A STUDY OF THE RESPONSE OF THE ORAL MUCOSA OF THE VERVET MONKEY TO MECHANICAL LOADING

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DECLARATION

I, Leslie Fleisch, hereby declare that this dissertation is my own work and has not been presented for any degree of another university.

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The work reported in this dissertation was performed in the Dental Research Unit of the S.A. Medical Research Council and the University of the Witwatersrand, Johannesburg.

To

Hilda, Margo and Brahm

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ABSTRACT

The oral mucosa can be divided histologically into the epithelium, the epithelial-connective tissue interface, the basal lamina, the lamina propria and the submucosa. Functionally, the oral mucosa is classified into masticatory and lining mucosa. The masticatory mucosa, which is keratinized, includes the attached gingiva and hard palate, and the lining mucosa, which is non-keratinized, includes the cheek, alveolar mucosa, floor of the mouth and soft palate. The epithelium of the tongue is a specialised type of keratinized epithelium.

Four regions of the oral cavity of the vervet monkey (Cercopithecus aethiops), namely the alveolar mucosa, hard palate, cheek and tongue were used in this study. A quantitative histometric investigation into the epithelial thickness and cell density in the above regions was carried out as well as an histological investigation. A study to ascertain the degree of shrinkage of tissues during histological processing indicated a shrinkage of 34,7%. The keratinized palatal mucosa had a well developed collagen system and no elastic fibres. The non-keratinized alveolar mucosa has a loose areolar collagen system and numerous elastic fibres. The non-keratinized cheek mucosa contained mucous glands and fine elastic fibres and the tongue epithelium consisted of keratinized filliform papillae. The results indicate that the differences which exist in the four regions described extend through all the layers of the epithelium and the sub-mucosa and may be considered to be based on the functional demands placed upon the various tissues.

Before investigating the effects of mechanical loading on the oral mucosa of the vervet monkey, a preliminary study was undertaken to develop a

satisfactory technique. The results indicated the feasibility of undertaking a comprehensive investigation. The effects of loading of tissues after fixation were studied, and the results obtained indicated that the results obtained in the main study are directly related to the fresh state of the tissues. The rate of fixation of tissues was also studied.

A pressure loading instrument was designed to deliver a reproducible load of 50 gm, to the four areas to be studied, namely hard palate, alveolar mucosa, cheek, and tongue. The palatal mucosa showed the greatest resistance to loading with little histological change and adaptation of the tissues. The results indicated that the histological changes in each area following loading were characteristic and consistent for that tissue, and were directly related to the histological structures of these tissues, such as presence or absence of keratin, elastic fibres and density of collagen in the lamina propria. The histometric analysis demonstrated a smaller degree of change in masticatory mucosa than in lining mucosa, indicating that masticatory mucosa tends to resist the loading force, whereas the lining mucosa appears to adapt to the forces applied to it.

A scanning electron microscopic study of the four areas investigated indicated that no changes were observed in the surface appearance of keratinized masticatory mucosa after loading. However, changes were apparent in the linear folds of the alveolar mucosa and in the intervals between the micro-plications of non-keratinized lining mucosa.

LIST OF PUBLICATIONS AND PAPERS DERIVED FROM MATERIAL IN THIS DISSERTATION

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Fleisch L. 1973.
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A Histological Study of the Response of Masticatory and Lining Mucosa to Mechanical Loading in the Vervet Monkey. 1976. J. Dent. Res. (In Press).

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INTRODUCTION

The oral cavity as the first part of the digestive tract serves a variety of functions. It is both the portal of entry and the place of mastication of food, and contains the taste organs. The oral cavity is lined throughout by a mucous membrane, the morphological structure of which varies in the different areas of the oral cavity in correlation with the functions of specific zones, and the mechanical influences that bear upon them. (Orban 1966).

This diversity in the structure of the oral soft tissues is wide, and varied, but it can be arbitrarily divided for the purpose of description into lining and masticatory mucosa. Lining mucosa includes the alveolar mucosa, cheek mucosa and the floor of the mouth. Masticatory mucosa is found in the attached gingiva and the hard palate.

The gingivae and hard palate consist of soft tissues firmly attached to bone which have to withstand the impingement of rough and hard foods, whereas other areas such as the cheeks, tongue and floor of the mouth may be similarly subjected to stress, but accommodate to the forces applied to them by adaptation. The histological differences between lining and masticatory mucosa, and the effect of mechanical loading on these tissues is the subject of this dissertation.

PART 1 - NORMAL TISSUE

CHAPTER 1 - THE ORGANIZATION OF THE ORAL MUCOSA

The oral mucosa can be divided into the oral epithelium which is the outer component; the epithelial-connective tissue interface including the basal lamina, the lamina propria and the submucosa.

1.1 The Oral Epithelium

Epithelium is generally described as having five layers or strata, one or more of which may be absent or modified according to the type of epithelium and the region where it is found.

1.1.1 The Cell Layers or Strata of the Epithelium

(i) Basal Layer or Stratum Germinativum

This is the deepest layer lying on the basement membrane and consisting of cuboidal or columnar cells from which the majority of the other cells of the epithelium originate. The nuclei are generally large and round and occupy more than half the area of the cells, which are 8-12 μm in diameter.

(ii) The Prickle Cell Layer or Stratum Spinosum

The cells of this layer, which constitutes one-half to two-thirds of the total epithelial thickness, are irregularly polyhedral in shape with flattened nuclei. The cells are larger than those of the basal cell layer.

Seen with a light microscope the 'prickle' appearance is produced by intercellular bridges of protoplasm. Under the electron-microscope these cells contain fine intra-cellular protein strands or tonofilaments which condense to form tonofibrils.

(iii) The Granular Layer or Stratum Granulosum

The cells of this layer are elongated horizontally and the cytoplasm contains keratinohyalin granules .

(iv) The Stratum Lucidum

This is a bright clear homogeneous line situated above the granular layer. It consists of several layers of closely packed cells with no nuclei. It is not usually seen in the oral epithelium. However, Meyer and Gerson (1964) have demonstrated the presence of this layer in the hard palate of humans.

(v) The Keratin Layer or Stratum Corneum

In this layer the cells have lost their nuclei, are markedly flattened into plates, or squames and contain keratin. The most superficial plates are continually exfoliating and are replaced by new ones from the lower layers.

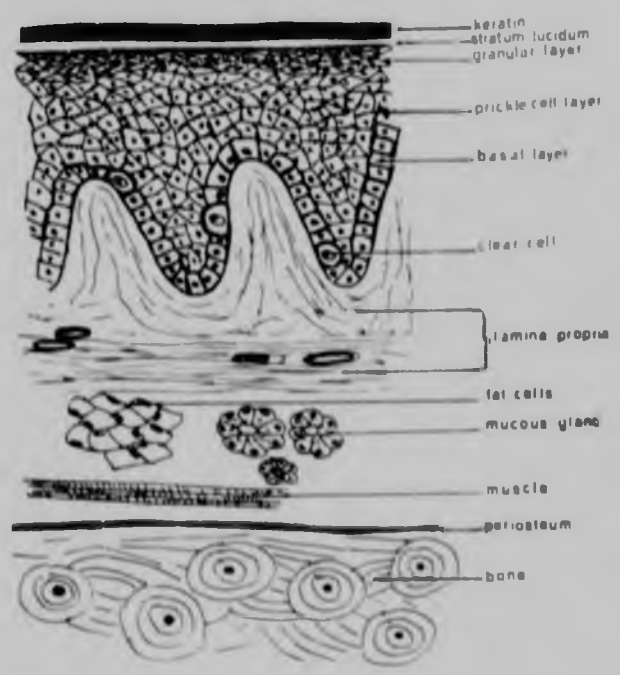

Fig. 1.1 Diagram of histological section through a typical area of oral mucous membrane

1.1.2 The Dendritic or Clear Cells (Figs. 1.1, 1.3)

These cells, which are found at various levels in the epithelium, exhibit a clear, unstained halo around the nucleus, hence the term clear cells.

Three types of dendritic cells are described by Sagebiel, Clarke and Hutchens (1971) namely, melanocytes, Langerhans, and non-specific cells. However, Squier, Johnson and Hopps (1976) classify the clear cells into melanocytes, Langerhans cells and the Merkel cell, and state that the latter is not dendritic in its morphology. Squier, Johnson and Hopps (1976) suggest that the lack of desmosomes in these cells allows the cytoplasm to shrink against the nucleus during histological processing producing the "clear" effect seen in these cells.

The melanocyte, which originates from the neural crest, is usually found in the basal layer of the epithelium. They produce the pigment melanin which, in the skin, characterises the latter's colour. They have long cytoplasmic processes extending between the other cells of the epithelium. According to Hutchins, et al.(1971) they tend to cluster around the bases of epithelial ridges. Barker (1967) estimated that the melanocytes constitute 70 per cent of the basal cells. The Langerhans cells do not produce melanin, and do not originate from the neural crest. Their function is unknown.

Fig. 1.2 Orthokeratinized stratified squamous epithelium from the hard palate showing numerous clear cells in the prickle cell and basal cell layers. Masson x 360

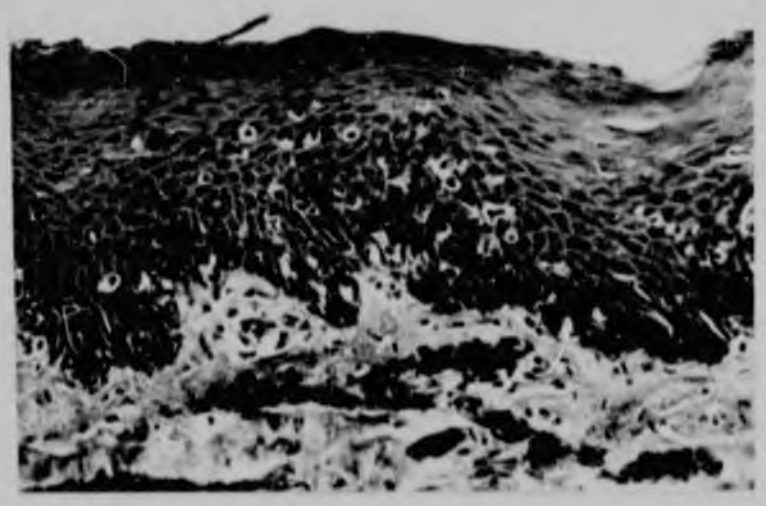

Fig. 1.3 Non-keratinized stratified squamous epithelium from the alveolar mucosa showing numerous clear cells in the prickle cell layer. Masson x 240

1.1.3 Types of Keratinization

Wentz et al.(1952) and Weinman and Meyer (1959) have described four types of keratinization in human gingivae.

1. Fully keratinized epithelium with no nuclei in the surface layer- stains pink with hematoxalin and eosin and bright red with picromallory. (Fig. 1.4).

2. Parakeratinized epithelium - Pyknotic nuclei present in the surface layer - stains pink with hematoxalin and eosin and bright red with picromallory. (Fig. 1.5).

3. Incompletely parakeratinized epithelium - With hematoxalin and eosin stains, it appears as parakeratosis. However, with the picromallory stain the surface layer is sharply divided into a bright red layer and a non-stained layer.

