairly definite broader membrane runs parallel with the dermal border of the basal

cell - the basement membrane. Several dense lamellae are also noted between the membrane and the "attachment plaques" or thickenings of the basal cell membrane.

Cahn et al (1962 I) describe features of the basement membrane. It is an amorphous layer, possibly of a mucopolysaccharide character, to which fine collagenous fibres staining as reticulin adhere.

Sicher (1962) states that biophysical and chemical studies have shown that the reticulin of the basement membrane is a collagen in a close association with a carbohydrate and a lipid component.

It may be assumed that the basement membrane is permeable to intercellular fluids which diffuse through this structure to provide an interchange of nutrients and waste products. The cytoplasmic processes of basal cells pass across the basement membrane to join reticular fibres in the stroma and provide anchorage for the epithelium.

Impermeability to large foreign particles is demonstrated by the fact that dyes injected intradermally beneath a subepidermal bulla will not appear in the fluid of the bulla, although it spreads rapidly through the dermis.

Histologic evidence seems to indicate that neutrophiles may cross the basement membrane and find their way into the region of the stratum germinativum and prickle-cell layers. In the pigmented naevus, cells (probably basal) drop through the basement membrane and are found in nests in the underlying corium.

Although note may be made of these special instances of cellular transgression, the basement membrane in general seems to be a limiting structure which marks the border of the epithelium even in the presence of extensive overlying hyperplasia.

The continuity or lack of continuity of the basement membrane may be of significance in the containment of epithelial downward growth. Routine haematoxylin and eosin stains do not demonstrate this structure, though a clear delineation between the epithelium and its stroma may be indicated by a continuous and well-defined basal layer.

With P.A.S. stain, basement membrane is delineated beautifully - it stains a vivid magenta colour.

From numerous experiments which show that the underlying mesenchymal tissue induces and maintains the different epidermal differentiations, there is a suggestion that the basal membrane itself could be the important factor. It appears to differ in thickness from site to site (200 - 600 Å), and there are differences in the types of mucopolysaccharides present. It is easy to picture such a continuous layer of colloids of this type, with their fixed network of charged sites, functioning as ion exchange resins, and exerting a selective effect on the transfer of signal molecules from the blood to the epithelial cell population. The idea, however, is very insufficiently explored experimentally as yet (Mercer).

Turesky et al (1951) found that in chronically inflamed areas of the gingiva, the basement membrane is thinned out, and tends toward disintegration.

8. Differentiation in the Epithelium.

The epithelial character of the epithelial cells of an embryo appears very early in life, and this may be due to the appearance of intercellular adhesion which continues thereafter to play an important morphogenetic role. There is evidence to suggest that in the next phase the factors responsible for localised specialisations arise in the underlying mesoderm. The epidermal cells at this stage may be described as being sandwiched between two environments: the external relatively free space, and the underlying mesodermal domain. In the first place, the external situation imposes a class of differentiations, and secondly, the mesodermal organisation further limits differentiation and gives rise to site-characteristic developments. As these develop and call into being an appropriate dermal organisation to support them, consisting in part of fibrous collagenous depositions and a blood supply, the situation is reversed. The epidermal tissue becomes dominant.

The dominant influence of the basement membrane has already been mentioned.

It seems possible to distinguish several successive stages in the establishment of the skin in which dominant control of the dermoepidermal partnership swings successively from one member to another. In the earlier phase the superficial cells, responding to their exposed position, begin to stick together, and thus bring about the epithelial pattern. Their, as yet unstabilised, free,

external surfaces produce a selection of responses from what Mercer has termed the "cell's surface repertoire". The establishment of a definite surface layer encloses the other cells in a different environment which diverts them toward synthesizing different products (mesenchymal substances), which, in their turn, react with the inner surfaces of the basal layer cells to form the basement membrane and to induce local variations in the epidermal layer. By their subsequent development, these epidermal variations make return demands for food and support on the underlying layers leading to the building up of a dermal organisation of fibrillar scaffolding and supply vessels.

There is much evidence to show that the determination is not irrevocable and that the cells of the germinal layer itself remain effectively multipotential. Evidence of persistent multipotency is provided by the epithelia of many internal surfaces which may exhibit a cyclic metaplasia under hormonal control with a well-defined physiological function. The best-known example is that of the vaginal epithelium which oscillates between mucin and keratin production, cells of a contrasted cytology being produced successively in the same basal layer. In other situations, keratin production oscillates with glycogen.

That the potentiality of producing both keratin and mucin can exist even in the same cell is indicated by some observations of Glucksman and Cherry (in Mercer) on mixed carcinoma.

It would seem that the several varieties of epidermal cells are examples of what Weiss (in Mercer) has preferred to term

cell modulations which are at first reversible and which require for their maintenance the presence of other elements of the cellular community. Modulations are to be contrasted with the perhaps irreversible differentiations which accompany embryogenesis, and which divide the total cell community into several major families of common descent. Support for such a view is given by direct experimental evidence of cell metaplasia produced by relatively simple chemicals. The work of Fell and Mellanby and their associates (in Mercer) has established that vitamin A disposes the epidermis towards mucin formation. Seven-day-old chick embryo ectoderm cultivated in vitro in a normal culture medium undergoes precocious keratinization, the two-layered epithelium being replaced by a stratified layer. When vitamin A is added to the medium (2000 - 3000 i.u. per 100 ml.), keratinization is prevented and a mucous secreting, often ciliated, epithelium appears. The change is not stable, for when the layered epithelium was transferred back to a normal medium, (i.e., lacking vitamin A) a typical mucous membrane containing ciliated cells and mucous cells at first appeared, but after a time this was replaced by a squamous keratinizing epithelium forming beneath it.

Jolly (1964) comments that this investigation conclusively proved that vitamin A is able to influence the differentiation of the basal cells of epithelium, and that its action is similar to that of a hormone. Excess of the vitamin causes the basal cells of squamous epithelium to differentiate into mucous-secreting cells.

Lasnitski (in Mercer) showed that the effect on mammalian skin (human embryo) was essentially the same. Embryonic epidermis (3-4 months foetus), in normal medium, formed a typical squamous keratinizing epithelium including a keratohyalin layer. In a medium containing vitamin A, several layers of large cuboidal cells appeared which contained mucin-like materials. Older skin is less responsive, but vitamin A suppressed keratinization.

The vitamin A induced metaplasia was correlated with changes in the uptake of sulphur detected by using radio-active sulphate. In explants of skin treated with S^{35} (as sulphate), mucous secreting material was intensely active; the keratinizing layers much less so. On the other hand, the uptake of radio-active cystine was greater in the keratinizing epidermis.

The effect of vitamin A is most apparent on the germinal layer cells as might be expected, since these are "uncommitted" (disputed by Gerson and Meyer, 1964), but cells in the process of keratinization can still be deflected in their course by the vitamin. The mucin forming cells, once formed, cannot however, revert when returned to a normal medium, but are shed.

Observations made on cells isolated and cultivated in the absence of other cells show that in these conditions, cells gradually cease to produce their characteristic products and assume a more generalised character. Further, when mixed cell

populations are grown together, differentiation again takes place. These very general findings are sufficient to prove that differentiation is maintained by restraints exerted by one cell type on another, either by direct contact or by exchange of their products through the medium of their common humoral pool.

It seems probable that each organ system itself produces changes which automatically lead to its limiting its own proliferation. Such a view is in harmony with modern theories of self-controlled mechanisms which envisage control, in very general terms, as being effected by a "feed-back" of information which introduces a limiting factor proportional to the deviation from a norm.

As emphasized already, the epithelium, because of its position on the outside of the cell system, constitutes a special case among the organs. Moreover, it is non-vascular, unevenly innervated and its cells grow outwards. Its cells have evidently only a limited possibility of communicating with each other and the rest of the system.

There is in the epidermis a vertical integration, but clearly only a rather limited lateral one. By postulating the same intracellular features, generative mass, differentiated mass, (keratin, mucin, etc.) and inhibitor production, the possibilities of control in a simple stratified epithelium may be considered. Division is largely confined to the basal layer and synthesis of specialised products takes place in more distal layers. The

inhibitor molecules, produced in the stream of outwardly-moving cells during these later reactions, are largely lost when these cells are shed, and can only feed-back to the germinal layer by back diffusion, and only by crossing the dermoepidermal junction can they reach the general circulation and thus be carried to distant parts of the system.

Certain possibilities may be made clear by considering a cell which has just been produced by division. Since division has occurred, we may suppose that inhibition is minimal. The cell leaves the germinal layer and, at a higher level, begins to differentiate, to synthesize both the differentiated product and also the inhibitor. The following conditions may arise:-

- (a) Before sufficient inhibitor is produced or diffuses back, the cell in the germinal layer again divides. This would be a condition permitting of uncontrolled, continuous growth.
- (b) Before division can occur again, sufficient inhibitor diffuses back to prevent it. After a further time, the concentration falls again, (with the decrease in synthetic activity in the differentiated layer and the decay of inhibitor molecules) and division again occurs. Here there is a condition of periodic division under the control of the events in the differentiated layer. The time elapsing between divisions will depend on the rate of synthesis in the differentiated layers and the rate of loss or decay of inhibitor at the germinal level.

Since a certain lateral diffusion from any cell is possible, a synchrony could develop in the germinal layers owing to the overlapping of the effects of several adjacent cells. This synchronisation may be favoured also by the possibility that a general systemic stimulation reaching a group of cells, in which the inhibitor concentration is already low, may cause them to enter division together.

- (c) Stimulation of growth may be caused by activities which facilitate the fall in inhibitor concentration. Perhaps one of the most characteristic properties of the epidermis, its adaptive response to the effects of hard work, may be produced by the dissipation of inhibitor resulting from friction and pressure.

An experiment by Bullough and Lawrence (quoted by Mercer) appears to have shown that inhibition rather than stimulation is the real growth controlling factor.

Another factor, which almost certainly plays a part in growth rates and through them morphogenesis, is competition between cells and organs and parts of organs for some essential requirement for cell growth.

9. The Life Cycle of the Epithelium.

There are various opinions about the detailed course of the process whereby the basal layer both maintains itself and supplies cells to form the differentiated layers. A common view is that there is some asymmetry in the division of a basal cell in the sense that two unlike cells result; one, referred to as a "stem cell", remaining attached and preserving a generalized character, the other free to move up and enter the stream of differentiating cells. This cell may also be capable of further divisions. Most observers agree now (according to Mercer) that nearly all dividing cells in epidermis are found in the basal layers and critical opinion holds that earlier observations were unreliable on the grounds that it is easy to be mistaken when examining oblique sections. It seems more likely that the widely accepted view, that cell differentiation and cell division are mutually exclusive, applies to the epidermis. Cell division appears to cease in cells in which cytoplasmic fibrils have commenced to accumulate, which, on the face of it, means that the cells' synthetic activities have swung over from producing materials needed for division to producing keratin precursors.

According to Silverman, the stratum germinativum and the stratum spinosum are together referred to as the germinal layers, since by mitotic division, they regenerate the epithelial cells lost at the surface.

Gargiulo et al (1961) found that mitosis occurred either in the basal-cell layer or in the deeper layers of the stratum

spinosum. Almost one-half of the total number of mitotic figures were in the basal-cell layers and the remainder were in the stratum spinosum.

Sicher reports that the rate of cell renewal may be expressed in the mitotic index, that is, the number of cells in mitosis of 1000 cells counted. The index increases with age. In individuals between 50-70 years of age, it is higher by 50% than in the 25-35 group. In the latter, it was calculated as 1.37, which is higher than the index in the epidermis. Presence or lack of a granular layer also influences the mitotic index. It is twice as high in the absence of the granular layer. There is however, no correlation of the M.I. to the variations of keratinization (Sicher).

McCarthy and Shklar, and most other authorities, assert that, as the width of the stratum corneum is increased, the stratum granulosum becomes wider and more obvious. If this statement were accepted unreservedly, it would allow one to deduce that the rate of cell renewal is inversely proportional to the degree of keratinization. This is in harmony with Mercer's view that cell division appears to cease when the synthesis of keratin precursors begins.