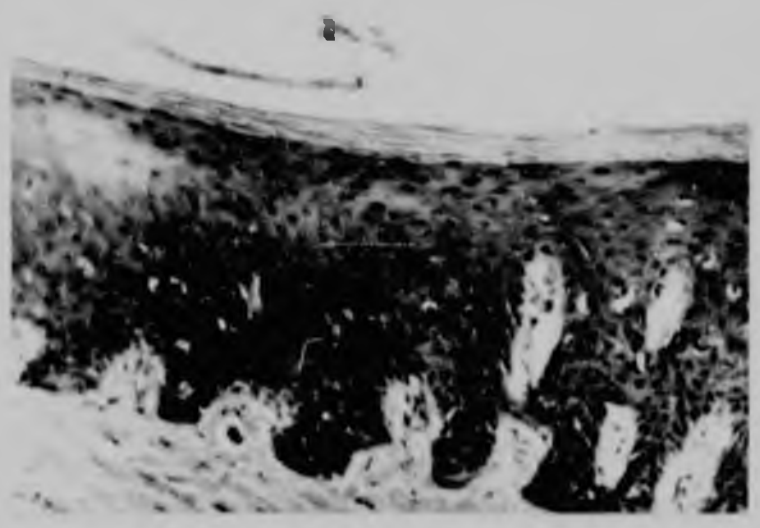

Fig. 1.4 Orthokeratinized epithelium from hard palate showing a surface layer of keratin without nuclei. H and E. x 240

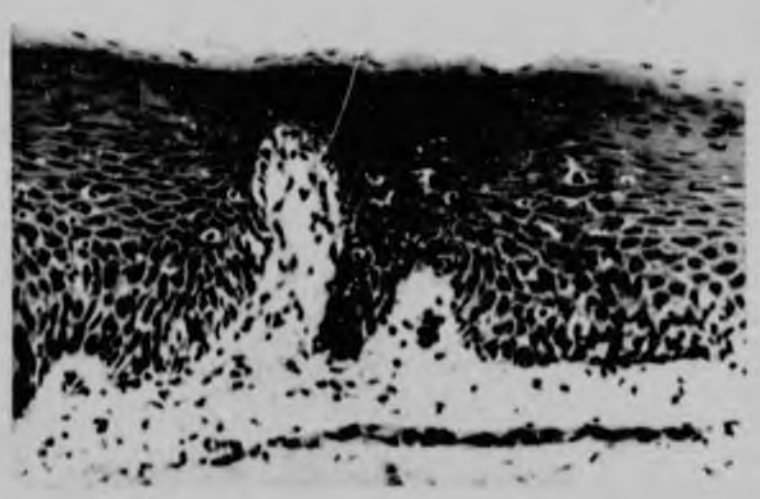

Fig. 1.5 Parakeratinized epithelium from hard palate showing keratin layer containing nuclei in superficial cells. H and E. x 240

4. Non-keratinized epithelium - The nuclei of the surface cells are present and there is no red layer when stained with picromallory. (Fig. 1.6).

Parakeratinization is described as the most common form of keratinization (53,8%) followed by incomplete parakeratinization (25,2%), full keratinization (13,3%), and non-keratinization (7,7%).

Fig. 1.6 Non-keratinized epithelium from alveolar mucosa showing nucleated cells on the surface. H and E. x 240

The thickness of the surface keratin layer varies. Meyer, Medak and Weinman (1960) found it to be 15,65 µm while Meyer and Gerson (1964) found it to be 32 µm. Meyer, Medak and Weinman (1960) felt that the thickness was inversely proportional to the mitotic activity of the epithelium.

1.2 The Epithelial Connective Tissue Interface

Dick (1947), Wentz, et al. (1952), Emslie and Weinman (1949), Meyer and Gerson (1964) and Karring and Löe (1970) and Scapino (1971) have variously described and discussed the epithelial-connective tissue interface. Their descriptions indicate that the functions of the interface are :

1. To provide a large surface area for the transport of nutrients and waste products to and from the epithelial cells.
2. To afford better anchorage of the epithelium to the underlying connective tissue.

In human tissue, conical connective tissue papillae project into the epithelium, which consists mainly of cords or ridges which frequently anastomose. In monkey specimens the epithelial-connective tissue interface displays an arrangement characterized by epithelial pegs. (Karring and Löe, 1970).

The extent and shape of the interdigitations varies in different regions according to the functional requirements of that specific region. As a rule the area of interface is much greater in masticatory than in lining mucosa. (Scapino, 1971).

1.2.1 The Basal Lamina

This is a dense layer usually less than 1 μm in thickness, which lies adjacent to the plasma membranes of the basal cells. It functions as a barrier in normal tissues against the free movement of cells across the junction and possibly secures the attachment of the epithelium to the connective tissue (Susi, 1971).

Melcher (1964) suggests that the basal membrane seen in light microscopy, is composed mainly of reticulin which is continuous with the reticulin of the submucosa (Fig. 1.7). Electron microscopic investigations, however, have shown that the basal lamina can be differentiated from reticulin and is a product of the epithelium and not connective tissue (Melcher, 1965). The thickness of this basal lamina is inversely proportional to the degree of keratinization of the overlying epithelium (Susi, 1971).

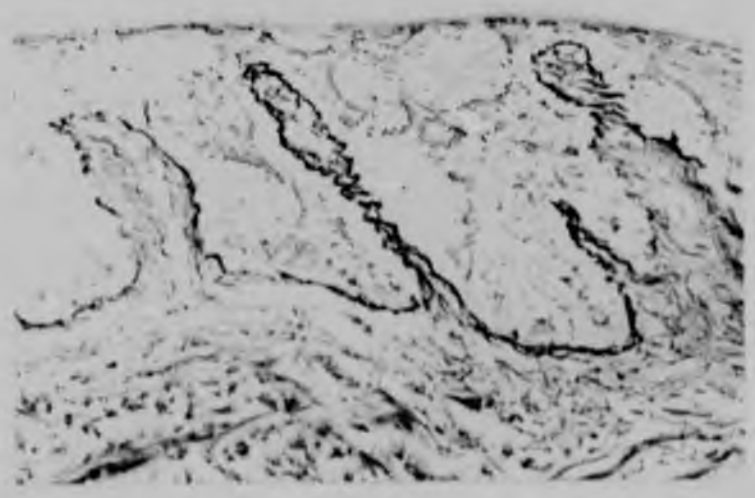

Fig. 1.7 Basal lamina of hard palate with associated reticulin fibres. Gordon and Sweet's. Reticulin Stain. x 360

1.3 The Lamina Propria

The lamina propria is a layer of connective tissue of varying thickness and density (Fig. 1.8). It consists of collagen fibres, elastic fibres, reticulin fibres and a ground substance, as well as blood vessels, nerves and lymph vessels. The cellular elements consist of fibroblasts, macrophages, mast cells, plasma cells and lymphocytes.

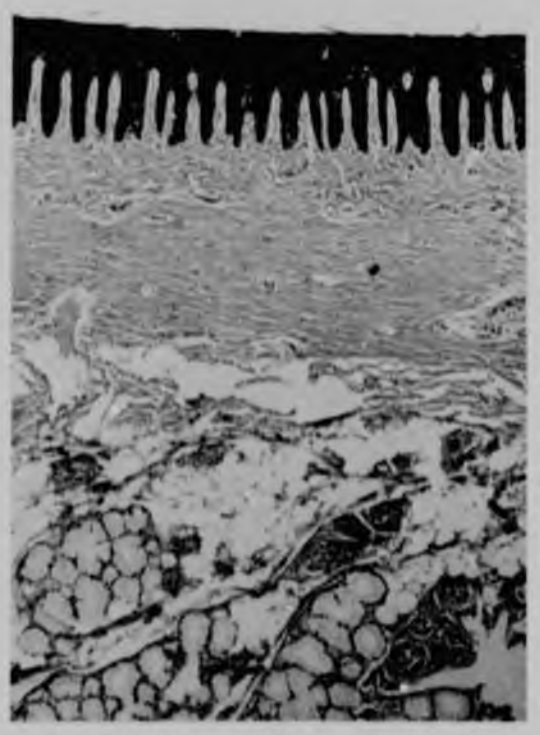

Fig. 1.8 Section of hard palate showing epithelium, lamina propria and submucosa. P.T.A.H. x 60

1.3.1 Collagen

The collagen network consists of long unbranched filaments disposed in a random manner and varying in thickness according to the type of oral mucosa (Figs. 1.9 and 1.10). Between the rete pegs of the epithelium, the fibres run more or less perpendicular to the epithelial surface and then pass into, what Stroud (1967) has described as the superficial papillary layer of the lamina propria, which is continuous with a deeper reticular layer. The papillary layer consists mainly of thin collagen fibres while the reticular layer contains larger and more densely packed collagen fibres. Melcher (1964) and (1965) in describing the relationship between the reticular and collagen fibres states that collagen fibrils in the lamina propria are bound into fibre bundles and that these bundles are bound in progressively larger bundles by a branching network of reticular fibres. Extensions of these reticular fibres into the sub-epithelial region, are the means by which continuity between the basement membrane and the lamina propria is achieved. This anchoring system between epithelium and connective tissue enables loads applied to epithelium to be transferred to the underlying lamina propria.

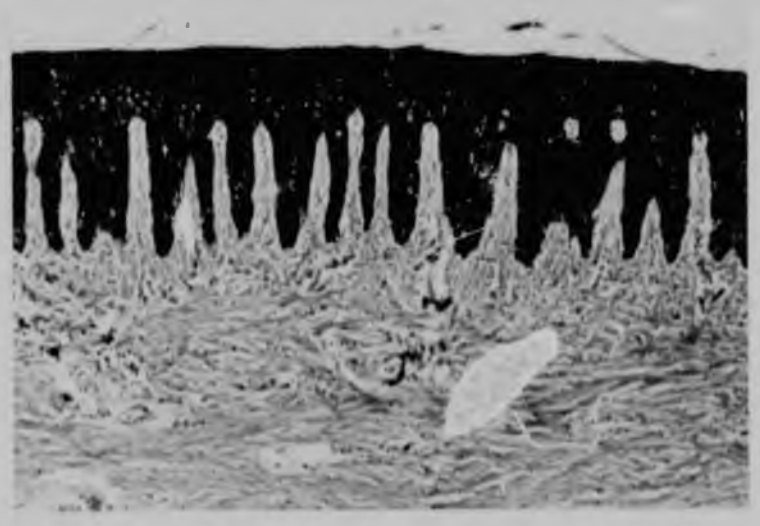

Fig. 1.9 Lamina propria of palatal mucosa showing papillary (P) and reticular (R) layers. Picromallory. x 90

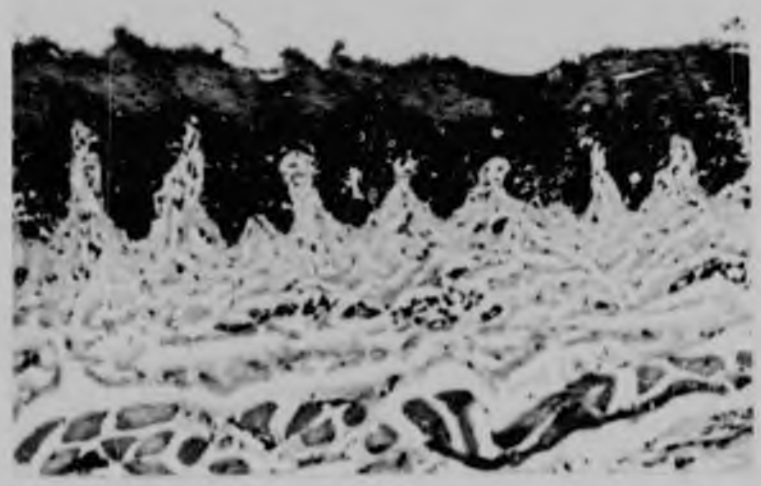

Fig. 1.10 Loose areolar arrangement of collagen fibres in lamina propria of alveolar mucosa. H and E. x 90

1.3.2 Elastic Fibres

These fibres are present in varying degrees in the oral cavity (Figs. 1.11 and 1.12). They appear to be closely associated with the collagen fibres in that they appear more or less perpendicular to the epithelial surface in the connective tissue papillae and parallel to the surface in the deeper layers (Fleisch, 1973). The initimate association of elastic fibres with collagen suggests that one function of the elastic fibres is to restore the collagen fibres rapidly to its original dimensions after extension. Dick (1947), in a study of elastic tissue in the skin, described two components, large fibres deep to the epidermis and a fine network of smaller fibres lying close to the epidermis. This was also seen in the oral mucosa (Fig. 1.13).