Gargiulo et al (1961) found an M.I. of 0.79 for a group of average age 25 years, and 1.69 for a group of average age 56 years. Thus, the average mitotic index was approximately twice as high in a group of older persons.

Two studies have been carried out in this field recently, in both instances mice being used in the experiment. Toto and Ojha (1962) estimated that the generation cycle of the oral epithelium in the tongue of mice was 100 hours (just over four days).

Beagrie and Skougaard (1963) found that the life cycle of the epithelial cells of the mouse from the basal layer to the surface was about 10 - 12 days duration in the oral epithelium, and only 1 - 5 days in the epithelial attachment.

Platt (quoted by Mercer), gives the renewal time for the epithelium for the tongue of the guinea pig as 8.4 days.

The mitotic rate is not the only factor which determines the thickness of the total epithelium. Clearly this depends on the renewal time, the time a cell takes to reach the surface and be shed. Ebling (in Mercer) showed, for example, that oestradiol, while increasing the number of mitoses four times, actually decreased the total thickness of the whole layer. The rate of differentiation and of exfoliation, about which less is known, influences the thickness of the intermediate layers and of the horny layer, respectively.

Gerson and Meyer (1964) found that the mitotic rate in buccal epithelium was 0.92 ± 0.24 , while there were no mitoses beyond the 10th row of cells and only 39% of divisions occurred beyond the 3rd row.

Hayes et al (1964) found no significant differences in

mitotic activity between boys and girls (ages 5 - 12). The spinous layer displayed the greatest mitotic activity, 66.14% followed by the basal layer, 33.86%. The average mitotic index decreased with an increase in cell density.

Silberkwett, Soni and Hayes (1963) found the average M.I. in children to be 0.545. The greatest activity occurred in deepest third of the spinous layer, then in the basal layer, middle and outer third of the spinous layer in that order. A change in the relation of metaphase/prophase ratio was noticed in inflamed gingival tissues. The average M.I. decreased with an increase in cell density.

Gargiulo et al (1961) list the following factors which will affect the daily variation in mitotic activity:-

- (1) Physical stimulation. Carleton found that continuous exposure of animals to light caused an alteration in the rhythmical periodicity of mitosis.
- (2) Hormonal. Bullough presented evidence that the oestrone levels in the female mouse effected the renewal rate of epidermal cells.
- (3) Temperature. Bullough considered that mitotic activity is high during lowered temperatures and low during higher temperatures.
- (4) Blood sugar levels. Bullough observed the existence of a direct relationship between mitotic activity and blood sugar levels.

- (5) Glucose oxidation. Bullough concluded that mitotic activity could be increased by stimulating the glucose oxidation. In order for this action to be effective, it had to occur just prior to prophase.
- (6) Stress. Bullough demonstrated that stress is an influencing factor in mitotic activity. The glucocorticoid hormones play a role in the antimitotic mechanisms, while testosterone was seen to induce mitotic activity.
- (7) Age. Marwah reported a marked increase in mitosis in older human gingival epithelium.

B. The Lamina Propria and Submucosa.

The lamina propria is a layer of dense connective tissue of variable thickness. The connective tissue is composed of dense bundles of collagen fibres, the characteristic cell being the fibroblast, a large spindle-shaped cell. These cells are normally sparsely scattered throughout the connective tissue. The deeper layers of the connective tissue are often referred to as submucosa, and the function is one of binding mucosa to underlying muscle or bone.

The papillae of the lamina propria, which indent the epithelium, carry both blood vessels and nerves. Some of the latter actually pass into the epithelium. The papillae vary considerably in length and width in different areas. The inward epithelial

projections between the papillae are described as epithelial pegs because of their appearance in sections. In reality, however, they form a continuous network of epithelial ridges. The arrangement of the papillae increases the area of contact between the lamina propria and epithelium, and facilitates the exchange of material between blood vessels and epithelium.

The submucosa consists of connective tissue of varying thickness and density. It attaches the mucous membrane to the underlying structures. Whether this attachment is loose or firm depends upon the character of the submucosa. Glands, blood vessels, nerves, and also adipose tissue are present in this layer. It is in the submucosa that the larger arteries divide into smaller branches, which then enter the lamina propria. Here they again divide to form a subepithelial capillary network in the papillae. The veins originating from the capillary network follow the course of the arteries. The blood vessels are accompanied by a rich network of lymphatic vessels. The sensory nerves of the mucous membrane traverse the submucosa. The nerves are myelinated but lose their myelin sheath in the mucous membrane before splitting into their end arborizations. Sensory nerve endings of various types are found in the papillae; some of the fibres enter the epithelium, where they terminate between the epithelial cells as free nerve endings. The blood vessels are accompanied by non-myelinated visceral nerve fibres that supply their smooth muscles; other visceral fibres supply the glands.

Histochemistry of the Oral Mucosa.

Information for this chapter has been gleaned from Mercer (1961), Sicher (1962), and McCarthy and Shklar (1964).

To a degree at the present time pure morphology has outrun knowledge of the chemistry and function of cell constituents. It is, for example, not always possible to state with certainty the chemical nature of the materials giving images in electron microscopes (Mercer).

In recent years, histochemical studies have shed some light on the metabolism of the oral mucosa. In the future, studies of enzyme distribution may serve to expand the knowledge of the nature of pathologic changes occurring in various diseases of the oral mucous membranes.

The following headings will be used:-

The Structure and Chemical Composition of Connective Tissue.

Epithelial Tissues and Derivatives.

Ground Substance in Normal and Abnormal Conditions.

Enzyme Histochemistry of the Oral Soft Tissues.

Microscopic histochemical techniques may be considered as extensions of routine staining procedures. True histochemical procedures, however, have a known chemical basis. Use of these techniques, only recently involved, enables direct microscopic visualisation of sites at which chemical reactions occur. The great advantage of microscopic histochemistry lies in accuracy of localization even at the cytological level.

Significant chemical constituents of the epithelial and connective tissue components of the oral mucosa are mucopolysaccharides, mucoproteins (glycoproteins), mucins (mucoids) and enzymes.

The Structure and Chemical Composition of Connective Tissue.

Connective tissue, which is derived from the mesenchyme, consists of specialised cells, fibres, and an amorphous ground substance.

The ground substance of the oral tissues stains with a number of histochemical procedures. Among these are techniques for the demonstration of carbohydrate groupings, the best known of which is the periodic acid-Schiff (pa-S) technique. The chemical basis of this method lies in the fact that periodic acid oxidises glycols with vicinal hydroxyl groups to aldehydes, which in turn are revealed as a coloured dye by leucofuchsin. This colourless reagent thus results in the formation of a red dye when reacted with aldehydes formed in tissues.

The pa-S method is believed to demonstrate under specific conditions the carbohydrate moiety of a glycoprotein complex.

Specific cell types appear to be implicated in the production of intercellular fibres and ground structure components. Fibroblasts are associated with the formation of collagenous fibres and mucopolysaccharides of the ground substance. The latter are hexosamine-containing polysaccharides. Ground substance also has proteins which contain carbohydrates, and these are referred to as glycoproteins. Mucopolysaccharides are of primary significance in

connective tissue and presumably act as binding and protective agents. The acid mucopolysaccharides of the ground substance are polymers containing acetylated amino sugars and hexuronic acids.

As previously indicated, glycoproteins are proteins containing protein, mucopolysaccharide, and in specific instances, hexuronic acid. Histochemically, glycoproteins stain with basic stains such as haematoxylin. They also stain metachromatically with dyes such as toluidin blue. This dye, which ordinarily stains tissue components blue, will stain certain tissue components a red-violet shade (metachromatic reaction). The glycoproteins have coiled chains of mucopolysaccharide which are attached to a central protein core. Each repeating disaccharide unit has two negative charges representing sulphate and carboxyl groups.

Many fine fibres (reticulin) associated with a ground substance form the basement membranes of epithelial surfaces. These fibres, which stain characteristically with silver, are also found in developing connective tissue. Biophysical and chemical studies have shown that the reticulin of the basement membrane is a collagen in close association with a carbohydrate and a lipid component.

Elastin is a relatively ill-defined protein characterised by its extreme insolubility. It is not considered to be an important constituent of the final product of tissue repair.

The nucleo-proteins also form an important class of conjugated proteins. The latter are combinations of protein and a

prosthetic or addition group. Nucleo proteins, for example, are composed of basic proteins in combination with nucleic acids. The latter are combinations of purine and pyrimidine bases, sugar, and phosphoric acid. Nucleoproteins constitute a major portion of nuclear protein. Desoxyribonucleic acid (D.N.A.) may be visualised by the so-called Feulgen method.

Recent concepts of ageing on oral connective tissue involve both morphological and biochemical changes. Morphologic changes take the form of decreasing cellularity and increased coarseness of collagenous fibres. Chemical changes involve decreasing mucopolysaccharide content, decreasing amounts of water, glycogen, hexosamine, tyrosine, and D.N.A. (Flieder, 1962).

Epithelial Tissues and Derivatives.

Epithelial glycogen is known to increase during inflammation and repair. Human attached gingiva, which is variable in keratinization, exhibits a variable glycogen content. On the other hand, the non-keratinized alveolar mucosa virtually always contains glycogen. Turesky et al (1951) found glycogen prominent in the cytoplasm of epithelial cells in zones of inflammation, but reduced or absent in the connective tissue.

Jolly (1964) found that in normal rats, the only convincing evidence of the presence of glycogen anywhere in the mouth was seen in the epithelium immediately adjacent to the margins of the experimental wounds made. In this location, there was evidence of intracellular deposition of considerable amounts of glycogen in the

spinous layer. At the wound margin this deposit generally occupied most or all of the width of the spinous layer, but further from the wound it tapered off until it occupied only the most superficial layers of cells, then finally disappeared.

Glycoproteins in human gingiva have been studied by means of the pa-S method. The reticular fibres comprising the basement membrane are discretely stained. The lamina propria exhibits reactive elements that include collagenous, reticular, and elastic fibres. The amorphous ground substance also exhibits a variable degree of staining in suitably fixed material.

Flieder's investigation of the chemical aspects of ageing on the oral mucosa revealed no significant changes in phospholipids, collagenous/non-collagenous nitrogen ratios with increasing age. Protein-bound hexoses and mucoproteins were reduced in amount with advancing age.

Klingsberg et al (1961) found that in the epithelium of rodents, glycogen was found to decrease in concentration with age, and was abundant in areas where there was no inflammation.

The nuclear contents of the basal cell are strongly basophilic and stain positively with the Feulgen technique for D.N.A. The nucleolus is Feulgen-negative, but gives positive tests for ribonucleic acid. The cytoplasm of the basal layer cells is strongly basophilic and rich in ribonucleic acid.

A decrease in the intensity of the Feulgen staining of the nuclei from the basal cells to the more superficial layer of the epithelium has been observed, followed by an increase in the

intensity of the stain in the flattened corneal cells of rats and hamsters (Cancellaro et al, 1961).

Phospholipids and polysaccharides have been demonstrated in the desmosomes, the polysaccharide possibly being located intracellularly as the adhesive "cement", and the phospholipid in the thickened cell membranes themselves.

Epithelial cells need a supply of energy for mitosis and division, for the synthesis of their specialised products and for keratinization when this occurs. Carbohydrates are the main source of energy, and these are probably supplied as glucose and stored as glycogen. Glycogen is not always found in the germinal layers, but may occur in the prickle cell layers (Mercer). The energy of the glucose probably becomes available in anaerobic glycolysis through the agency of the tricarboxylic Krebs acid cycle. Rothman (1954, quoted by Mercer) believes there may be other pathways specific to skin, while Snitzer (1961) carried out studies which supported the hypothesis that the tricarboxylic acid cycle and the Embden-Meyerhof pathway are functional in the rat oral mucosa.

The work of Bullough, and Bullough and Lawrence (quoted by Mercer) seems to indicate that glycogen is necessary for the energy required for mitosis, that diurnal variations in the mitotic activity are linked with rhythmic changes in adrenal activity, as the high adrenalin associated with muscular activity inhibits the mitotic activity of the epithelium.

From a consideration of the composition and reactions of proteins in general, and of the keratins in particular, the following kinds of bonds might be supposed to participate in the consolidation

of an insoluble protein (from Mercer):-

- (a) Hydrogen bonds, i.e., associations between neighbouring CO and NH groups mediated by the hydrogen atom.
- (b) Salt bridges, i.e., salt-like linkages formed between acid groups ($-\text{COOH}$) and amino groups ($-\text{NH}_2$).
- (c) Weaker and less well-defined forces referred to as Van der Waal's forces.
- (d) Disulphide bridges ($-\text{S}-\text{S}$).
- (e) Other bonds have been proposed, e.g., between phenolic OH groups and acid groups, but are not known to exist for certain.

In keratin the covalent disulphide bonds appear ultimately to prohibit solution, and next in importance on account of their number are the hydrogen bonds. These appear particularly to influence the dry hardness and extensibility. The H-bond may sustain the structure in the early stages of keratinization before the closure of the disulphide bonds.

In rat and hamster oral mucous membrane, Cancellaro et al found a strong reaction in the intercellular substance for protein, sulphhydryls, and disulphides, in contrast to a weak reaction in the connective tissue ground substance. They also found that the parakeratotic zone of one-day old animals stained identically with the keratinized area of the older animals for sulphhydryl, disulphide and protein groups. They therefore suggested that an alteration in protein groups, other than sulphhydryl and disulphide, are involved in the transformation of parakeratotic to completely keratinized tissue.

Ground Substance in Normal and Abnormal Conditions.

Alterations in ground substance in various physiological and pathological states have been stressed by Gersh and Catchpole (quoted by Sicher). One concept suggests that depolymerization of mucopolysaccharides is reflected in a number of physiologic and pathologic changes. It is also possible that enzymatic hydrolysis of the protein component of the polysaccharide complex may be responsible for tissue alterations.

Turesky et al (1951) found that glycogen and ground substance normally present in the connective tissue of the gingivae is markedly reduced in chronic gingival inflammation.

Enzyme Histochemistry of the Oral Soft Tissues.

Enzymes may be considered as living molecular catalysts. They are proteins which catalyse a series of vital complex chemical reactions at body temperature. Among these are synthetic, hydrolytic and molecular transfer processes.

Alkaline Phosphatase.

In human gingiva, alkaline phosphatase has been observed in the capillary endothelium of the lamina propria. Phosphatase activity ascribed to collagenous fibres may be genuine or may be a staining artifact. On the other hand, polymorphs exhibit a genuine activity.

The oral epithelium of the rat exhibits an increased alkaline phosphatase activity during the oestrous cycle, which also can be correlated with phosphatase changes in the vaginal epithelium.

Although alkaline phosphatase may be related to keratinization, its precise role cannot be ascertained until more is known about the function of this enzyme.

Turesky et al (1951) found that the alkaline phosphatase content is increased in chronically inflamed areas of the gingiva.

Cabrini and Carranza (1951) stated that the hydrolysis of nucleic acid showed the presence of alkaline phosphatase in the gingival epithelium, especially in the basal layers.

Phosphamidase.

Phosphamidase is known to hydrolyse the phosphorus nitrogen bond (Burstone, 1961).

Acid Phosphatase.

The entire epithelium is moderately reactive, with notable reactivity in the upper part of the stratum spinosum and in the stratum granulosum. Susi (1964) confirmed the presence of acid phosphatase in the stratum granulosum. The stratum corneum is nonreactive or mildly reactive. In the corium there is a weak reaction in collagen fibres. Connective tissue cells are non-reactive. High activity is seen in macrophages in chronic inflammation. Burstone (1961) states that acid phosphatase hydrolyses phosphoric acid esters at an acid P.H.

Santis et al (1964) state that evidence has been presented indicating that acid phosphatase is related to the process of keratin formation.

Nonspecific Esterase.

A reaction is usually observed in the more superficial layers of the gingival epithelium, including the keratinizing zone.

Santis et al report that nonspecific esterases are involved in numerous enzymatic processes. They are utilised in the metabolism of short carbon chain fatty acids, in various acetylative processes and in the metabolic processes linking the glycolytic and citric cycles.

In the connective tissue, there is intense activity in leucocytes and also in fibroblasts. There is a weak reactivity in the collagen fibres.

Aminopeptidase.

There is reactivity only in the stratum germinativum of epithelium. The connective tissue presents moderate reactivity of collagen fibres and fibroblasts. Polymorphs also exhibit activity.

B-Glucuronidases.

This term represents a group of enzymes capable of hydrolyzing the B-glycoside linkage of a number of naturally occurring and synthetic glucuronides. The enzyme is believed to have three roles:- the conjugation of steroid hormones, the hydrolysis of conjugated glucuronides, and a role in cellular proliferations.

The epithelium presents moderate activity in the stratum germinativum. The stratum spinosum is relatively free of activity,

but the stratum granulosum presents intense activity. The stratum corneum is moderately reactive. In the connective tissue, fibroblasts and endothelial cells are positive for activity.

B-D-Galactosidase.

The entire epithelium is nonreactive, or presents low to moderate activity, with some decrease in activity in the stratum granulosum and stratum corneum.

According to Santis et al, B-D-Galactosidase is one of the glycosidases - a group of enzymes that are not restricted simply to hydrolysis of their respective substrates, but catalyze the transfer of glycosyl groups by which short-chain oligosaccharides are formed.

Cholinesterase.

There is intense activity in the nerve fibres of the connective tissue.

Cytochrome Oxidase.

A major respiratory enzyme, widely distributed in tissue with high metabolic activity (Burstone, 1961). With the application of new histochemical techniques, the precise sites of oxidase activity in normal and inflamed gingiva have been observed. Cytochrome oxidase activity was found to be low and was observed in the crevicular epithelium, epithelial attachment, and basal cell layer of the free and attached gingiva.

Succinic Dehydrogenase.

Sicher, and McCarthy and Shklar, differ on the distribution

of this enzyme in the stratum germinativum - the former states that the basal cell exhibits the highest reducing activity, while McCarthy and Shklar say that the stratum germinativum presents little activity. They later describe moderate activity in the stratum spinosum and a relatively strong reaction in the stratum granulosum, while the stratum corneum is nonreactive, as is the connective tissue.

Klingsberg et al found the maximum dehydrogenase activity, in the oral epithelium of rodents, in the basal cells, with diminishing activity in the deeper layers of the stratum Malpighii. Susi (1964) confirmed these findings.

Gibb and Bradley (1964) found that significant differences appear between normal and inflamed gingival tissue, especially in the female, when succinic dehydrogenase and diaphorase enzyme systems are studied quantitatively.

Ribonuclease and Deoxyribonuclease.

Droust and Haruko Amano (1964) confirmed the presence of high ribonuclease and deoxyribonuclease activities beneath the horny layer in human epidermis. Apparently the high nuclease activity seen in the keratogenous zone would be responsible for the disappearance of nuclear and cytoplasmic nucleic acids occurring in that region. This view is further supported by the fact that decreased deoxyribonuclease activity of the keratogenous zone is associated with retention of nuclei in parakeratotic horny layers.

These authors state that perinuclear regions of oval,

crescent or ring shapes were found to be the sites of ribonuclease and deoxyribonuclease activities in the Malpighian layer, and the nuclease activities in these sites was observed to increase as the cells move away from the basal layer. In electronmicrographs, the perinuclear zones appear as regions free of tonofibrils. They contain the Golgi apparatus, the centriole and possibly the lysosomes; endoplasmic reticulum, mitochondria and pigment granules are found in perinuclear regions, as well as in other parts of the cytoplasm. Zelickson described R.N.P. granules on the cytoplasmic surface of the nuclear membrane, on the cytoplasmic surface of the endoplasmic reticulum, and these act as building blocks which synthesize different proteins. These accumulate in the endoplasmic reticulum, pass to the Golgi Complex for modification, and thence to the exterior of the cell.

Changes taking place in the nucleases and nucleic acids at the upper limit of the Malpighian layer appear to be closely connected. Abrupt changes occur in both the distribution of nuclease activity and the nucleic acid content of cells at the limit between the non-keratinized and keratinized cell layers. The nucleases give a strong band reaction at that level instead of showing a multifocal distribution, and nucleic acids rapidly disappear in the same area. A sudden change in the intracellular distribution of the enzymes is probably responsible for the rapid digestion of deoxyribonucleic acid and ribonucleic acid which accompanies other radical changes occurring in the transitional

cells. Nucleases thus seem to play an important role in the keratinization process and disturbances in nuclease activity or distribution may likely result in abnormal keratinization.

Droust and Haruko Amano are of the opinion that their research does not support the view that a positive relationship exists between ribonuclease or deoxyribonuclease activity and cell proliferation.,

KERATINIZATION.

Introduction.

Preparation for the Keratinization Process in the Stratum Germinativum.

Keratinization in the Stratum Spinosum.

Keratinization in the Stratum Granulosum.

Keratinization in the Stratum Corneum.

Differences between Keratinizing and Non-Keratinizing Epithelium.

Summary.

Present State of Knowledge of the Chemical Structure of the Keratins.

Further aspects of the Keratinization Process.

Material for this section has been condensed from
Zelickson and Hartmann (1963) and Mercer (1961).

Introduction.

An idealized, generalized, keratinizing epidermis might be said to consist of at least the following layers:-

(a) a germinal layer of cells whose proliferation maintains the entire cell population; (b) a differentiating layer in which the protein is synthesized and keratinized; and (c) the dead and hardened layer. If the tissue is to be of constant average thickness, the cells formed over a given time must equal those lost by exfoliation in the same time. The thickness varies with the total number of cells in each of the layers, and structures with a variety of properties may be produced by variations in the proportion of differentiating cells and hardened cells. The ease of exfoliation determines the thickness of the horny layer, and obviously a thick, hard layer will result if the exfoliation is slowed down (Mercer, 1961).

Rothman described keratinization as the transformation of living epithelial cells into horny material, while Flesch considered it a consolidation of fibrous precursors of keratin. Unna in 1925 suggested two types of protein in epidermal cells - a fibrillar and a globular, lying between the fibrous elements. Astbury studied x-ray diffraction spectograms of mammalian keratin and found that ordinary keratin showed an alpha-keratin pattern, but when stretched produced a beta-keratin pattern. Montagna has emphasized that x-ray diffraction patterns for alpha-keratin are obtained whenever tonofilaments are present, whether in the basal layer or the stratum

corneum; therefore the tonofibrils are probably responsible for the characteristic diffraction pattern of alpha-keratin seen in the epidermis.

Barnett and Seligman and later Montagna, using a histochemical technique, studied the localization of sulphhydryl (thiol) and disulphide groups in human epithelium. They showed that the - S H groups were present throughout the epidermis, being greatest in number in the basal cells and least in the stratum granulosum and stratum corneum. Disulphide groups, though at a minimum, were present in all layers of the epidermis. Flesch pointed out that there is no specific evidence that sulphur-containing amino acids are attached to the fibrous pre-keratin and the possibility exists that they might be localized in a non-fibrous precursor surrounding the fibrous unit. It was his opinion that keratinization is a two-stage process, the first step being the formation of a fibrous pre-keratin, poor in sulphur, not as solid as keratin, and occurring in the lower levels of the stratum malpighii. Second is the combination of this precursor with a sulphur-containing protein, thus forming a consolidated keratin with disulphide bridges.

Selby, in a study of the fine structure of the epidermis, pointed out that tonofilaments are present throughout the epidermis. She described the stratum corneum as a structureless mass with no apparent internal composition. Others suggested that keratohyalin granules may form directly into keratin fibres, or that keratohyalin is a substance which is precipitated from the cytoplasm and deposited around tonofibrils.

Preparation for Keratinization Process in the Stratum Germinativum.