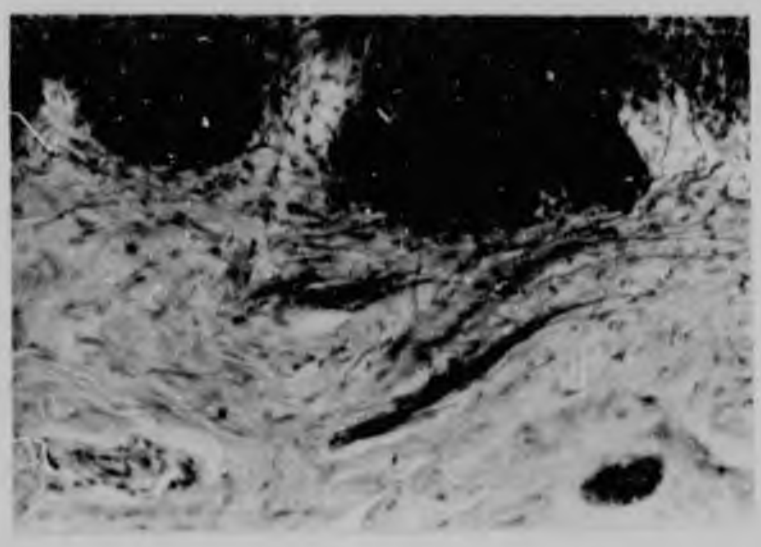

Fig. 1.11 Fine elastic fibres in the cheek mucosa. 25 μm section. Orcein. x 240

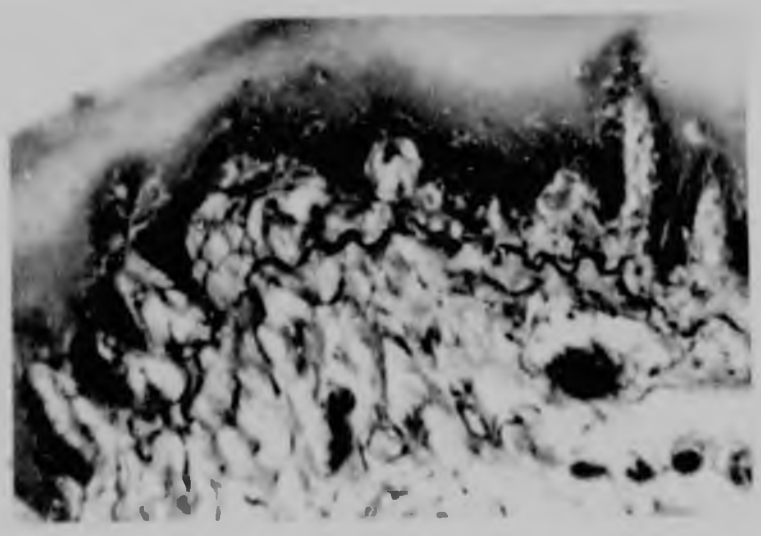

Fig. 1.12 Thick elastic fibres running parallel to the epithelium in alveolar mucosa. 25 μm section. Orcein. x 240

Fig. 1.13 Elastic fibres in alveolar mucosa showing subepithelial plexus (P) and longitudinal fibres (L) 25 µm section. Orcein. x 240

1.3.3 Reticulin Fibres (Fig. 1.14)

Little is known of the mechanical properties of reticulin. They are thinner than collagen but show a similar periodicity of 700 Å (0,07 µm). Melcher (1964) described three groups of fibrils: Interfibrillar reticulin related to the collagen fibres; a subepithelial reticulin and reticulin in the walls of blood vessels.

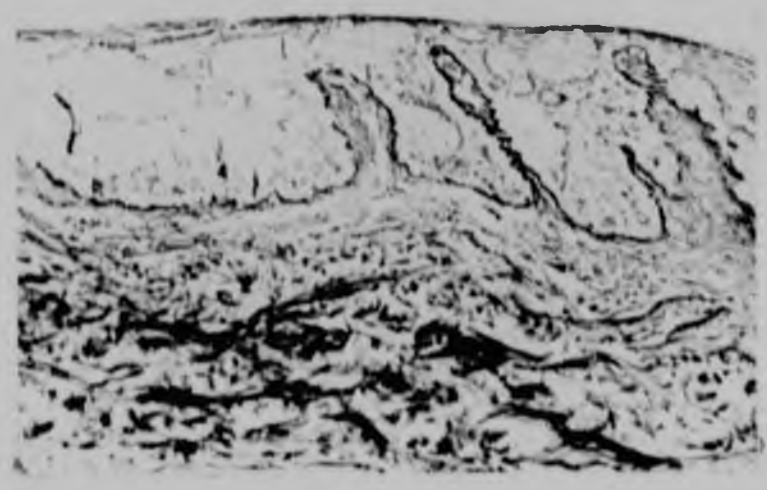

Fig. 1.14 Reticulin fibres in lamina propria of hard palate. Gordon and Swect's. Reticulin stain. x 150

1.3.4 Ground Substance

This is a term used to describe the amorphous semi-fluid substance contained in the spaces between the fibres. It contains no free fluid although it has a large water content. The major constituents are mucopolysaccharides, hyaluronic acid, chrondroitin sulphate B and glycoproteins.

1.3.5 The Submucosa

Consists of collagen fibres of varying density and thickness. It contains blood vessels, nerves, lymphatics and various inclusions which vary in amount depending on the particular region. These inclusions consist of adipose tissue, mucous glands and muscle fibres. The submucosa attaches the mucosa to the underlying structures (Fig. 1.8).

1.4 Regional Differences in the Oral Mucosa

The differences which exist between the various regions of the oral cavity extend from the keratin layer through all the other layers of the epithelium into the submucosa. The subject has been discussed by various workers. Cohen (1967) described the oral mucosa of the Macaque Iris monkey. Meyer and Gerson (1964) compared human palatal and buccal mucosa. Meyer, Medak and Weinman (1960), Demetriou and Ramfjord (1971) and Cleaton-Jones (1976) studied the mitotic activity and rate of growth in various regions of the oral cavity. Weinman et al. (1959), Meyer and Gerson (1964) and Fleisch et al.(1976) have reported the differences in cell density and size. Weinman (1940), Weinman and

Meyer (1959), Cohen (1967) and Meyer and Gerson (1967) have discussed the degrees of keratinization, while Cutwright and Hunsuck (1970) have detailed the micro-circulation of the peri-oral tissues.

1.4.1 Degrees of Keratinization

Cohen (1967) described the hard palate of the Macaque Iris as having a well developed keratin layer and the buccal mucosa as being non-keratinized. Alvares and Meyer (1971) and Cleaton-Jones and Fleisch (1974) stated that small islands of para-keratinization exist between the rugae of the palatal epithelium. Cohen (1967) described the dorsum of the tongue to exhibit ortho-keratosis, para-keratosis and non-keratinization. The surfaces of the papillae were keratinized. Meyer and Gerson (1964) described the keratin layer to be 32 μm thick on the buccal epithelium and the buccal epithelium to be non-keratinized. Meyer and Gerson (1964) stated that, whereas the four layers of the epithelium were easily distinguishable in the palatal epithelium, it was not possible to differentiate the layers in the buccal epithelium.

1.4.2 Cell Density

According to Alveres and Meyer (1971) there is an inverse relationship between the average cell size and the degree of keratinization. Cells of non-keratinized cheek epithelium average twice the size of the cells of ortho-keratinized palatal epithelium, and the cells of the epithelium of the alveolar mucosa are more than twice the size of the adjacent attached gingivae. This confirms the findings of Weinman et al.(1959) who stated that the cells of epithelium of the alveolar mucosa are

much larger than the attached gingiva, which had a slightly greater cell density, 12,3 cells per 100 μm^2, as compared to 10,5 cells per 100 μm^2. Although the cells were more closely packed in the alveolar mucosa due to the absence of intercellular bridges, the small nuclei per unit area is due to a larger average size of the cells. Meyer and Gerson (1964) state that the average cell size in buccal epithelium was nearly twice that in the palate. Fleisch, Cleaton-Jones and Austin (1976), in a histometric analysis comparing alveolar mucosa to attached gingiva, found more cells in the alveolar mucosa than in the attached gingiva.

1.4.3 Dendritic Cells

Sagebiel, Clarke and Hutchens (1971) describe three types of dendritic cells: Melanocytes, Langerhans cells and non-specific cells.

Hutchens et al.(1971) showed that the number of dendritic or clear cells is higher in keratinized tissues and the dorsum of the tongue contains the largest number. Barker (1967) estimated that the dendritic cells constitute approximately 10 per cent of the basal cell layer.

1.4.4 Lamina Propria and Submucosa

Cohen (1967) described the corium underlying the epithelium of the buccal mucosa as consisting of loose networks of collagenous fibres with numerous fibroblasts and connective tissue cells and numerous blood vessels. There were collections of mucous and serous glands and some of these glands were found in the striped muscle layer. In the palate the corium was described as containing bundles of collagen fibres

between which many blood vessels and mucous glands were prominent along the lateral margins of the palate. Cohen (1967) also suggested that the oral mucosa of the Macaque Iris was identical to that of man.

Scapino (1967) reported the connective tissue of the lamina propria of the palatal tissue to consist of two layers : a superficial or reticular layer and a deeper longitudinal layer which ran at right angles to the more superficial layer.

CHAPTER 2

DETERMINATION OF NORMAL EPITHELIAL PARAMETERS IN THE VERVET MONKEY BEFORE TISSUE LOADING

2.1 Introduction

Prior to studying loaded tissue it is necessary to determine, quantitatively, normal parameters in the tissues to be loaded. In the choice of the experimental tissue there are many problems, both practical and ethical, in experimental investigations, which preclude the use of human tissue. Other primates such as the Vervet monkey (Cercopithicus aethiops), Macaca fascicularis, and Rhesus (Macaca mulatta) are therefore often used because of their similarity, both morphologically and histologically, to humans. In South Africa the Vervet Monkey (Cercopithicus aethiops) has been extensively used in dental research, (Ockerse, 1959; Cleaton-Jones and Fleisch, 1972; Fleisch, 1973; Retief and Austin, 1973; Volchansky and Cleaton Jones, 1973). This animal was therefore chosen as the experimental model for the study. The monkeys used in the present study were obtained from the National Institute for Virology. They were caught in the Northern and Western Transvaal, and after a period of not less than six weeks quarantine, were killed to provide kidney tissue for the manufacture of poliomyelitis vaccine.

The aim of this experiment was to determine quantitatively the regional differences in the oral mucosa prior to loading for a comparative study.

2.2 Materials and Methods

Forty adult Vervet monkeys with clinically healthy oral tissues were selected post-surgically from batches of nephrectomised animals. The

anaesthetized monkeys were killed by decapitation, and the heads immediately immersed in 10 per cent buffered neutral formol saline solution. The solution was changed after 24 hours (Lillie,1965). After five days of fixation, specimens were taken from the following areas :

1. Cheek - sections of cheek approximately 10 mm^2 of full thickness, that is, oral epithelium through to skin, were taken from areas approximately 10 mm distal to the commissure of the mouth.

2. Tongue - sections of tongue approximately 10 mm^3 were taken 10 mm posterior to the tip of the tongue in the mid-line.

3. Palate - sections of hard palate approximately 10 mm^3 with the bone attached, were taken from areas midway between the centre line and the teeth opposite the first and second maxillary molars.

4. Alveolar mucosa - sections of mandible approximately 10 mm^3 complete with soft tissues, were taken from the area of the first and second molars.

In the cheek and tongue the specimens were removed using a scalpel. In the palate and alveolar mucosa the specimens were removed, attached to underlying bone, with a bandsaw. Care was taken not to distort the tissue.

In an earlier study, Cleaton-Jones and Fleisch (1973) classified the oral epithelium into orthokeratinized (hard palate), parakeratinized (valleys between rugae of hard palate, attached gingiva, free gingiva and lip), and non-keratinized (soft palate, cheek and floor of the mouth).

The above areas were chosen because each tissue had different characteristics. Two tissues were keratinized (tongue and palate), but one was backed by bone (palate), and one was not (tongue). Two were non-keratinized (cheek and alveolar mucosa); one backed by bone (alveolar mucosa) and one not (cheek). These combinations cover the majority of variations which occur in the oral cavity.

2.2.1 Histological Preparation Techniques

The blocks of tissue containing bone and teeth as in the palatal and alveolar mucosal tissues were decalcified in five per cent nitric acid until no calcified material was radiographically evident. Dehydration was in graded ethanol series followed by clearing in chloroform and embedding under vacuum in Paraplast (Sherwood Medical Industries, St. Louis, Missouri) under vacuum. Serial sections were cut at 7 µm intervals on a Leitz rotary microtome or Reichert sledge microtome. The sections were floated out on a distilled water bath onto clean glass slides and dried on a heated drying plate.

Twenty-five µm step serial sections were cut at every hundredth section and stained with orcein (Shellow and Kligman, 1967), to demonstrate the presence and orientation of elastic fibres in the lamina propria. The first five slides were stained with haematoxylin and eosin, phosphotungstic acid haematoxylin, Masson trichrome stain, picromallory and reticulin stains respectively. The following five slides were left unstained. This sequence was followed until the block was complete.

2.2.2 Staining Techniques

The following methods were employed :

1. Harris' haematoxylin and eosin (Culling, 1963) to demonstrate the cellular elements of the epithelium

2. Masson's trichrome stain (Culling, 1963) to demonstrate the collagen fibres.

3. Taenzer Unna's orcein (Culling, 1963) to demonstrate the presence of elastic fibres.

4. Gordon and Sweet's reticulin stain (Culling, 1963) to demonstrate the presence of reticulin fibres.

5. Picromallory (Royal Post-Graduate Medical School 1958) to demonstrate the presence of keratin.

6. Phosphotungstic acid haematoxylin to demonstrate the cellular elements plus the collagen fibres.

The sections were examined with a Reichert Univar light microscope and photographed with a Kodak Panatomic [illegible] mm film developed in a 1:1 dilution of Ilford ID33, for four minutes at 20°C. Filters were used where necessary to improve photographic contrast.