The plasma membrane of the basal cell is well defined, and numerous desmosomes dot its surface. These are specialized areas of apposed cell walls with several lamellae embedded in an opaque material located between them. The desmosomes facing the dermis (half-desmosomes) are also noted to be apposed by several dense lamellae which lie between the basal cell and the basement membrane. The basal cell contains numerous cytoplasmic tonofilaments approximately $50 \text{ \AA}$ in diameter (Mercer states that the tonofilaments in the basal cells are $100 \text{ \AA}$ in diameter), all oriented in the same plane, and perpendicular to the surface. Bundles of tonofilaments stream into the cytoplasm from their attachment point at the cytoplasmic surface of the desmosomes, but do not cross from cell to cell. On cross section, some of these osmiophilic appearing filaments are found, with high magnification, to consist of a non-staining central unit or filament which is coated with an osmiophilic substance, thus having a "tube-like" structure.

Keratinization in the Stratum Spinosum.

The cells at this level differ from those in the stratum germinativum, in that the tonofilaments are not well oriented. The filaments also gradually increase in diameter until they reach a diameter of approximately $100 \text{ \AA}$. Cross sections of bundles of filaments in this region again show the unstained filaments coated with an osmiophilic material. According to Brody, individual tonofilaments can be observed only in the stratum germinativum. In

the stratum spinosum, the tonofibrils appear as compact structures in which individual filaments can no longer be distinguished. He notes that the filaments which are loosely arranged in the stratum germinativum become densely aggregated in the stratum spinosum.

Keratinization in the Stratum Granulosum.

Characteristic electron-dense keratohyalin material appears in significant amounts in this region. Keratohyalin granules are located throughout the cytoplasm, and increase in size and number as the cells approach the stratum corneum. With higher magnification one discerns an internal structure in some of the "granules", apparently due to the presence of filaments within the keratohyalin material. Usually the keratohyalin material is closely associated with the tonofilaments. The keratohyalin is distinctly more electron-dense than that material which ensheaths the filaments in the lower levels, and in some areas the keratohyalin material is deposited over this original material. Although there is a significant relationship between the filaments and the keratohyalin material, no such relationship is noted between the keratohyalin and the nucleus, nucleolus, or their membranes. Thus the keratohyalin is apparently cytoplasmic in origin and is deposited around and between the tonofilaments.

Brody found the intensely stained parts of the cytoplasm to always be located on the tonofibrils. He also suggested that the intensely stained regions qualified as keratohyalin granules by their shapes and their gradual increase in size during keratinization.

The conventional view that keratohyalin granules are discrete cellular inclusions clearly separated from the tonofibrils, has been based, he felt, on light microscopic investigations. Results obtained by electron microscopy suggest that this is incorrect, and that at least most of the keratohyalin material corresponds to regions in the tonofibrils which show particular staining properties. Zelickson agrees.

The keratohyalin material is surrounded by dense particles of R.N.P. approximately $125 \text{ \AA}$ in diameter, and at some points there is a suggestion that these particles are incorporated into the keratohyalin material.

Mercer (1961) supports the view that the keratohyalin granules are a direct precursor of the fibrous keratin, so that the final fibrillar contents of the stratum corneum would seem to be derived from two sources: (a) a small early contribution of fibrils and (b) a larger amount produced later by the transformation of the non-fibrous precursor, keratohyalin.

Eichel et al (1964) consider that triphosphopyridinenucleotide and diphosphopyridinenucleotide dependent reducing systems may be the prime mechanism for the reduction of the prekeratin (tonofilaments and keratohyalin) in the stratum granulosum. They picture the process as follows:-

- (1) The high reductive capacity of the keratohyalin granules may reduce the disulphide bonds of the tonofilaments grouped about the granules.

- (2) In this fashion, the tonofilaments and keratohyalin are combined, possibly held together by hydrogen bonds, and it is in this zone of epithelium that the high concentration of sulphhydryl groups is disclosed.
- (3) The tonofilaments then, may be regarded as the normally occurring acceptor of the hydrogen produced by the reductases that may, but need not necessarily, be present in the keratohyalin granule.
- (4) Once the tonofilaments and keratohyalin combine, the terminal oxidising system converts the excess sulphhydryl groups back to the disulphides with the formation of keratin.
- (5) In this manner, keratohyalin may be regarded as the key source of hydrogen, a probable essential requirement for the process of keratinization to occur and/or
- (6) keratohyalin and the tonofilaments may be regarded as hydrogen carriers between the reductases of the stratum granulosum and the keratin of the keratin layer.

Keratinization in the Stratum Corneum.

The stratum corneum is usually 4 - 8 cells thick, and the cells are flat and undulating with their long axes parallel to the surface. The final pattern of "filament embedded in matrix" is seen only in the cytoplasm of the cells of the stratum corneum. This appearance is the result of the presence of numerous round, unstained filaments (100 Å) embedded in an electron-dense cement substance.

The filaments are oriented parallel to the surface. In sections, some are cut in a longitudinal pattern while others, cut in cross-section, have a "tube-like" appearance. In some cells, the typical structural pattern of development of the stratum corneum is irregular, with individual cells at lower levels at times being in a more keratinized state than some cells nearer the surface.

In the stratum corneum, the cell membrane changes in appearance. In the lower levels, it consists of two electron-dense layers separated by a less opaque region; in the upper levels the two layers fuse, forming a single, thick, opaque membrane (approximately 125 $\bar{A}$ thick). The desmosomes also change in a characteristic manner. An osmiophilic body (200 $\bar{A}$ thick) forms in the intercellular space between the apposed cell membranes which make up the desmosome. The intercellular body is separated from the adjacent cells by a less opaque zone. When the cell is shed, the break in the desmosome occurs in one of the less opaque zones rather than through the "body". Often these intercellular bodies are quite extensive in length rather than being limited to the "desmosome" region.

Differences between Keratinizing Epithelium and Non-Keratinizing Mucosa

are that in the mucosa:-

- (1) Fewer tonofilaments (50 $\bar{A}$) are present, and they do not go on to form the wider filaments (100 $\bar{A}$) seen in keratinizing epithelium.
- (2) Fewer desmosomes (or nodes of Bizzozero) are present, and those seen do not change internally as the cell nears the surface.

- (3) The cell membrane does not change structurally as the cell nears the surface.
- (4) Keratohyalin "granules" are not formed; therefore no transitional cell layer is seen.
- (5) The stratum corneum is absent and no keratin is formed.

Summary.

A combination of events is important in the morphologic process of keratinization. First, tonofilaments must be present. Secondly, a cementing substance is needed to bind the filaments. Both are needed in adequate amounts for normal keratinization to take place.

As is shown in electronmicrographs, tonofilaments are present in great numbers in basal cells, and thus at the onset of the keratinization process. Often they are unstained by the osmium used in fixation and thus contain very little, if any, sulphur, a finding which corresponds to the biochemical evidence for low sulphur content of fibrous epidermal protein. The filaments are coated with an osmiophilic sheath, and this substance which attracts the osmium probably contains most of the sulphur groups. The tonofilaments increase in diameter from approximately 50 $\bar{A}$ to 100 $\bar{A}$ as the cells move upward in the epidermis. In the higher epidermal levels the wider filaments are also unstained and ensheathed in an osmiophilic material. The final structure of the horny cell therefore consists of unstained filaments cemented together in a random manner by electron-dense material.

The second important component in the keratinization process is the matrix or cementing substance. Because it reacts strongly with osmium tetroxide, it probably contains sulphur. As was mentioned, some osmiophilic material is present in the basal layer and ensheaths the filaments. More cement substance is laid down in the stratum granulosum. This keratohyalin material is extremely electron-dense, much more so than that material which coats the filaments in the lower levels of the epidermis. It ensheaths the tonofilaments as it is laid down and brings the filaments together in larger and more compact bundles.

Mercer comments that it is sometimes said that keratinization is a degenerative phenomenon, a consequence of poor nutrition, of desiccation or other deleterious factors. That this is not true is shown by observations on cells cultivated in vitro where conditions are under closer experimental control. Skin cultivated in vitro readily undergoes keratinization with the production of histologically-normally keratinized cells. The same sequence of histochemical events as in vivo occurs, and the product is also birefringent. Keratinization in vitro does not take place in all types of cell regardless of origin, nor is it initiated or promoted in epithelial cells by poor nutritive conditions or low oxygen tension or lower temperature. The phenomenon is properly to be regarded as the final stage of an intrinsic differentiation of epithelial cells, as well as adapted to the function of the tissue as in, for example, the production of collagen by fibrocytes. This not to say, of course,

that it cannot assume an abnormal, perhaps degenerative form. That the epithelial habit with the potentiality of keratinization is a fundamental type of cell behaviour is shown by the fact that it is one of the forms to which cells revert when cultured in vitro for some time.

Cancellaro et al (1961) found parakeratin in one-day-old rats and hamsters in areas which showed keratin in older animals.

McFall and Kraus (1963) in their study on human foetuses, imply that keratinization occurs between the tenth and twentieth weeks on the palate and gingiva, but do not specify whether parakeratin or keratin occurs. The illustrations show nuclei in the stratum corneum.

The Present State of Knowledge of the Chemical Structure of the Keratins.

(After Mercer, 1961).

The chemical composition and constitution of a protein may be considered established when the following are known:-

- (a) the number of separate polypeptides composing the molecule, and the nature of any covalent cross-linkages uniting them;
- (b) the amino acid sequence in each of the polypeptides;
- (c) if a prosthetic group is present, its relation to the polypeptide moiety.

Complete solutions to (a) and (b) are available for three proteins; for the keratins, which are far more complex, there is no immediate prospect of even partial solutions. There is no evidence, however, to show that there is anything in the nature of a prosthetic group to complicate the position further. Essential information

concerning a protein is provided by a knowledge of its total amino acid composition and its end-group composition, i.e., the groups which terminate the main polypeptide chains. For many keratins there are adequate, though not complete, determinations of both end-group and total amino acid composition from which may be inferred a general picture of the overall chemical reactivity of the molecular complex.

Mercer gives Ward and Lundgren's figures for the reactive groups of epidermal keratin (gramme equivalents per 10^5 g. of keratin); free carboxyl 27-83, amide 83, carboxyl plus amide 110-166, phenolic hydroxyl 19-32, aliphatic hydroxyl 186, total basic 59-120, amino 21-47, aromatic nuclei 23-38, half-disulphide 19-32, oxidizable 207-280.

He also gives Ward and Lundgren's figures for the composition of the keratin of human epidermis as follows (g. of component from 100 g. of dry keratin):-

Nitrogen, total.	14.2 - 15.5
amide nitrogen	1.16
sulphur	1.9
Amino acids with hydrocarbon side chains;	
glycine	6.0
alanine	-
valine	4.2
leucine	8.3
isoleucine	6.8
phenylalanine	2.8
proline	3.2

Hydroxy;

serine	16.5
threonine	3.4
tyrosine	3.4

Acid (free and as amide);

aspartic	6.4
glutamic acid	9.1

Basic;

arginine	5.9
lysine	3.1
hydroxylysine	-
histidine	0.6

Heterocyclic;

tryptophan	0.5
------------	-----

Sulphur-containing;

cystine	2.3
methionine	1.0

Further Aspects of the Keratinization Process.

Klingsberg et al (1961) found that a possible correlation between glycogen deposition and the rate of formation of keratin in rodents is suggested by its presence in greater amounts where tissue abrasion during mastication may occur - that is, on the palate or on the oral surface of the crestal gingiva. Glycogen was found to be most abundant in areas of diminished succinic dehydrogenase activity.

Papic and Glickman (1950) found no correlation between keratinization of the gingiva and various stages of the menstrual cycle.

Montgomery (1951) showed that age, sex and the menstrual cycle did not significantly alter the pattern of keratinization of the oral mucosa, and also that the oral p H has no noticeable effect on the staining properties of the exfoliated cells.

Cancellaro et al (1961) found a decrease in intensity of the Feulgen staining of the nuclei for D.N.A. from the basal cells to the more superficial layers, followed by an increase in the intensity of the stain in the flattened corneal cells. They found, too, that the staining reaction for R.N.A. decreases in intensity from the basal layer of the epithelium to the stratum corneum.

Rothman (1954) states that before keratinization ensues in the keratogenous zone (the granular layer and above) there is a sudden increase in the sulphhydryl content (cf. Eichel et al's study). As the keratinization is complete, the sulphhydryls disappear.

Rothman comments on the strong hydrogen bonding in keratins (as does Mercer), and also that the disulphide bridging process is catalyzed by copper. All other biologic agents which modify keratinization, such as vitamin A or oestrogenic hormone, modify the potentialities of germinative epithelial cells by metoplasia, and thereby influence the production of keratin indirectly, but copper acts the most directly on the cornifying cells at the moment of keratinization.

The glycogen which accumulates in the keratogenous zone disappears with the onset of keratinization. The energy liberated by glycogenolysis is thought to be utilised for disulphide closure.

McFall and Kraus (1963), in referring to their study on human foetuses, state that the apparent decrease in glycogen concomitant with the development of keratinization during the foetal period seems to agree with the adult finding, which shows an inverse relationship between glycogen and keratinization of epithelium.

Trott and Gorenstein (1963) studied mitotic rates in the rat, and found that the hard palate, cheek, crevicular gingiva and attached gingiva had daily mitotic rates of 6.76, 7.25, 6.21, and 2.27% respectively. They feel that the low figure for the attached gingiva was because "it was not as much under the influence of function as the hard palate and cheek". (This reviewer takes the direct contrary view that greater function stimulated greater keratin production, resulting in reduced mitotic activity in the attached gingiva.)

Bennett et al (1964) carried out a histochemical study of glucose - 6 - phosphate dehydrogenase in human oral mucosa and found:-

1. In non-keratinizing mucosa, G - 6 - P.D. is low in activity, being present in highest concentration toward the stratum germinativum.
2. In keratinizing mucosa, the activity is low in the stratum germinativum, and increased in the upper spinosum.

Intermediate patterns of activity are found in para-keratosis and incomplete parakeratosis. In the given disease categories, the activity is variable; however, a consistent enzyme pattern related to the degree of keratinization is seen. It is concluded that the basic enzyme pattern is related to the degree of keratinization; however, both degree and pattern of activity may be modified by disease states.

Pathologic Processes Involving the Oral Mucosa.

Introduction.

Histopathology of Mucosal Lesions,

(a) Epithelial Alterations;

Hyperkeratosis

Parakeratosis

Acanthosis

Spongiosis

Hydropic degeneration

Acantholysis.

Extension of rete pegs

Pseudoepitheliomatous hyperplasia

Dyskeratosis

Carcinoma in situ.

(b) Connective Tissue Alterations;

Inflammatory infiltration

Hyperplasia

Collagen degeneration

Vascularity.

(c) Proliferation of Epithelial Cells under Stimuli.

Introduction.

The oral mucous membrane reacts to various forms of injurious stimuli and develops lesions if the stimuli or noxious influences are sufficient to produce disease. Lesions occurring on the oral mucosa may be of several distinct clinical forms, and a similar type of gross lesion may be produced by a large variety of entirely different agents (McCarthy and Shklar, 1964).

In the majority of tissues, the vascular supply is diffusely distributed throughout, and injury or irritation thus provokes an inflammatory response of such a degree as to dominate the picture completely. Only in those tissues where the blood supply is not so closely integrated does the associated inflammatory response remain relatively separate, thereby allowing the tissue cells to contribute significantly to the histologic and clinical picture without being obliterated by the inflammation. Epithelium is one such tissue because, despite the fact that it is adjacent to the underlying vascularised connective tissue stroma, the inflammatory response is largely limited to this site. Thus, the irritation or injury experienced by the epithelial cells themselves produces a picture which can be clearly seen, both clinically and microscopically, on the epithelial surface (Spouge and Diamond, 1963).

The basic response of any tissue cell to injury is strictly limited. Where this damage principally involves nuclear function, a mild stimulus may result initially in proliferation of the individual cell or tissue; if more severe or prolonged, it may bring about their

ultimate degeneration and death. In addition, the predominantly cytoplasmic functions may be affected, and thus may alter the degree of differentiation of the cell, producing a metaplasia in the tissue.

The appearance of gross lesions is generally insufficient for adequate diagnosis. The distribution of the lesions, clinical history, and post history may be of some diagnostic value, but microscopic examination is often necessary. The microscopic features may be nonspecific in nature, so that the diagnosis is dependent upon an evaluation of both clinical and microscopic features.

Histopathology of Mucosal Lesions. (After McCarthy and Shklar, 1964).

(a) Epithelial Alterations.

Hyperkeratosis. This condition is characterised by a widening or thickening of the stratum corneum. It results in a white lesion clinically. The opacity and whiteness of the lesion are related to the amount of keratinization.

Silverman, Renstrup and Pindberg, (1963) use the term hyperorthokeratosis, describing it as a pathological condition in which superficial layers of epithelium are cornified, contain no nuclei, and appear homogeneous and strongly acidophilic. In areas where orthokeratosis normally occurs, it has to exceed the expected thickness to be called hyperorthokeratosis.

Parakeratosis. In parakeratosis there is a persistence of nuclei in the stratum corneum. In parakeratosis, the stratum corneum presents varying degrees of thickening,

so that it may equal that of hyperkeratosis. This reaction may represent an abnormally rapid keratinization process. Parakeratosis usually results in a white clinical lesion.

Silverman, Renstrup and Pindborg (1963) use the term hyperparakeratosis, describing it as a pathologic condition in which the outer cells and cell layers are flattened, contain pyknotic nuclei and exhibit a marked acidophilia. In areas where parakeratosis normally occurs it has to exceed the expected thickness to be called hyperparakeratosis. (This seems to be semantically correct - Reviewer).

There is no agreement in the literature as to the significance of parakeratosis. It has already been mentioned that Sicher (1962) found parakeratosis in 50% of normal gingiva, and only 15% full keratinization. Spouge and Diamond state that, in the absence of additional signs of pathologic change, it would not be taken as necessarily indicative of disease.

Cahn et al (1962) say that the significance of parakeratin as opposed to keratin is not known; nor is it clear which reaction is more normal or more sinister. They give the incidence in their study of white lesions as being 39% keratin and 61% parakeratin. In some instances keratosis and parakeratosis were seen side by side, changing abruptly under some unknown influence from

one to the other, with the accompanying appearance and disappearance of the keratohyalin layer.

Cooke (quoted by Stones, 1962) points out that where the epithelium is normally keratinized, then hyperkeratosis is seen, but where non-keratinized epithelium normally exists, either parakeratosis, or hyperkeratosis, or both alongside each other, may be seen in the early lesion. Stones continues with the observation that hyperkeratosis is seen in the well established lesions, with a thick keratin layer and a well marked granular layer. In parakeratosis, there is absence of the granular layer.

Bernier (1959) states that in parakeratosis the cells show imperfect cornification and cohesion. This reaction results from the rapidity of the cornification process, the cells being produced so rapidly that the transformation to horn cells (keratin) is incomplete. Its outstanding features are persistence of the nuclei within the horn cells, incomplete production of keratin, and occasional oedema, causing the cells to be slightly swollen.

Waldron and Shafer (1960) describe alternating areas of hyperkeratosis and parakeratosis in the same section, and state that the significance of this finding is not known.

Again, Turesky et al (1961) say that it is difficult

to account for the structural and histochemical differences between the hyperkeratotic and parakeratotic lesions other than in terms of the rate of epithelial proliferation. In the parakeratotic lesions the overall thickness of the epithelium was invariably greater, and hyperplastic epithelial pegs occurred more frequently than in the hyperkeratotic lesions. They feel that in parakeratotic lesions the rapidity of cellular activity does not permit sufficient time for the structural and chemical differentiation required for hyperkeratosis.

Acanthosis. In this condition there is a widening of the stratum spinosum. Acanthosis signifies epithelial hyperplasia. It may exist with or without hyperplasia.

Spongiosis. This term is used to signify intercellular oedema of the epithelium. Intercellular bridges of the stratum spinosum become more prominent.

Bernier (1959) comments that oedematous changes within the corium may cause this reaction within the epithelium, it in turn being partially responsible for disturbances in cornification, resulting in parakeratosis.

Hydropic Degeneration. Because of oedema and degeneration of cells of the stratum germinativum, the nuclei are replaced by clear spaces. The entire cells gradually degenerate, and the epithelial-connective tissue boundary is poorly defined.

Acantholysis. This lesion is caused by a separation of cells in the stratum spinosum so that an intraepithelial split occurs, leading to the formation of an intraepithelial vesicle.

Extension of rete pegs. Because of the elongation, the rete pegs extend into underlying connective tissue.

Pseudoepitheliomatous hyperplasia. In this condition, there is extensive downgrowth of rete pegs, usually accompanied by marked acanthosis, so that a tissue pattern similar to carcinoma is seen. However, the cells are essentially normal in size, shape and chromatically.

Dyskeratosis. This condition shows an abnormal orientation or development of epithelial cells. Dyskeratosis, or malignant dyskeratosis signifies a premalignant epithelial change, and the cells present such alterations as hyperchromatism, changes in polarity, and increased nuclear size with prominence of the nucleoli. Increase in mitotic figures may also be noted.

Almost every author studied uses the above meaning for dyskeratosis as described by McCarthy and Shklar, that is, they include under dyskeratosis, hyperchromatism, changes in polarity and increased nuclear size, mitotic figures, and also formation of keratin in the prickle cell layer.

Hansen (1959), for instance, gives the criteria for dyskeratosis as some or all of the following:-

1. Pleomorphism - the variation in size and shape of the cells.
2. The loss of polarity, e.g., the formation of keratin deep in the epithelium near the basal layer.
3. Prominent nuclei.
4. Prominent nucleoli.
5. Nuclear hyperchromatism.
6. Vacuolization of nuclei and cytoplasm.
7. Increased and abnormal mitotic activity.

Shafer and Waldron (1961) add the following alterations in the nuclear-cytoplasmic ratio, poikilocarynosis - division of nuclei without division of cytoplasm, and basilar hyperplasia.

Incidentally, distinction must be made between "malignant dyskeratosis", characterised by these features, and so-called benign dyskeratosis, such as seen in Darier's disease and molluscum contagiosum.

Pindborg et al (1963) use the term "epithelial atypia" for a disordered maturation of the epithelium, giving the following criteria, of which one or more are present:-

Irregular epithelial stratification,

Increased density of basal layer or spinous cell layer, or both,

Thickening of basal cell layer,

Increased number of mitotic figures,

Alterations in nuclear-cytoplasmic ratio,

Loss of polarity of cells, hyperchromatism,

Nuclear atypism.

Abnormal mitoses are never observed in epithelial atypia and the basement membrane is always intact.

Bernier (1959) is of the opinion that, since dyskeratosis is an acknowledged disturbance in maturation, it is reasonable to assume that dyskeratosis involves areas of epithelial tissue, not only single cells, as the involved single cells must be related to the presence of abnormal cell units in their immediate environment .

Two notable exceptions in using the term dyskeratosis are Orban and Wentz (1960) and Rushton and Cooke (1963). Both of these authorities refer to individual cell dyskeratosis and describe the other changes separately.

Spouge and Diamond (1963) also describe dyskeratosis as the production of keratin in abnormal situations (in the stratum spinosum) and do not use the term to describe mitotic figures, etc.

Dyskeratosis, meaning "disordered formation of keratin", cannot be applied logically to changes which probably do not play any direct part in the keratinization process - mitotic figures, increased nuclear size and prominence of the nucleoli. Electron microscopy seems to indicate that keratin production is a function of the cytoplasm of cells, and therefore could only be indirectly

affected by nuclear changes. For these reasons, the term "dyskeratosis" appears to be unsuitable to convey the true meaning of all the ten changes in the criteria described by Shafer and Waldron. It is this reviewer's opinion that the disorder in the production of keratin is incidental to, and one facet of, the changes which take place in the epithelium of the oral cavity when the more advanced white lesions develop. Pathologic reports would convey a more precise meaning if the use of the term "dyskeratosis" was confined to changes which appear to be directly related to the keratinization process, while other changes were individually described.

Carcinoma in situ or Intraepithelial Carcinoma.