2.2.3 Determination of Epithelial Thickness

2.2.3.1 Introduction

A review of the literature reveals that there are several methods used to compare the morphology and thickness of the epithelium and/or mucoperiosteum. Wentz et al.(1952) described a non-histometric method using two parameters in grading the morphology of the epithelium namely :

The supra-papillary width and the length of the rete pegs or ridges.

Each was divided into three gradings, thick, medium and thin for the supra-papillary width, and short, medium and long for the rete peg length, the criteria being the length or width of the tissue concerned. They also classified the epithelium into two histiotypes according to the combination of the length of the ridge and the supra-papillary width. A method comparing the connective tissue qualitatively according to texture was also described. Turesky, Glickman and Fisher (1959) used an occular micrometer and classified the epithelium according to measurements of rete peg length and supra-papillary width. They measured the three longest epithelial ridges in each section and the narrower adjacent supra-papillary width.

Meyer, Nedak and Weinman (1960) used a combination of occular micrometry and planimetry. In the floor of the mouth and the palate, the micrometer was used to measure the thickness of the epithelium, and the measurements averaged to obtain a representative thickness. The thickness of the epithelium of the cheek was determined by planimetry. The planimeter readings were made from projected sections magnified 100 times.

Fleisch (1973),using micro-photographs enlarged 150 times, measured the thickness of the mucoperiosteum at three levels of the attached gingiva using vernier calipers to the nearest 1 mm. The length of the rete pegs and the adjacent supra-papillary widths were measured.

After carefully considering all these methods the technique to be described in the next section, was used.

2.2.3.2 Materials and Methods

A Bausch and Lomb eyepiece graticule was standardised against a Zeiss graduated slide and used at a 10 times magnification so that the

ocular units could be converted into units of measurement. Two measurements were taken (Fig. 1.15) :

(A) the length of the rete peg defined as the mean of the three longest pegs in a randomly selected field, and

(B) supra-papillary widths defined as the distance from the surface to the base of the epithelium at the tip of the connective tissue papilla. The mean of three narrower widths adjacent to those measured for (A) was determined for each section.

From each specimen 10 slides with approximately 5 - 8 sections of 7 μm on each slide were selected, and from each slide five sets of measurements (A) and (B) as described above were obtained. Each specimen therefore, produced 50 sets of measurements and for each type of tissue there were 10 specimens producing 500 sets of measurements. As there were four types of tissue, the total number of measurements were 2,000 sets, that is 2,000 of (A) and 2,000 of (B) (Fig.2.1). These measurements were then transferred onto I.B.M. Punch Cards and analysed in an I.B.M. 370/518 Computer using the Statistical Package for the Social Sciences (Nie et al., 1970).

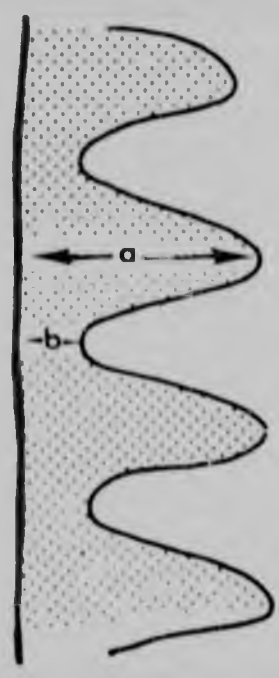

Fig. 1.15 Diagram showing the sites for measurement of epithelial width

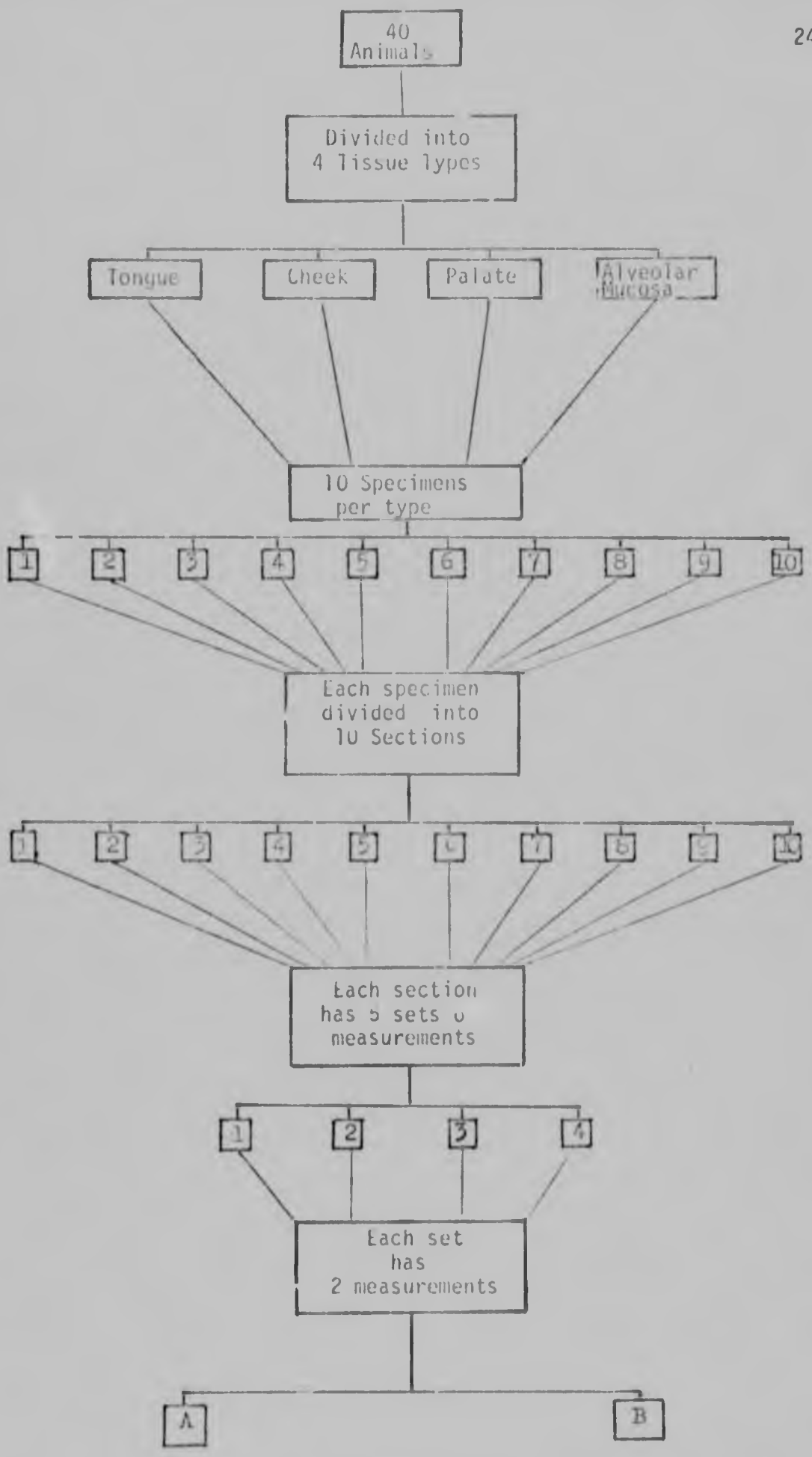

Fig. 2.1 Scheme showing subdivision of specimens for histometric analysis

2.2.3.3 Results (See Table I)

The tongue had a mean rete peg length (A) of 1081 µm, the cheek 390 µm, palate 427 µm and the alveolar mucosa 278 µm. The corresponding suprapapillary width (B) measurements were 95 µm, 57 µm, 35 µm and 28 µm. This gave epithelial widths $\frac{(A + B)}{2}$ of 692 µm for the tongue, 261 µm for the cheek, 281 µm for the palate and 186 µm for the alveolar mucosa.

Table I - Comparison of Epithelial Widths in µm

	Rete peg length (A)			Suprapapillary width (B)			Epithelial width $\frac{(A+B)}{2}$		
	n	$\bar{x}$	± SD	n	$\bar{x}$	± SD	n	$\bar{x}$	± SD
Tongue	500	1081	±147	500	304	± 95	500	692,0	± 121,0
Cheek	500	390	± 87	500	132	± 57	500	261,0	± 71,9
Palate	500	427	± 74	500	135	± 35	500	281,0	± 54,5
Alveolar Mucosa	500	278	± 49	500	94	± 28	500	186,0	± 38,4

2.2.4 Determination of Tissue Shrinkage

2.2.4.1 Introduction

A problem associated with the processing of tissues for histological examination is that of shrinkage associated with the infiltration of paraffin wax (Brain, 1966). The degree of shrinkage in this phase of processing depends on several factors. Two major factors are, the consistency of the tissues, whether they be bone, soft tissues or both and the properties of the reagents used. Shrinkage takes place at various stages of preparation of the tissues.

1. Fixation
2. Dehydration
3. Clearing and embedding

Stroud (1967) states that the average shrinkage during fixation was 15,8 per cent. According to Brain (1966) the total shrinkage varied considerably depending upon the dehydrating and clearing agents used. For example tissue fixed in 10 per cent aqueous formalin, dehydrated in ethyl alcohol and cleared in chloroform shrank 29,3%. Tissue fixed in 10 per cent aqueous formalin, decalcified in 4N Formic acid, dehydrated in ethyl alcohol and cleared in methyl salicylate shrank 31,4%. Tissue fixed in 10 per cent formalin dehydrated in ethyl alcohol and cleared in Xylene shrank 31 per cent (Brain, 1966).

In order to relate the histometric findings to in vivo conditions, a study was made to determine the degree of tissue shrinkage from the fresh (in vivo) state to the stained sections used for histometric analysis.

2.2.4.2 Materials and Methods

Sixteen adult Vervet monkeys were obtained immediately post-nephrectomy from the National Institute for Virology. The animals were decapitated and the maxillae sectioned with a bandsaw in the coronal plane distal to the canine teeth, using the inferior border of the midline palatal suture as the fixed point of reference. The distance between the latter and the palatal epithelium was measured with Vernier calipers. The tissues were then fixed in 10 per cent neutral buffered formol saline, embedded, cleared and sectioned as described in section 2.2.1 and stained with haematoxylin and eosin (Culling, 1963). The sections were again measured with Vernier calipers and the results calculated. The mean percentage shrinkage was shown to be 34,7% (Fig. 2.2).

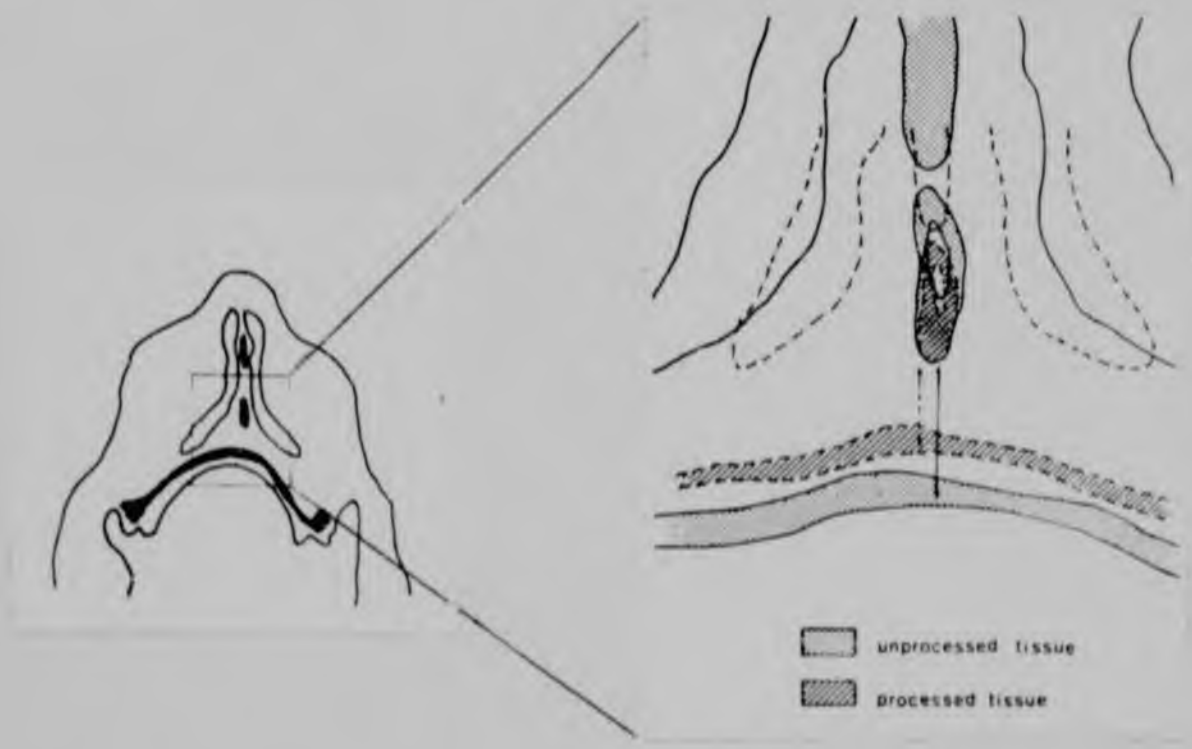

Fig. 2.2 Diagram showing degree of shrinkage during histological processing

2.2.4.3 Discussion

The percentage shrinkage of 34,7% of tissue after fixation, embedding, sectioning and staining indicates that in order to equate the histometric results with the actual measurements in vivo, one would have to increase the measurements obtained by 52,1% to obtain the true measurements. However, in the comparative studies such absolute figures are unnecessary, relative values being acceptable. The results obtained in the histometric analysis have not been corrected to allow for this shrinkage.