A definitive distinction cannot always be drawn between dyskeratosis and carcinoma in situ. Certain of the criteria listed for dyskeratosis, when encompassing an even greater area than could be considered focal, when extremely severe in degree, or when exhibiting "top to bottom" change, particularly with respect to basilar hyperplasia, must undoubtedly be diagnosed as carcinoma in situ, provided of course, that it has not progressed to the point of true invasion of the connective tissue (Shafer, Hine and Levy, 1963).

Two other terms, erythroplasia of Queyrat and Bowen's disease, are sometimes given to lesions which

microscopically represent carcinoma in situ.

Clinically, erythroplasia of Queyrat is limited to the mucous membranes and appears as a red velvety lesion.

Bowen's disease, on the other hand, is seen primarily on the skin. Since microscopically both of these in essence represent carcinoma in situ, the terms are superfluous (Bhaskar, 1961).

Bernier (1959) objects to the use of the term carcinoma in situ, as the implications of the term are obviously multiple. If invasion is the hallmark of malignant behaviour, then in its absence cancer cannot exist, since it is well recognized that histological patterns, however bizarre, do not establish the presence of malignant neoplasia unless clinical behaviour parallels the altered histologic appearance. Instead, Bernier uses the term dyskeratosis to convey the changes for which most other writers use the term carcinoma in situ.

Shafer, Hine and Levy point out that some intraoral lesions exhibit "top-to-bottom" basilar hyperplasia, loss of polarity, increased mitoses, hyperchromatism, dyskaryosis and alterations in nuclear-cytoplasmic ratio, without any evidence of thickening of the epithelial layer or, more significantly, without any evidence of a disturbance of the keratinization process. Thus, in half the lesions described as carcinoma in situ in Shafer and Waldron's (1961) series,

there was no individual cell keratinization, no epithelial pearl formation, and no hyperkeratosis. Inevitably these lesions were described clinically as being flat, red, velvety, and having a granular appearance. Shafer and Waldron's experience suggested that carcinoma in situ did not inevitably present as a clinically hyperkeratotic or leukoplakic lesion. In some specimens, however, areas of flat, thin, red carcinoma in situ alternated with areas showing hyperkeratosis or parakeratosis, with or without acanthosis and focal atypia.

If these observations are accepted, the use of dyskeratosis as suggested by Bernier would seem to be illogical.

It seems to this reviewer that pathological reports would, for preference, list the various changes taking place in the section under study, rather than use the "shotgun" and rather inaccurate term carcinoma in situ. If used for the sake of brevity, it may have some value.

(b) Connective Tissue Alterations.

Inflammatory Infiltration. In chronic inflammatory lesions the connective tissue is infiltrated by lymphocytes, plasma cells, histiocytes, and scattered polymorphs. The inflammatory exudate may be homogeneously spread throughout the connective tissue, may be localised in a broad band

close to the epithelium, or may be perivascular. In acute inflammatory lesions, the primary cell of the infiltrate is the polymorph.

Hyperplasia, of connective tissue is characterised by a markedly increased density of collagen fibres, as in dilantin reaction.

Collagen Degeneration, is characterised by a "coagulation" or clumping of collagen bundles so that the fibrous texture is absent. This condition is seen in radiation lesions and in lupus erythematosus.

Vascularity. There may be an increase in the number of capillaries and engorgement with erythrocytes.

(c) Proliferation of Epithelial Cells under Stimuli.

The mucosa itself is normally maintained in health by a natural physiologic level of stimulus in which the desquamation caused by friction and salivary cleansing is just balanced by the replacement proliferation of the epithelium. A mild increase in this basic level of stimulation may produce a corresponding degree of irritative proliferation. If proliferation is orderly, then this can result in increased thickness of the prickle-cell layer (acanthosis), while in keratinized areas, the cells may go on to complete maturation and result in increased thickness of the stratum corneum (hyperkeratosis), (Spouge and Diamond, 1963).

Increased stimulation may result, however, in a disorderly

proliferation, This may be of a relatively mild form, manifested by a faulty formation of the keratin and the presence of nuclei in the stratum corneum (parakeratosis). A more severe and ominous degree of disorientation is indicated by proliferation which results in the production of keratin in abnormal situations ("dyskeratosis") as, for example, when it is seen to be formed by individual cells and groups of cells in a surrounding stratum spinosum which shows histologic indications of irregularity. In non-keratinized tissue, a mild degree of disorderly proliferation may result in a metaplasia involving production of mature keratin in areas where it would not normally occur.

An important study relating to "Metaplastic Keratinization in the Human Buccal Mucosa," has been reported recently by Gerson and Meyer (1964). They found that the metaplasia of the mucosa covering fibrous lesions of the cheek (normally unkeratinized) resulted in a keratinized mucosa, which shares all the characteristics of a normally keratinized region. It has undergone structural and chemical changes that enable the epithelium to synthesize keratin, and in so doing, it has lost the distensibility that is an outstanding trait of the buccal mucosa.

Narrow epithelial ridges and high connective tissue papillae seem to be a characteristic by which keratinized mucosa differs from non-keratinized. Mercer, emphasizing the high rate of metabolic activity of keratinized epithelium, draws attention to the ease of metabolite transfer brought about by the large surface thus provided.

A smaller average cell size also seems a consistent difference. This difference may be related to the fact that reticulum of cytoplasm and the deposition of glycogen occur only in quantity in unkeratinized regions.

Gerson and Meyer found the mitotic rate in metaplastic epithelium to be 1.80, about double that in the normal areas, while 82% of the mitoses occurred in the basal cells and lowest two rows of spinous cells. The basal cells were found to increase in height and size.

Silverman, Renstrup and Pindborg (1963) reported that:-

1. Hyperparakeratotic lesions exhibit a definitely higher mitotic activity than hyperorthokeratotic lesions.
2. Acid phosphatase was found in hyperorthokeratoses and not in hyperparakeratoses.
3. "Epithelial atypia" was demonstrated only in cases of hyperkeratoses classified as hyperparakeratoses.

(The criteria for "epithelial atypia" and "dyskeratosis" are very similar. The exclusive association of "epithelial atypia" and hyperparakeratosis is not pointed out elsewhere in the literature - Reviewer).

Renstrup (1963), using Marthaler's technique (mitotic activity is expressed in terms of mitotic figures per unit length - 10 mm - of basement membrane), found that the average mitotic activity of the hyperparakeratotic group was more than four times that of the hyperorthokeratotic group.

Renstrup then links this conclusion with conclusion 3. of

the previous study, and states that it may be assumed that there exists a correlation between high mitotic activity and malignant potential. (Although the validity of the reasoning whereby this conclusion is reached is open to serious doubt; the conclusion itself is most likely acceptable, particularly in view of the fact that abnormal and excessive mitotic activity are universally accepted criteria for malignancy - Reviewer).

Gigoux (quoted by Gerson and Meyer) found that a reduction in thickness was a striking feature in the keratinization of buccal epithelium of rabbits induced by vitamin A deficiency. Gerson and Meyer also found a proportionate decrease in thickness related to the specific area of the mouth. They suggest that the requirement of metabolite supplies to superficial cells imposes a limit to the possible thickness of the cellular layer in a keratinized epithelium. The maximum may be much below that of unkeratinized epithelium.

The findings of Gigoux are substantiated partially by those of Jolly (1964) in his study of vitamin A deficient rats, but Jolly suggests different reasons than do Gerson and Meyer for the striking reduction in thickness of the oral epithelium:-

1. The greater part of this reduction occurred in the cellular part of the epithelium and was due to;
 - (a) atrophy in the regions which normally exhibit "complete orthokeratinization" (hard palate and papillary regions of tongue),
 - (b) a combined effect of metaplasia and atrophy in those regions which normally exhibit "incomplete ortho-

keratinization" (soft palate, cheek, interpapillary regions of tongue).

2. The large reduction in the cornified layer of the soft palate was principally due to the epithelial metaplasia with the production of a thinner, more compact stratum corneum. Reduced replacement rate of this cornified layer also contributed.
3. Reduction of the cornified layer of the hard palate was due to reduced rate of production of new epithelial cells and reduced rate of replacement of the cornified layer.
4. The deficient female rats showed an increase in thickness of the cornified layer and this was probably due to a great extent to the uninhibited keratinizing effect of oestrogen.

Jolly feels that oestrogen seems to favour cornification, whilst vitamin A opposes it, and that in the rat when oestrogens are present and vitamin A is withdrawn, it is reasonable to expect that the general tendency will be for cornification to predominate. A further point of explanation lies in the fact that the cellular layer suffered less reduction in thickness in the female than in the male. Therefore the rate of new cell production (and therefore the rate of cornification) was probably less reduced in the female.

Jolly found a vastly reduced rate of cell division in his vitamin A deficient rats. For the epithelium to be maintained in a state of equilibrium, cell loss must be balanced by cell replacement.

If the rate of cell production is reduced whilst oral function (and therefore wear and tear) is maintained, then equilibrium can only be maintained if the rate of exfoliation at the surface is correspondingly reduced. Obviously this could not be achieved fully in the deficient rats, and the whole epithelial layer was reduced in thickness and the cornified layer showed excessive wear and tear. In the hard palates of the males the stratum corneum also was reduced in thickness, but in the females this layer was actually increased in thickness, as though, in these animals, there was a more effective mechanism for reducing the rate of exfoliation of the squames. It was quite evident that the response of the soft palate, cheek, and interpapillary regions of the tongue was to replace their normally loose, flaky cornified layers with a denser and presumably wear resistant layer. These findings also explain the absence of a clinically observable hyperkeratosis in the rats.

"With the establishment of a new and greatly reduced rate of mitosis, the whole nature of the epithelium must change to reduce the rate of exfoliation at the free surface.

It must be conceded of course that the reduction in mitotic rate may not have been the primary result of vitamin A deficiency with a secondary adjustment of the maturation process to reduce the rate of surface loss. The alternative is that the change in mode of maturation was the primary response, and that the reduced rate of exfoliation was then balanced by a reduced rate of mitosis. The possibility of the latter being correct seems less likely" (Jolly).

According to Gerson and Meyer, the lamina propria and basement membrane of the buccal mucosa participated in the metaplastic changes. This is in harmony with the findings of Mercer, who described the complex sequence of interaction of epithelium and connective tissue in epidermal development, which begins with an inductive action of the epithelium on the underlying connective tissue, continues with dermal dominance, and ends with epidermal dominance. In the transitional zone between normal buccal mucosa and full-blown keratinization, the changes next to the normal region were limited to the deepest cell layers, and toward the summit of the lesion they involved a progressively wider layer. "This gradual extension from the deepest to all rows of cells is probably a facsimile in space of the time sequence of the metaplastic changes at a given point".

Gerson and Meyer suggest the following sequence of events. Following exposure of the epithelium to surface friction, the first induced change might be one in the transfer properties of the basement membrane, for example, such as to cause vitamin A deficiency. The first effect of this change might be the altered specialization of the basal cells, which is microscopically visible as changes in size and shape. These basal cells, in due sequence, would give rise to a progressively widening layer of metaplastically altered cells that eventually comprise the entire thickness of the epithelium, and a layer of keratin.

Basal cells are usually described as being undifferentiated, but Gerson and Meyer dispute this, as they found definite metaplastic changes in the basal layer.

The studies of Gerson and Meyer on the one hand, and Jolly on the other, are not parallel, as the former studied human mucous membrane not influenced by vitamin A deficiency, normally non-keratinized, but keratinized due to chronic irritation, while the latter studied rat mucous membrane influenced by vitamin A deficiency. However, Gerson and Meyer suggest that vitamin A may still play a part through a change induced by surface friction in the transfer properties of the basement membrane. It is notable that both found a reduction in thickness in the epithelium during metaplasia.

LEUKOPLAKIA.

Introduction.

Historical Review.

Clinical Features,

1. Incidence,
2. Sites of Predilection,
3. Clinical Appearance of Lesions.

Histopathology.

Histochemistry.

Aetiology.

Diagnosis.

Differential Diagnosis.

Therapy and Management.

Incidence of Carcinoma in Leukoplakia.

Incidence of Leukoplakia in Carcinoma.



Introduction.

The term "leukoplakia" as used in this review will imply only the clinical feature of a white plaque on the mucosa, (specifically excluding all definite entities also manifesting as white lesions, such as lichen planus, syphilitic mucous patches, white sponge naevus, moniliasis, lupus erythematosus, chemical burns, and other stomatitides) and will carry absolutely no histologic connotation, although it is characterized inevitably by some form of disturbance of the surface epithelium. This definition follows that of Shafer, Hine and Levy, (1963).

Kollar et al (1954) urged that the term leukoplakia should be discontinued as a diagnosis, classified white lesions as hyperkeratosis simplex (without dyskeratosis) or hyperkeratosis complex (with dyskeratosis), and presented a histopathologic classification. Boyle (1955) divided leukoplakia into hyperkeratosis simplex and complex according to the clinical picture, while Cheraskin and Langley (1956) state that the term is generally used to designate a premalignant white patch on the mucous membranes.

Bernier (1959) introduced (at an earlier date) the terms pachyderma oris (showing no dyskeratosis) and leukoplakia (showing dyskeratosis) to replace the clinical term leukoplakia. He has been followed in this classification by Shira (1957), Thoma and Goldman (1961) and Bhaskar (1961).

Russ (1957) in his review used "leukoplakia" as a clinical term, but suggested acceptance of Kollar's classification of maturation

disorders, and of dyskeratosis, as a prerequisite for a pathological diagnosis of leukoplakia.

Orban and Wentz (1960) followed the classification of Kollar et al into hyperkeratosis simplex and complex. (Orban was one of Kollar's co-workers).

Chomet et al (1962) suggested redefining leukoplakia as "the histologic triad of hyperplasia, hyper- or parakeratosis, and inflammation", also that proliferative and neoplastic changes of the oral epithelium be classified as follows:-

1. Hyperplasia, 2. Leukoplakia, 3. Dysplasia,
4. Carcinoma in situ, 5. Early invasive carcinoma, 6. Carcinoma.

Smith (1962) seemed to avoid the term leukoplakia, and described the lesions as hyperkeratosis.

Rushton and Cooke (1963) used the term in the limited sense of a reaction to external irritation.

Hellinger et al (1963) suggested that the term be reserved for those cases in which the lesion is clinically abnormal in appearance and/or occurs at an unusual site. They believed that a term such as "nonspecific leukokeratosis" when applied to a clinical impression, should denote a clinical "white plaque" suspected of exhibiting microscopic alterations consistent with a hyperplastic type of response.

The following authors use the same meaning for leukoplakia as Shafer, Hine and Levy; Sharp et al (1956), Gorlin (1957), Renstrup (1958), Fasske (1959), Sutherland (1959), Silverman and Ware (1960), Shafer and Waldron (1961), Golby, Kerr and Robinson (1961), Turesky et al (1961), Burket (1961), Robinson (1962), Stones (1962), McCarthy and Shklar (1964) and Kruger (1964).

Silverman, Renstrup and Pindborg (1963) define white lesions as being leukoplakias if they;

1. cannot be removed by scraping,
2. cannot be reversed by removing obvious irritants,
3. cannot be classified clinically or microscopically as another diagnosable disease.

Hansen (1959) listed different classifications, and stated that if leukoplakia and other terms are used, their meaning must first be defined.

In deciding how to define leukoplakia, the first step would surely be to find its literal meaning. The derivation has been given by Cheraskin and Langley as leukos, meaning white, + plax, meaning plaque.

It would seem illogical to apply this term to lesions showing "dyskeratosis", numbers of which do not appear white clinically. The arguments advanced by the proponents of this usage are:-

1. The term has for so long carried the implication of malignancy that this implication should be conformed to by limiting its use to premalignant lesions.

2. Leukoplakia is a term which may frighten cancerophobic patients.

The answer to the first argument would seem to be to educate gradually the dental profession to the correct use of the term and in the description of the histopathologic features of white lesions. There is some point in the second argument, and here the solution would seem to be to use the alternative "white patch" when speaking to the patient. At the same time, the clinician should take the opportunity of preparing the way for the psychological shock when the almost inevitable biopsy becomes necessary.

Complete elimination of the use of the word leukoplakia would seem to be impossible, so the only rational meaning to use for it is its literal meaning.

In communication between various members of the health team, as Hansen suggests, the meaning to be used for leukoplakia should be first defined.

By following these principles, confusion should be avoided, and treatment may be planned on a rational basis.

Historical Review.

Schwimmer in 1877 suggested the term leukoplakia to describe white raised lesions involving the oral mucosa. Paget in 1851 had previously described white lesions and used the name smoker's patch. Other names used were leucoma, leucokeratosis, and ichthyosis, but the term leukoplakia was considered most suitable as a clinical descriptive

term, and has remained in use over the years. Butlin related these lesions to smoking and considered a smoker's patch to be an early stage of a more advanced white raised lesion which he called leucoma, following Hutchinson's use of the term. Numerous case reports followed these original descriptions. Gradually some experimental observations were presented, and clinical and microscopic surveys on large numbers of cases were carried out. Roffo in 1930 presented experimental evidence in rabbits, demonstrating that keratotic lesions on mucosa were produced by tobacco smoke after 25 days. Wolbach (1937) demonstrated that vitamin A deficiency produced hyperkeratosis on the mucosa of experimental animals.

Sturgis and Lund in 1934 discussed a series of 312 cases, and found syphilitic involvement in 17 per cent. Furthermore, 12 per cent of the lesions were found to develop into carcinoma. McCarthy in 1936 presented a series of 316 cases, and graded the lesions on the basis of their clinical and histologic patterns. This approach to a gradation of lesions in leukoplakia, with the Grade IV lesions representing early malignant changes, was of great significance in pointing to a differentiation of leukoplakial lesions into various types, based upon a correlation of clinical and microscopic features. McCarthy also suggested four main aetiological factors; faulty occlusion, chronic irritation, smoking, and syphilitic involvement.

Eichenlaub in 1938 reported on 327 cases of leukoplakia found among 16,802 persons. The buccal mucosa was found to be

involved in 217 cases, lips in 38 cases, tongue in 8 cases, gingiva in 3 cases, and palate in 2 cases.

Nathanson and Weisberger in 1939 suggested a possible endocrine factor in the cause of leukoplakia. Of 38 cases that were treated with oestrogenic hormone, the lesions disappeared in 42 per cent and improvement was noted in 39 per cent. Following cessation of therapy, the lesions reappeared in 3 to 6 months. In 25 of the females, the leukoplakia was found to be associated with menstrual disturbances.

The percentage of cases of leukoplakia that undergo malignant alteration has not been set down with clarity, in that statistical surveys approach the problem either from a large number of leukoplakial lesions without a careful follow-up, or from a group of malignant lesions apparently arising in areas of leukoplakia. Weisberger (1957) reported that 60 per cent of 275 patients with oral carcinoma had leukoplakia adjacent to the cancer. Hobaek (1946) found that 10 per cent of a series of 1,272 patients with oral cancer had a history of pre-existing lesions of leukoplakia. Meyer and Shklar (1960) found that 26 per cent of a series of multiple malignant lesions of the oral cavity were associated with leukoplakial lesions.

Clinico-histopathologic surveys have been presented by Renstrup (1958), Shafer and Waldron (1961), while Fasske et al (1959) examined a series of 103 patients with leukoplakia of the cheeks, gingivae and tongue, clinically, histochemically, electron-microscopically and biochemically.

Recently, the term leukoplakia has been suggested for use as a histologic description of lesions with dyskeratosis as well as hyperkeratotic changes (notably, Bernier, Shira, Bhaskar, and Thoma). The simple hyperkeratotic lesions would be called pochyderma oris clinically. This change in the use of leukoplakia from a clinical term to a microscopic term suggesting malignant potentiality is confusing and has not gained widespread acceptance (McCarthy and Shklar, 1964).

Clinical Features.

1. Incidence.

- (a) Frequency. Leukoplakia is a very commonly occurring lesion, particularly beyond the age of forty. This is agreed on by most writers, but actual statistics of incidence in the population as a whole are very scarce. In a series of 2,300 consecutive studies, McCarthy (1941) found leukoplakia present in 14 per cent. Burket (1961) gives the incidence as 1 in 500 of the general population, but does not give details of the means whereby he arrived at this figure, while the figure given by Kephart (quoted by Bernier, 1959) is 1 in 50 patients over the age of forty. Shafer and Waldron's series (1961) shows that 332 (4 per cent) specimens of leukoplakial lesions were found in a series of 8,554 biopsy specimens of oral lesions.

Firstly, this does not allow one to deduce the incidence of leukoplakia in the general population, and secondly, the figures are not completely reliable, in that all leukoplakial lesions were biopsied, whereas other oral lesions were biopsied only if necessary for diagnostic purposes.

Schaffer (1952) stated that leukoplakia occurred in 50 per cent of all men, and 10 per cent of all women over the age of 45 years.

Hunter (quoted by Orban and Wentz) showed that about 1 of 800 people has leukoplakia (hyperkeratosis, simplex and complex). Orban and Wentz state that hyperkeratosis complex (showing dyskeratosis and inflammation) comprises about one-half of all dystrophic, hyperplastic and hyperkeratotic lesions.

- (b) Incidence according to sex. Hobaek (1946) gave the male : female ratio as 73.6% : 26.4%; Cheraskin and Langley (1956), 9 : 1; Renstrup (1958), 66% : 34%; Fasske et al (1959), 6 : 4; Bernier (1959) states that pachyderma oris (his name for a white lesion without dyskeratosis) is common in females, but that the ratio for leukoplakia (the term is only applied by Bernier if the lesion shows dyskeratosis) is 95 : 5. Orban and Wentz (1960), who divide leukoplakia into hyperkeratosis simplex and complex, state that it is predominantly a disease of males, and that 75 per cent

of cases of hyperkeratosis complex (showing dyskeratosis and inflammation) occur in males. Bhaskar (1961, and Stones (1962), agree that leukoplakia occurs predominantly in males, while Burket (1961) states that the incidence in males has decreased in the last two decades from 95 per cent to 65 per cent. Shafer and Waldron (1961) give the incidence as 68 per cent in males, 32 per cent in females, with a slight trend for earlier occurrence in females.

McCarthy and Shklar (1964) give the ratio of male : female as about 2 : 1.

- (c) Incidence according to age. Hobaek (1946) gives the average age of patients with leukoplakia as 60.1 years, while Cheraskin and Langley state that it occurs chiefly in the older age group. Renstrup found that 90 per cent of sufferers were older than 40 years, and Fasse et al in their study found the average of men 57, women 55, while 12 of 103 patients were under 40. Weisberger (1957) found the highest incidences in fourth to sixth decades. Bernier states that pachyderma oris is more prevalent with age, and that leukoplakia (with dyskeratosis) is more prevalent after the fourth decade. Orban and Wentz give the age of incidence for hyperkeratosis simplex as 40-60 years, and for hyperkeratosis complex as 50-70 years, while Bhaskar gives the age of occurrence as 40 years and later. Burket says leuko-

plakia occurs mainly in the fourth and fifth decades, but can occur even in teenage people, while McKown (quoted by Burket) gives an average age of 54 years. Shafer and Waldron's figures are that 81 per cent of the occurrence is in patients over 40 years of age, and Stones states the disease occurs predominantly over the age of 40.

2. Sites of Predilection.

The following orders of frequency in the sites of predilection are, according to;

- Hobaek (1946) - Tongue and floor of mouth, lower lip, buccal mucosa, palate, gingivae, multiple areas of involvement being common.
- Cheraskin and Langley (1956) - Lower lip, buccal mucosa, anterior portion and sides of tongue.
- Sharp et al (1956) - Tongue, buccal mucosa, gingivae, hard palate and lips.
- Renstrup (1958) - Cheeks and commissures, alveolar mucosa, tongue, lip, hard and soft palate, floor of mouth and gingiva. Lesions on the cheeks and commissures are generally bilateral, while the commissure lesions form just inside the junction of the lips as triangular areas. Cheek lesions are usually diffuse, or are bands opposite the occlusal line, or near teeth likely to cause

irritation. Lip lesions are generally near where a cigarette is held. The palate may show local or general involvement, while the tongue lesions may be at the borders or tip near a local irritant, or variable sized lesions on the dorsum.