2.2.5 Method Used for Cell Density Calculations

Sections of each type of tissue (palate, alveolar mucosa, cheek and tongue), were examined using an eyepiece graticule. The number of cells in a

700 μm^2 area was determined in 50 randomly selected fields for each type of epithelium. The findings were computerized as described in Section 2.2.3.2.

2.2.5.1 Results (Table II)

The alveolar mucosa had the highest cell density of 5,6 cells/700 μm^2. The tongue had a cell density of 4,1 cells/700 μm^2, the palate 3,5 cells/700 μm^2 and the cheek 3,4 cells/700 μm^2. The nuclei of the alveolar mucosa and cheek epithelial cells were one-half to two-thirds in size of those of palatal and tongue epithelial cells.

Table II - Cell Density - Normal Tissue

	Mean cell densities/700 μm^2		
	n	$\bar{x}$	$\pm$ SD
Tongue	50	4,1	$\pm$ 1,0
Cheek	50	3,4	$\pm$ 0,9
Palate	50	3,5	$\pm$ 0,8
Alveolar Mucosa	50	5,6	$\pm$ 1,2

2.3 Results

2.3.1 Keratin

The presence of keratin was observed only in the palatal and tongue epithelia. In the palate the epithelium was predominantly ortho-keratinized with areas of parakeratosis seen, particularly in the valleys between the rugae. The tongue exhibited a variable amount of ortho-keratinization on the filliform papillae.

A granular layer indicating the presence of keratinohyalin granules was only observed in the palatal region (Fig. 2.3). No keratin was observed in the alveolar mucosa or cheek.

Fig. 2.3 Ortho-keratinized epithelium of the palate showing granular layer (arrowed) beneath the keratin layer. H and E x 240

2.3.2 Configuration of the epithelium

The regions differed strikingly in the configurations of the epithelial-connective tissue interfaces. The palatal region exhibited narrow rete pegs with correspondingly narrow connective tissue papillae which penetrated deeply into the lamina propria to produce a regular pattern (Fig. 2.4). The epithelium of the tongue also exhibited narrow deep rete pegs but produced a much more irregular pattern than the palatal epithelium (Fig. 2.5). The epithelial surface was very irregular with numerous projections which produced the filliform papillae. The cheek epithelium had broad, blunt rete pegs (Fig. 2.6), with a very irregular epithelial connective tissue interface and no suggestion of a particular pattern (Fig. 2.7). The epithelial surface was smooth. The epithelium of the alveolar mucosa had blunt, shallow rete pegs with connective tissue papillae narrower than the corresponding rete pegs. The epithelial connective tissue interface exhibited a relatively even pattern and the epithelial surface had an irregular appearance (Fig. 2.8).

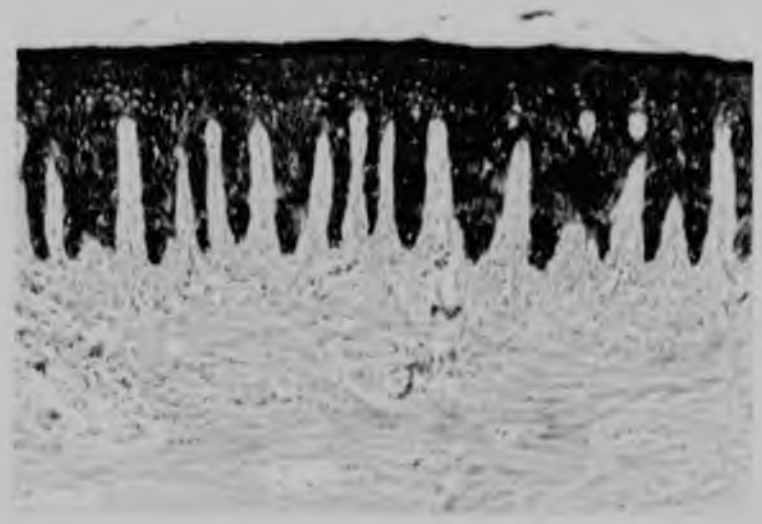

Fig. 2.4 Section of hard palate showing deep regular rete pegs and narrow connective tissue papillae. Picromallory x 90

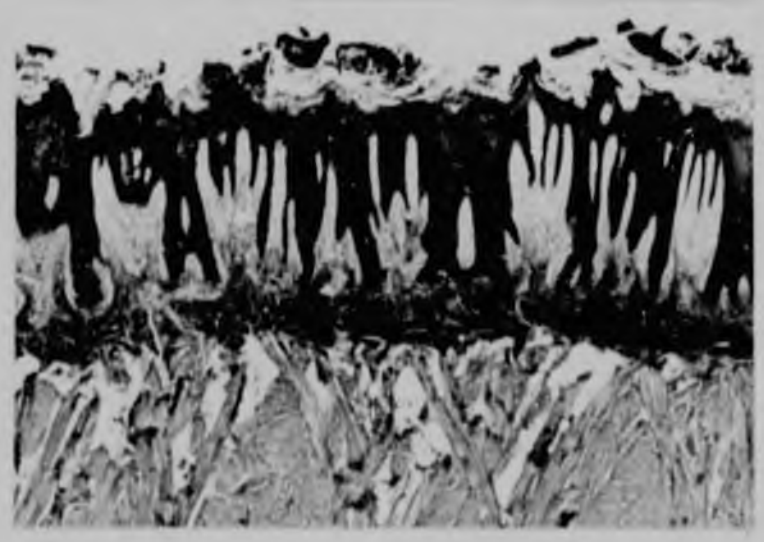

Fig. 2.5 Section through tongue showing irregular rete pegs of varying length. P.T.A.H. x 140

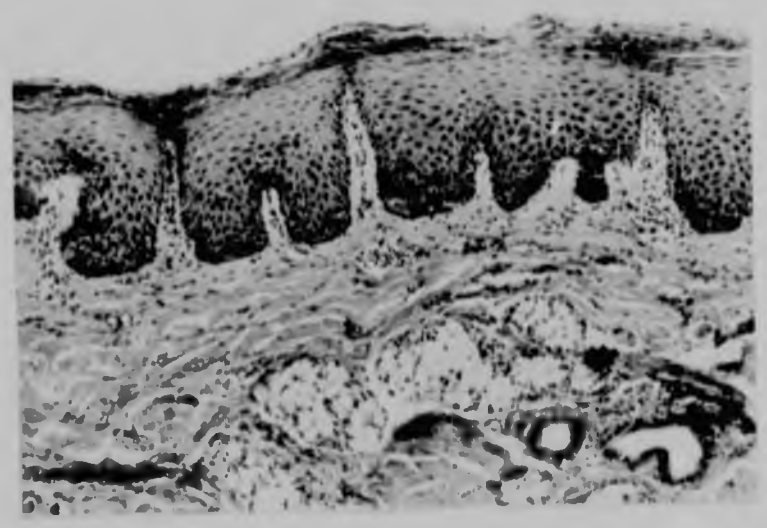

Fig. 2.6 Section through cheek mucosa with broad irregular rete pegs
Masson x 90

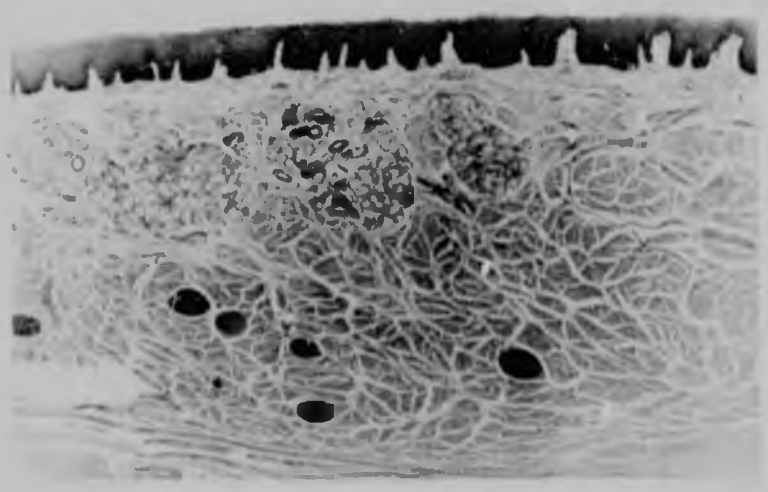

Fig. 2.7 Section through cheek mucosa showing irregular pattern of the interface. H and E x 38

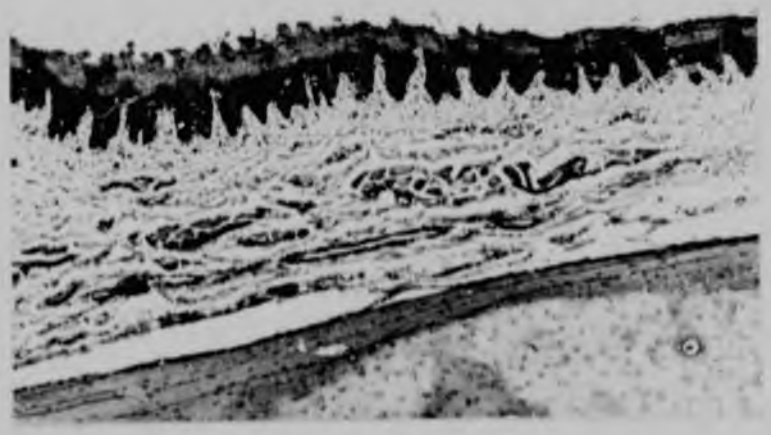

Fig. 2.8 Section of alveolar mucosa showing narrow connective tissue papillae and irregular epithelial surface. H and E. x 38

2.3.3 Cell Morpnology

The cells in the basal and prickle cell layers in the palate and tongue appeared angular and irregularly polygonal. In the more superficial layers they appeared flattened and lay parallel to the surface. Intercellular bridges were only seen in the palatal epithelium (Fig. 2.9). In the alveolar mucosa the cells of the basal and prickle cell layer appeared to be more rounded but still possessed some angularity (Fig. 2.10). In the cheek the cells were rounder with little angularity (Fig. 2.11).