Fasske et al (1959) - Cheek, jaws, lips, tongue, alveolar process, soft palate, hard palate.

Bernier (1959) - lips, cheeks, gingiva, tongue and palate.

Orban and Wentz (1960) - Hyperkeratosis simplex - floor of mouth, ventral surface and dorsum of tongue, cheeks, palate, lips and gingiva.

Shafer and Waldron (1961) - Mandibular ridge, gingiva and mucobuccal fold, 28.6 per cent (greater frequency in women due to snuff held there), buccal mucosa 19.6 per cent, palate 10.5 per cent, maxillary ridge, gingiva and mucobuccal fold 9.3 per cent, floor of mouth 8.1 per cent, lower lip 7.8 per cent (1% females, 11% males), retromolar area 6.3 per cent, tongue 4.8 per cent, unspecified 4.8 per cent.

Turesky et al (1961) - Buccal mucosa 32, gingiva 18, hard and soft palate 17, lip 6, tongue 5, floor of mouth 2.

Bhasker (1961) - (a) Pachyderma oris - not dyskeratosis.

Lips and cheeks, but anywhere on the oral mucosa. (b) Leukoplakia (with dyskeratosis) - usually lip, tongue, cheek, floor of mouth, but anywhere.

Burket (1961) - Cheek (near commissures and along the occlusal line), tongue, floor of the mouth, palate, undersurface of the tongue, edentulous ridges.

McCarthy and Shklar (1964) - Buccal mucosa, edentulous alveolar ridge, hard palate, tongue and lips, gingiva, soft palate and floor of mouth. The occlusal line of the buccal mucosa is very commonly involved, the lip mucosa is more frequently involved in pipe smokers, while the hard palate is a common site in both pipe and cigarette smokers.

The above and other authors emphasize that leukoplakia can occur anywhere in the oral cavity. Sometimes a white area can be related to a specific stimulus, but often no such relation can be found, though it is impossible, when dealing with a chronic lesion, to be sure what stimuli have been present in the past.

It was shown in the earlier chapter on anatomy that a well-defined stratum corneum (i.e. keratin) normally occurs only on the gingiva and the hard palate. Therefore, it would be logical to assume

that a white area in these locations would be of less significance, and possibly less threatening than, a white area occurring where keratinization does not normally take place to any degree. While agreeing that there is significance in this observation, it would be unwise to overemphasize this factor, in view of the persistent multipotency of the basal layer of the epithelium, which is in constant readiness to produce a protective layer at the free surface when a stimulus is applied.

It seems to be a valid observation that the incidence of tongue lesions has decreased since Hobæk's study of 1946. The better control of syphilis may be significant here.

3. Clinical Appearance of Lesions.

In general terms, the clinical appearance of the lesions of leukoplakia presents considerable variation, both in the severity of the lesions and the degree of involvement of the oral mucosa.

A classification of leukoplakia according to clinical and histologic appearance by McCarthy in 1936 is quoted by Cheraskin and Langley (1956) and Archer (1961). It is as follows:-

- Grade I. - Red, granular, sharply defined, sensitive area for a short time, then a slightly whitish area. Inflammatory infiltration without epithelial proliferation was seen microscopically.
- Grade II - Smooth, tessellated type. Pearly white discolourations or diffuse opacity having a bluish tint. Sharply outlined, no induration. If extensive, the mouth is dry and the tongue hindered in its movements. The lesions

revealed hyperkeratotic changes and inflammation on microscopic evaluation.

Grade III - Indurated plaques of milky white, or pearly, silvery appearance, raised, may cover a large area. Harsh, horny surface with wrinkles, may form fissures. Microscopically, marked hyperkeratosis and inflammation were noted.

Grade IV - Indurated, leathery - verrucous or papillomatous formations. Thick, keratinized epithelium often coated with a heavy fur. Fissures form with downgrowth of epithelium causing localised induration. The tongue is handicapped in its movements, and the speech thick. Desquamation and ulceration may occur. Microscopically, early malignant changes were noted.

Hobaek (1946) described two stages - first stage; "leukoplakia plana" - a smooth non-elevated type (stationary in development for up to 15 years): second stage; "leukoplakia verrucosa", a thicker and verrucous type.

Lyman (1948). In earlier stages, leukoplakia appears as a nonpalpable, faintly translucent, white discolouration. Next, it turns white and develops a palpable papillary growth, and finally, the mucous membrane becomes thick and stiff, and erosion and ulceration takes place. There is variation in size.

Sharp (1948) describes three stages; earliest - nonpalpable, faintly transparent, white discolouration. Later, localized or diffuse, slightly elevated plaques of irregular outline develop, opaque

white in colour, with a fine, granular texture. Third stage - markedly thickened white lesions, with induration, fissuring and ulceration.

Ward and Hendrick (1950). 1. Acute leukoplakia - a history of only weeks or months. Lesions develop rapidly, may be thickened, ulcerated or papillomatous. 2. Chronic - up to 10, 15, 20 years. More diffuse, a thin, white film over the surface of the tongue or buccal mucosa. Rarely cancerous. 3. Subacute, between 1. and 2.

Boyle (1955) - divides leukoplakia into two classes according to the clinical picture - hyperkeratosis simplex and hyperkeratosis complex, the latter being verrucous, rough and fissured.

Sharp et al (1956): Two varieties; a thin, lacy, translucent or grey film, tending to be generalized (termed leukoedema), a forerunner of the second type, which is an opaque patch, thick or thin - a white, hornified, leathery plaque, sometimes quite irregular and roughened.

Shira (1957) points out that separation of the harmless and dangerous on clinical basis is almost impossible.

Renstrup (1958). Chronic lesion, develops gradually, may be diffuse or ill-defined, slightly elevated areas of varying extent. The lesions are whitish, greyish or yellowish-white in colour, or stained brownish-yellow by tobacco. In early stages, the lesion is usually smooth or slightly rough and wrinkled, but sometimes a completely smooth pearly patch. In advanced stages, there may be ulcerations and fissures, or verrucous formations. On palpation,

there is dry, rough and leathery thickening sometimes showing infiltration in depth.

Bernier (1959) - For pachyderma oris, the lesions are white, irregular and of varying sizes. The small lesions may be situated in direct relation to trauma, while the larger lesions may not be so. Discolouration may be caused by pigmentation by food and bacteria. For leukoplakia (showing dyskeratosis), the clinical appearance varies from a dull white to grey, at times pink (no keratin being produced). The keratin is in irregular heaps with crevices in between. The size of the lesions varies greatly.

Silverman and Ware (1960) - Lesions show extreme variation in size and appearance.

Waldron and Shafer (1960) - There is considerable variation in size and appearance, from a nonpalpable, faintly translucent white area, to thick, fissured, papillomatous, indurated lesions. The surface is often finely wrinkled or shrivelled in appearance, may feel rough on palpation. The colour is whitish-grey or yellowish-white, or if tobacco-stained, brownish-yellow.

Orban and Wentz (1960). In hyperkeratosis simplex, there are sharply outlined white patches showing no induration and no red margin. In hyperkeratosis complex (inflammation and dyskeratosis), there are irregular, hard, yellowish-white patches; leathery texture; indurated margins.

Colby, Kerr and Robinson(1961). White, opaque, leathery appearing plaque. Variation in appearance was described; a diffuse area, thick, extensive area; and a white, thick, extensive, leathery and fissured area.

Bhaskar (1961). For pachyderma oris - white, flat or raised, may be rough, usually single, of short duration. For "leukoplakia" (showing dyskeratosis) - white, flat or raised, rough or smooth, of any size, sometimes ulcerated.

Burket (1961). Great variation, from small to extensive involvement, is seen. The lesion is yellowish-white, the margins are usually well-defined. A typical feature of leukoplakia of the tongue is the lack of papillae at the site of the lesion.

Chomet et al (1962) noted three types of small lesions (5 mm in diameter).

1. The simple leukoplakias - white to greyish-white plaques, without hyperaemic margins, could not be wiped off.
2. The suspicious lesions - curdled plaques with peripheral hyperaemia. They could be wiped off, but the plaques soon reappeared.
3. The probable early carcinoma. The mucosa showed scuffing or, more frequently, superficial ulceration with peripheral hyperaemia.

Stones (1962). The colour is usually greyish-white, but is influenced by individual habits. Frictional keratosis - on the cheeks it may appear as a whitish band, about 1 cm wide, extending

the length of the interdental line, and may involve the commissures. The lesion starts as an erythematous area, which is replaced by a heaping up of ridges of keratin. Cracks and fissures may be formed and, on palpation, the lesion feels rough and has a well-defined margin. Smoker's keratosis (after Cooke, 1956) - the cheeks show a diffuse, faint bluish whiteness of the mucosa limited to the areas that are exposed to smoke. The keratin becomes thickened and the surface rougher. Similar lesions may affect the lips and tongue. In the tongue there is atrophy of the papillae, and areas of desquamation may be seen between the keratotic lesions. On the lips the so-called smoker's patch is seen as a tessellated, bluish-white patch on each side of a groove in which the cigarette is usually held. There may be a marked heaping up of the keratin which, when associated with ulceration and induration, is indicative of neoplastic change. When associated with syphilis, there may be areas of desquamation between the keratotic patches which may be verrucous in nature, or confluent raised patches.

Pindborg et al (1963 V); A study of 185 white lesions disclosed that 35 lesions in 29 patients (6 were bilateral) had characteristics of white patches on an erythematous background, giving a "speckled" appearance. Of 35, (average age 54; 59% being men) 23 were at the commissures, 4 at alveolar sulcus, 1 alveolar process, 2 dorsum of tongue, 2 buccal mucosa, 2 floor of mouth, 1 palate. They commented that the preponderance of lesions at the commissures was remarkable, as that location is not a common site for oral carcinomas. Histological examination disclosed, in all 35 cases,

the simultaneous occurrence of hyperparakeratosis, epithelial hyperplasia and epithelial atrophy (defined as reduction of the prickly cell layer). Five of the 35 lesions on histologic examination disclosed carcinoma, while 18% showed epithelial atypia.

McCarthy and Shklar (1964) discuss the clinical appearance under the following headings:-

Diffuse Initial Involvement; A reddened, finely granular area, with a slightly grey or grey-white coat, becoming somewhat more coarsely granular and more obviously grey-white, with indistinct boundaries. The central area may be more distinctly white, fading into grey and finally into the normal pink. This picture is seen characteristically on the buccal mucosa, lateral borders of the tongue, and occasionally, the labial mucosa.

Diffuse Moderate Involvement; On the tongue the entire dorsal surface and lateral borders may be involved by a pebbly white pattern. The lingual papillae also appear to be involved, and may be clearly distinguished.

On the gingiva, this type of reaction appears as a grey zone on the attached gingiva. The marginal gingiva tends to be free of leukoplakial involvement, and may be characterised by inflammatory changes.

Diffuse Severe Involvement; The lesions are thick and leathery, firm, hard, and often verrucous in localized areas. This type of involvement often leads to malignant alteration. In addition to the white and grey areas, zones of erosion may be noted.

Localized Initial Involvement; A localized zone of pink-white discolouration is noted. This may be seen in the mucobuccal fold area, floor of the mouth, buccal mucosa, and other areas. The margins are indistinct and no induration is apparent.

Localized Moderate Involvement; A white patch or plaque is the typical lesion, and most descriptions of leukoplakia refer to this type. The plaques are raised, and the margins are sharply defined. There may or may not be obvious induration, and the mucosa at the margins of the lesion is erythematous. The surface is pebbly and often wrinkled or fissured.

Localized Severe Involvement; The white plaques here are indurated, sharply outlined, raised, and wrinkled. However, surface alterations are usually apparent. The surface texture is not smooth, but rough and granular. Deep fissures and erosions are apparent, with areas in which the mucosa appears ulcerated. Occasionally warty proliferations are seen to arise in these lesions, giving a warty texture to the lesion. The colour in these lesions may be brown in areas, although the bulk of the lesion is chalky or dull white. Malignant tumours often arise in this type of lesion, and appear as an area of tissue proliferation, or as a zone of increased induration.