Fig. 2.9 Prickle cells in the hard palate showing angularity and irregularity of cells. Note intercellular bridges. H and E. x 600

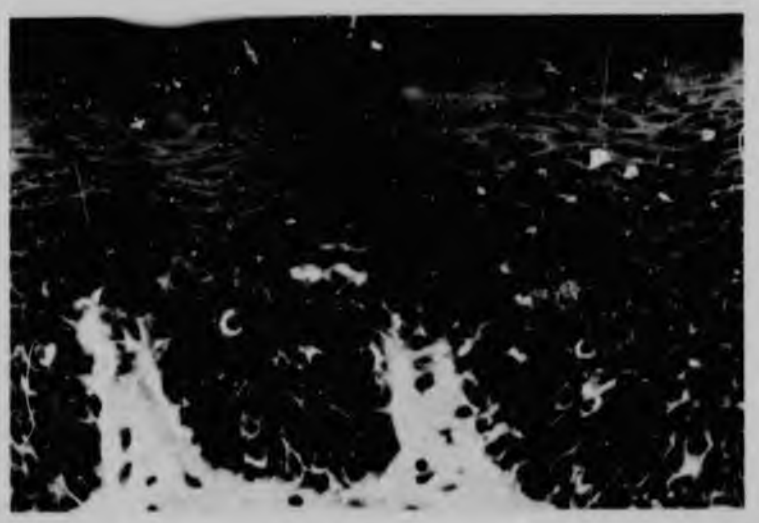

Fig. 2.10 Section of alveolar mucosa showing angularity of the prickle cell and basal cell layers Masson x 360

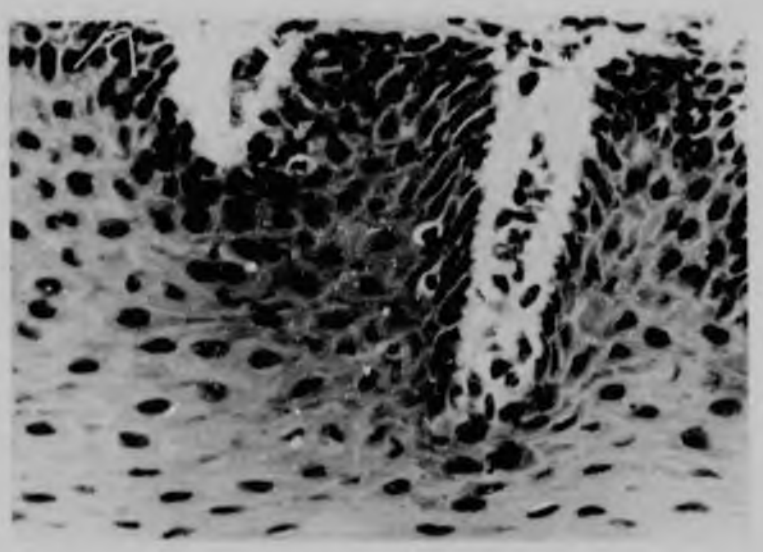

Fig. 2.11 Section through cheek epithelium showing more rounded cells than in Fig. 2.9 and 2.10. H and E. x 360

2.3.4 Dendritic Cells (Figs. 2.12, 2.13, 2.14 and 2.15)

Clear cells were seen in all four regions examined. They appeared to occur mainly in the basal or spinous layers with relatively few seen in the more superficial layers. They appeared to be most numerous in the tongue epithelium and least in the cheek epithelium. The clear

cells did not conform in either shape or size to the adjacent epithelial cells and varied considerably in size. It was not possible, with the technique used, to differentiate between the types of dendritic cells.

Fig. 2.12 Clear cells in the alveolar mucosa. H and E x 360

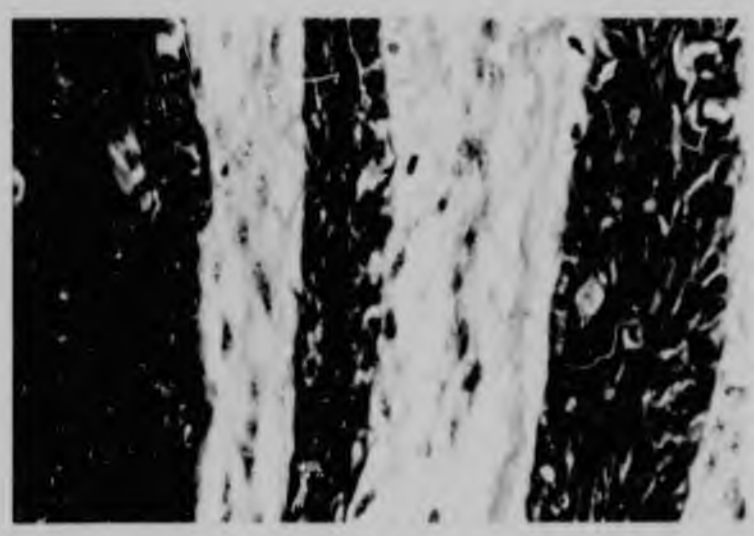

Fig. 2.13 Clear cells in tongue epithelium. P.T.A.H. x 500

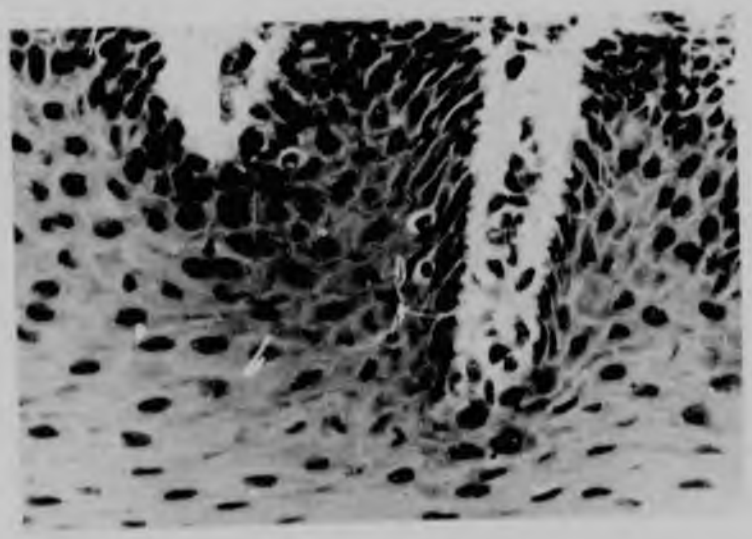

Fig. 2.14 Clear cells in the epithelium of the cheek. H and E. x 360

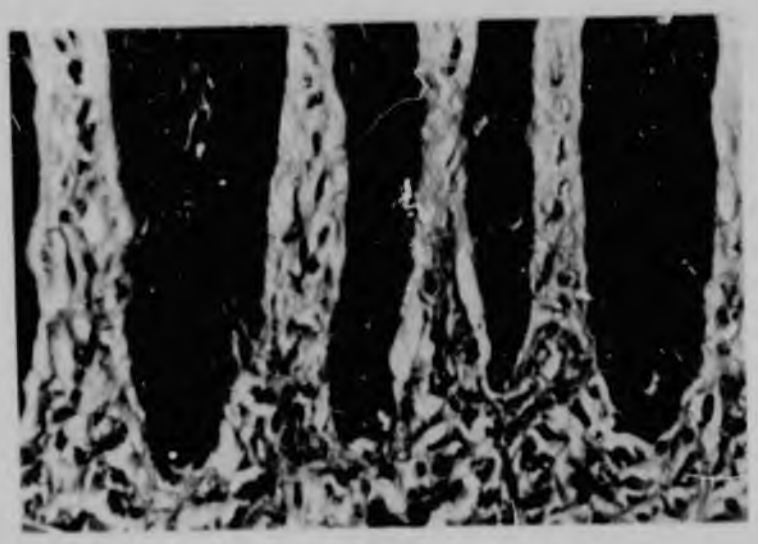

Fig. 2.15 Clear cells in the palatal epithelium. Picromallory. x 360

2.3.5 Lamina Propria and Submucosa

The collagen fibres in the lamina propria of the pala - appeared extremely dense and thick and were arranged in two layers, a superficial or papillary layer just beneath the epithelium and a deeper longitudinal or reticular layer running at right angles to the reticular layer (Fig.2.16).

A similar appearance was seen in the tongue, with the longitudinal layer being very prominent (Fig. 2.17). In the cheek the collagen fibres were finer and less densely packed together without differentiation into layers (Fig. 2.18). In the alveolar mucosa the collagen fibres were finer still, and arranged in a loose areolar network (Fig. 2.19). The reticular fibres appeared to follow the configuration of the collagen fibres.

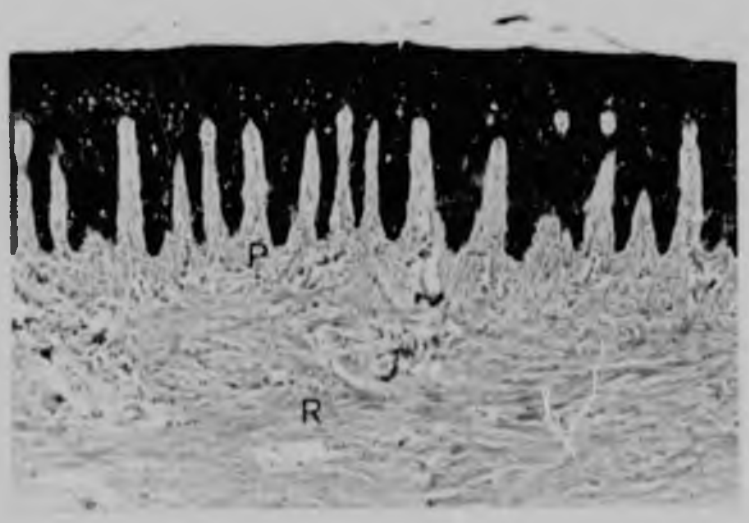

Fig. 2.16 Lamina propria of palatal epithelium showing papillary (P) and reticular (R) layers of collagen fibres. Picromallory. x 90

Fig. 2.17 Lamina propria of the tongue showing the papillary layer (P) and prominent longitudinal layer (L). P.T.A.H. x 150

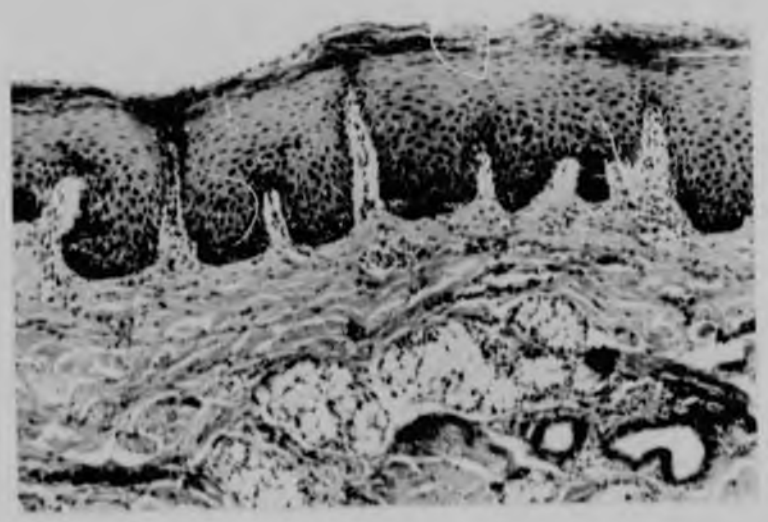

Fig. 2.18 Lamina propria of cheek showing collagen fibres without differentiation into layers. Masson x 90

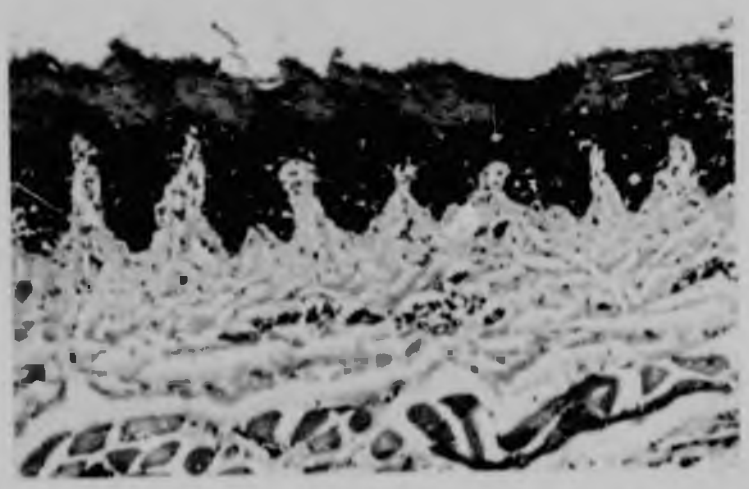

Fig. 2.19 Lamina propria of alveolar mucosa showing loose areolar arrangement of collagen fibres. H and E x 90

2.3.6 Elastic Fibres

These were seen in all the tissues examined except in the palate. In the alveolar mucosa the fibres were extremely numerous and were variable in diameter. They were arranged in a sub-epithelial plexus parallel to the epithelium and a longitudinal collection approximately midway

between the epithelium and the underlying bony structure (Fig. 2.20). In the cheek the elastic fibres were fine and scanty and also ranged roughly parallel to the epithelial surface (Fig. 2.21). In the tongue the fibres appeared to be present mainly in the connective tissue papillae and around the rete pegs (Fig. 2.22 and 2.23).

In the submucosa of the palate, blood vessels, mucous glands and fat cells were often seen. In the tongue the submucosa consisted of muscle fibres arranged in bundles held together with collagen and the submucosa of the cheek contained muscle fibres and mucous glands.

Fig. 2.20 Elastic fibres in alveolar mucosa showing subepithelial plexus (P) and deeper longitudinal layer (L) 25 μm section Orcein x 240

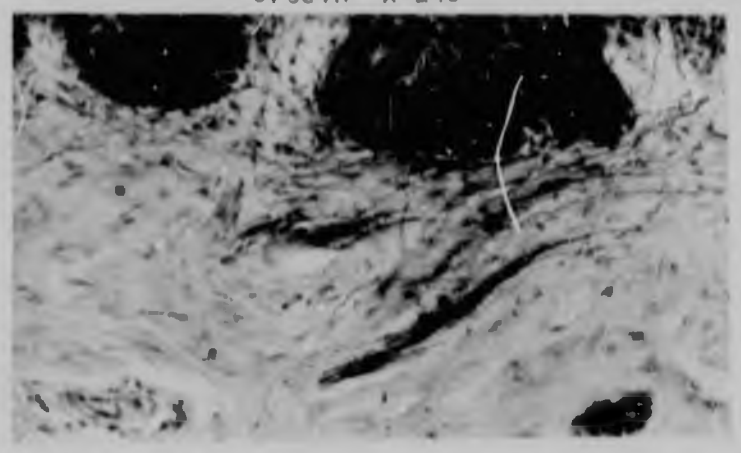

Fig. 2.21 Fine elastic fibres in the mucosa of the cheek. 25 μm section Orcein x 240

Fig. 2.22 Elastic fibres in the connective tissue papillae in the tongue 25 µm section. Orcein x 150

Fig. 2.23 Higher magnification of elastic fibres in the tongue similar to those shown in Fig. 2.22

2.4 Discussion

The four areas described have each been shown to have features specific to that tissue. Although the palate and tongue both have predominantly keratinized surfaces, the surface characteristics were extremely varied.

In some sections the areas between the rugae in the palate and between the filliform papillae in the tongue were para-keratinized. This is in agreement with the findings of Meyer and Gerson (1964), Cohen (1967) and Cleaton-Jones and Fleisch (1974). The palate had a smooth keratinized surface well suited to resist abrasive and tensile forces. The surface of the tongue, however, has been adapted for the functional demands required of it, such as an increased surface area for tactile nerve endings (Ham, 1969) and to aid in the manipulation of the bolus of food. The irregular "saw tooth" appearance of the epithelial surface of the alveolar mucosa may be related to the linear folds described by Whittaker and Adams (1971) and Fleisch, Cleaton-Jones and Austin (1976), which run parallel to the muco-gingival junction and are probably due to the orientation of the underlying elastic fibres. According to Meyer and Gerson (1964), in the palatal epithelium, the combination of a keratin layer and tonofibrils, give the cells rigidity and cohesion to provide the capacity to withstand stress. Such properties are not required by the other tissues. In fact, the absence or scarcity of tonofibrils and the rounder, less angular cells of the non-keratinized epithelium of the cheek and alveolar mucosa suggest that these cells may move over each other along their contact surfaces (Meyer and Gerson, 1964). Osmanski and Meyer (1967) suggested that, apart from the smaller nuclei, and the size of the tonofibrils, the highly convoluted cell borders of the cells of lining epithelium, aid in allowing the cells of non-keratinized epithelium to move over one another. According to Osmanski and Meyer (1967), Meyer and Gerson (1964) and Chen and Meyer (1971), there is a direct relationship between the concentration of tonofibrils and the degree of keratinization.

The width of the epithelium varied from 185,99 µm in the alveolar mucosa to 692,16 µm in the tongue. The tongue, however, should be excluded in this instance, as it has a specialized epithelial configuration which cannot be favourably compared with that of the other three tissues. The palatal epithelium had the greatest overall thickness, of 281,02 µm. and the longest rete pegs 426,77 µm as compared to the cheek and alveolar mucosa. This highly convoluted epithelial connective tissue interface of the palate has a dual function of providing a large surface area to provide for a voluminous flow of nutrients and to afford greater anchorage between the epithelium and underlying connective tissue (Scapino, 1971). In contrast, the epithelial-connective tissue interface of the cheek and alveolar mucosa allow for a greater degree of mobility of the epithelium. The epithelial width shown in Table II shows that the greatest thickness is in the tongue followed by the palate, cheek and alveolar mucosa. This is almost exactly the opposite of the figures given by Gigoux (1962) in a study on the rabbit. Meyer, Medak and Weinman (1960) in a study on mice showed the greatest epithelial thickness to be in the cheek. A possible explanation of these differences is that firstly, the presence and degree of keratinization varies in the different species, and secondly, that functional demands in relation to the diet of these animals is different. These two factors could modify the morphology of the tissues considerably. In comparing cell densities, the highest density was found in the alveolar mucosa, 5,6 cells/700 μm^2 and the lowest in the cheek, 3,4 cells/700 μm^2. This again is in contrast to that found in humans (Alvares and Meyer, 1971). The high cell density in the alveolar mucosa in the vervet monkey may be associated with the high cell turnover in this tissue as a result of trauma and cell loss during mastication

Dendritic cells were seen in all the tissues examined. The greater number of these cells seen in the tongue and palate confirms the work of Hutchens et al.(1971) who suggested that the presence of an increased number of dendritic cells may be correlated with a slow turnover rate and a high degree of keratinization. It was impossible to ascertain which layers of the epithelium contain the most dendritic cells.

The wide variations in the morphology, content and density of the lamina propria of the four areas described above, makes it difficult to correlate a specific type of epithelium with specific features in the submucosa. However, the presence of a keratin layer as in the palate and tongue, appears to be consistent with a dense collagen network running approximately parallel to the epithelial surface. The collagen in the palatal lamina propria can be described as consisting of two layers, a superficial reticular layer and a deeper longitudinal layer. This is similar to that described by Scapino (1967) in the dog. This arrangement of the collagen fibres in the palate is consistent with the need for this tissue to withstand the forces applied to it during mastication. In the lamina propria of the alveolar mucosa, the collagen fibres are thin and loosely orientated. This is once again consistent with the reaction of the tissue to forces applied to it.

The submucosa of the tongue and cheek have several characteristics in common. The collagen fibres run parallel to the surface. The orientation of these fibres is generally uniform and consistent and in each tissue the submucosa contains muscle fibres.

The high density of elastic fibres in the alveolar mucosa can be explained as follows. The three tissues which contain elastic fibres are the

alveolar mucosa, cheek and tongue, with the latter two showing considerably less elastic fibres than the alveolar mucosa. However, both the cheek and tongue have muscle fibres in the submucosa whilst the alveolar mucosa has none. If we consider Treagar's (1966) statement that the elastic fibres generate the motor force that restores the deformed tissue to its resting state, and then maintains the unloaded tissue in that state (Dick, 1951), then the elastic fibres in the alveolar mucosa take on the role of the muscle fibres in the other tissues. This would explain the greater density of elastic fibres in the alveolar mucosa.

It is apparent from the foregoing discussion that the differences which exist in the four tissues described, extend through all the layers of the epithelium and submucosa. Such differentiation of the various components of the mucosa may be considered to be the end result of different pathways of histo-differentiation based on the functional demands placed upon the various tissues.

PART II

THE EFFECTS OF MECHANICAL LOADING ON THE ORAL MUCOSA

CHAPTER 3

DEVELOPMENT OF THE LOADING TECHNIQUE

3.1 Introduction

Before proceeding with the main study, a preliminary investigation was carried out to ascertain the feasibility of the project. A review of the literature indicated that the only studies which had been done were on dogs by Scapino (1967), Stroud (1967) and Kydd *et al.*(1969). The *in vivo* response of lining mucosa did not appear to have been investigated.

The aim of this experiment was to develop apparatus which would reproducably and accurately apply a known load to a defined area of the oral mucosa.

3.2 Materials and Methods

Sixteen adult Vervet monkeys were obtained immediately post-nephrectomy from the National Institute for Virology. They had been anaesthetized with hexabarbitone sodium administered intravenously. A hollow brass cylinder was fixed to the upper pre-molar distal to the canines using self-curing acrylic resin and cyanoacrylate as adhesive (Fig. 3.1). Using the hollow brass cylinders as a base, adjustable brass loading dowel pins 3,17 mm in diameter were adjusted so that the pins would

Fig. 3.1 Fixation of apparatus to teeth with acrylic resin and cyanacrylate. Plunger has been placed against alveolar mucosa

apply a force at right angles to the tissue to be studied, which were hard palate, cheek, alveolar mucosa and attached gingiva (Fig. 3.2) Using a Dontrix orthodontic-strain gauge, the dowel pins were loaded with a force of 60 gm. The dowel pins were fixed in position by tightening a set screw (Fig. 3.3). The pressure produced was therefore 7,5 gm/mm^2. Within two minutes of the application of the load the animals were killed, decapitated, and the heads immersed in 10 per cent neutral buffered formol saline for five days, after which the appliances were removed and the tissues beneath the dowels, together with some surrounding tissues, were removed and processed as described in Section 2.2.1. The sections were stained with haematoxylin and eosin and Masson stains.

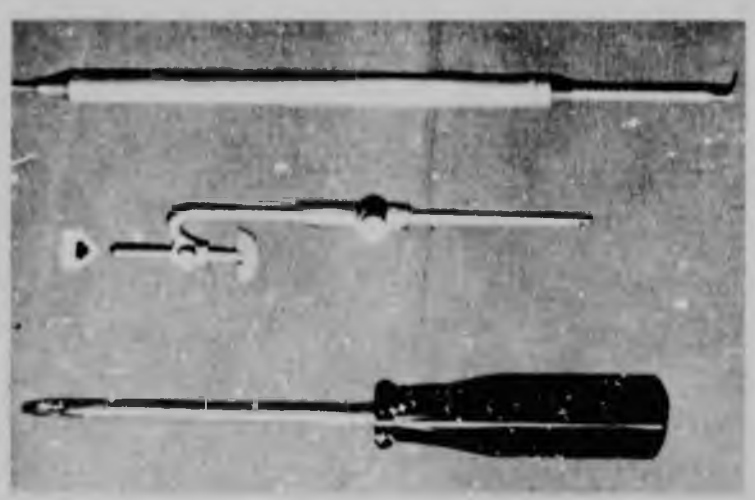

Fig. 3.2 Adjustable loading dowel in hollow brass cylinder and Dontrix strain gauge

Fig. 3.3 Dowel pin in position loaded with 50 gm force onto cheek mucosa

3.3 Results

3.3.1 Palatal Mucosa

Slight narrowing of the epithelium and reorientation of the collagen fibres in the lamina propria were seen. The rete pegs appeared rounder with narrowing of the connective tissue papillae. There was a tendency for the more superficial layers to move away laterally from the area of maximum load to produce an angulation of the rete pegs, but there was little observable change in the cells themselves.

3.3.2 Attached Gingiva (Figs. 3.4 and 3.5)

In some sections there appeared to be a greater degree of compression of the epithelium than in the palatal tissue. In other specimens the appearance was similar to that seen in the palate. The collagen fibres of the lamina propria also exhibited considerable variations ranging from complete reorientation of the fibres to lie parallel to the epithelium, to sections where no reorientation was seen. The cells and nuclei of the various layers of the epithelium, except the basal layers, exhibited some flattening.

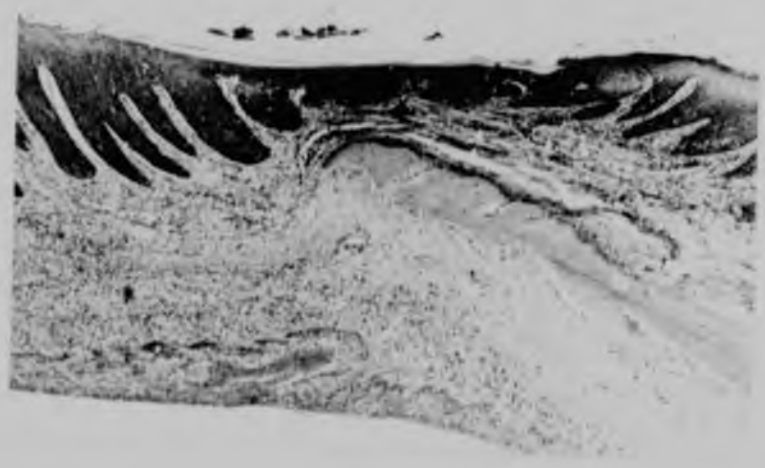

Fig. 3.4 Loaded attached gingiva overlying prominent alveolar bone
H and E. x 50

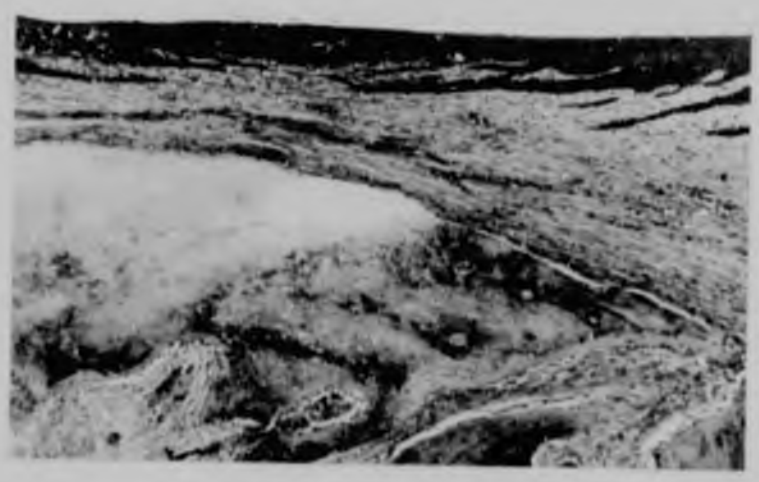

Fig. 3.5 Loaded attached gingiva overlying flat alveolar bone
H and E. x 60

3.3.3 Alveolar Mucosa (Fig. 3.6)

There was considerable distortion of the overall morphology of the tissues with the indentation produced by the edges of the dowel pins clearly visible. The epithelium had become markedly flattened with the complete disappearance of the rete pegs in connective tissue papillae. The cells and nuclei had been very flattened.

Fig. 3.6 Loaded alveolar mucosa showing indentation produced by dowel. Note thinning of the epithelium. H and E. x 15

3.3.4 Cheek (Fig. 3.7)

The appearance of this tissue was similar to that seen in the alveolar mucosa but with even more overall distortion and even some degree of tearing of the epithelium.

Fig. 3.7 Loaded cheek epithelium showing sharp edges produced by dowel with some tearing of the tissues. H and E. x 60

3.4 Discussion

The tissues of masticatory mucosa, namely, palatal mucosa and attached gingiva, offer resistance to mechanical loading with comparatively little distortion of the tissues. The tissues of lining mucosa however, adapted to the mechanical stress by distortion.

The response of the attached gingiva varied from extreme compression and distortion confined to a small area overlying a prominent protion of alveolar bone (Fig. 3.4) to an even compression of the soft tissues over a wide area overlying relatively flat alveolar bone (3.5). Thus the attached gingiva was not considered suitable as consistent results were not obtainable.

A further point emerging from the study was that the sharp right angle edge of the dowels produced a pattern which is not consistent with physiological forces which may be encountered in normal mastication.

3.5 Modifications of the Experimental Apparatus Required Before Proceeding with the Main Study

1. The method of attachments of the apparatus to the monkey heads needed to be improved as the self-curing acrylic and cyanoacrylate adhesives frequently separated.
2. The use of brass had to be discontinued due to the corrosion of the metal after five days in formol saline.
3. The use of a Dontrix orthodontic-strain gauge on each occasion was cumbersome and difficult.

4. The production of a sharp right-angled edge in the tissues was to be avoided to produce a more physiological load.
5. The inconsistencies in the results seen in the attached gingiva excluded this tissue as suitable for use in future studies.

CHAPTER 4

THE EFFECT OF LOADING OF THE TISSUE AFTER FIXATION

4.1 Introduction

Fixation, according to Brain (1966), is defined as "the process of preserving the tissues in a state simulating that which exists during life". Following this statement the question then arises - What is the effect of applying a mechanical load to tissues after they have been fixed, and if there are any changes, will they be similar to those found in tissues subjected to mechanical stress before fixation? In order to demonstrate the effect of the loading of tissue after fixation the following procedure was carried out.

4.2 Materials and Methods

Eight adult Vervet monkeys were used in this experiment. The animals were decapitated post-nephrectomy and the heads immersed in 10 per cent neutral buffered formol saline for 24 hours. The solutions were then changed. After five days a loading apparatus, modified according to the recommendations in Section 3.5, was attached to the heads and a 50 gm force applied as follows. In four of the animals the load was applied to the hard palate approximately 10 mm medial to the second premolar. In the other four animals the load was applied to the alveolar mucosa two to three millimetres below the muco-gingival junction beneath the second mandibular premolar. The modified apparatus and technique of application will be fully described in Section 6.2. After a further five days in the formol saline, the blocks of tissue to be processed were examined macroscopically and then removed and processed as described in Section 2.2.1. The sections were then examined microscopically.

4.3 Results

4.3.1 Macroscopic

Palatal tissue

On removal of the loading device no depression or other alteration in the tissues was discernible. The appearance was similar to the adjacent tissues.

Alveolar mucosa

A slight indentation of the tissue beneath the plunger was visible.

4.3.2 Microscopic

The palatal mucosa showed no changes in the morphology of the epithelium or lamina propria. The keratin layer appeared normal and there was no reorientation of the collagen fibres. In the palatal mucosa the slight indentation seen macroscopically was visible (Fig. 4.1). The folds or tags normally seen on the surface of the alveolar mucosa appeared to have been flattened. However, there was no alteration in the thickness of the epithelium or flattening of the cellular components of the tissues nor any reorientation of the collagen fibres of the lamina propria (Fig. 4.2).

Fig. 4.1 Post fixation loading of alveolar mucosa showing only slight indentation of epithelial surface. H and E. x 90

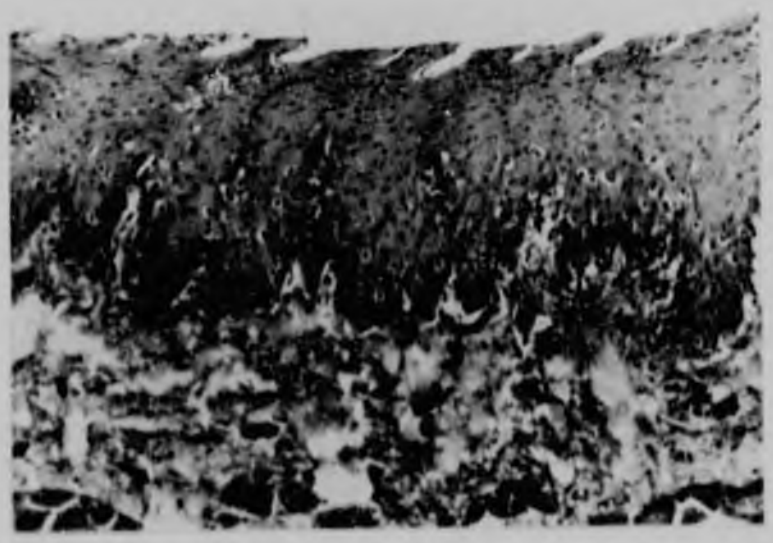

Fig. 4.2 Higher magnification of Fig. 4.1, showing no change except for flattening of the epithelial folds. H and E. x 150

4.4 Discussion

The effect of loading tissue after fixation appears to be minimal, if any. We can therefore consider that the results obtained in the present experiment when the tissues are loaded before fixation, are directly related to the fresh state of the tissues. The slight change seen in the alveolar mucosa in this study is in no way similar to those seen in the alveolar mucosa fixed after loading.

CHAPTER 5

RATE OF FIXATION OF SOFT TISSUES

5.1 Introduction

In all the experiments in this study the monkeys were decapitated and within two minutes of this, the heads were immersed in 10 per cent neutral buffered formol saline. This point in time has been taken as the time of fixation. However, in order to ascertain

1. when the fixation actually does take place, and
2. whether the contact of the plunger on the tissues inhibits or slows down fixation, the following study was carried out.

5.2 Materials and Methods

Eight mandibles were obtained from decapitated Vervet monkeys as described in Section 2.2. To four of the mandibles metal discs 5 mm in diameter were placed over the attached gingiva. All eight specimens were immersed in 10 per cent neutral buffered formol saline. After five minutes one specimen with the disc attached and one without, were removed and washed with normal saline. This was repeated after 10 minutes, 15 minutes and 30 minutes. After embedding and clearing and sectioning as described in Section 2.2.1, the tissues were stained with haematoxylin and eosin. The sections were then examined under light microscope.

5.3 Results

All the sections examined microscopically showed complete fixation of the tissues. No differences could be found at any of the time periods between the appearance of the control tissues and those under the disc (Fig. 5.1).

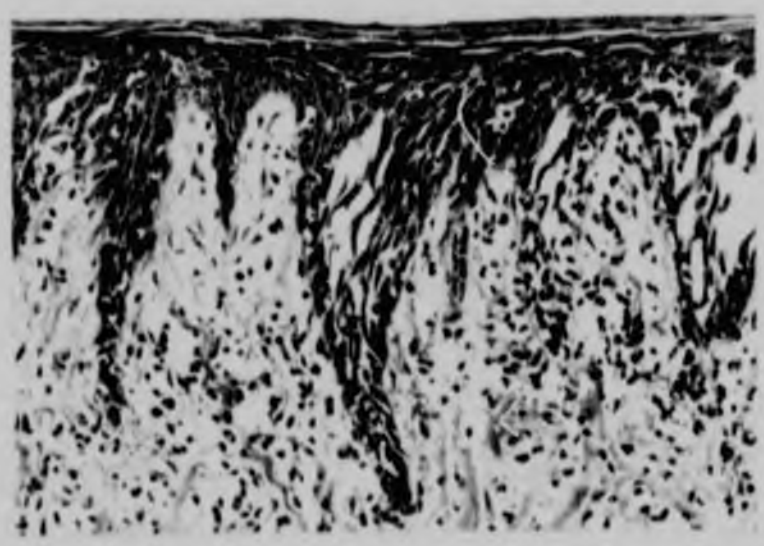

Fig. 5.1 Section of attached gingiva which was covered with a metal disc and fixed for ½ hour showing complete fixation. H and E. x 240

5.4 Discussion

The normal appearance of all the cellular components indicate that autolysis had not taken place in any of the eight specimens examined and that the fixation was not affected by the application of the plunger to the tissues. It was therefore assumed that the moment the heads were immersed in 10 per cent buffered formol saline, fixation took place. This was not affected by the presence of the disc on the tissues.

CHAPTER 6

THE RESPONSE OF THE ORAL MUCOSA TO MECHANICAL LOADING

6.1 Introduction

The relationship between histological structure and function of oral masticatory and lining mucosa has long been of interest to oral biologists. Whilst the micro-anatomy of these and other soft tissues at rest is well documented, only a few studies have been reported on the histological changes which occur, in tissues subject to mechanical loading.

Cattell (1936) carried out experiments to demonstrate the physiological effects of pressure on isolated tissues and unicellular organisms. He showed that the initial displacement is in the fluid portion of the cell and that, provided the pressure is not too extreme, all changes are reversible. Kosiak (1961) demonstrated that there was a marked susceptability of tissue to relatively low constant pressures for short periods of time. Gibson, Kenedi and Craik (1965), in a study on the mobile micro-architecture of dermal collagen stated that all the data in stress studies are time dependent and that a certain period in time must elapse before the dimensional changes become stable. Craik and MacNeal (1966) described the reorientation of the collagen fibres under mechanical loading. Elastic and reticulin fibres were reported to be largely unaffected by stretching. Stroud (1967), in experiments on the palatal tissue of dogs extending over a period of four hours, demonstrated that pressures on the muco-periosteum causes flattening out of the epithelial ridges and obliteration of the connective tissue papillae.

Cytologic alterations were only produced after 30 minutes with a pressure of 20 gm/mm^2 and after four hours with a pressure of 5 gm/mm^2. He deduced therefore, that the duration of the applied force is more important than the intensity of the pressure. Scapino (1967) stated that, in compression of the palatal mucosa of dogs, there was a general diminution in intercellular spaces but that the collagen network in the lamina propria showed little change and the epidermal and connective tissue ridges were slightly deformed. Kydd et al.(1969) and Kydd and Mandley (1967) in a study of the stiffness of the palatal epithelium and the effect of mechanical stress on the oral muco-periosteum of dogs, showed that the epithelial ridges were flattened and that the variable pressure produced little immediate difference in the morphology. Philipsen and Pejerskov (1973), studying the effects of acute mechanical stress on the oesopnageal epithelium of the guinea pig, found that the epithelium connective tissue inter-digitation was completely obliterated with only occasional connective tissue invaginations. Kydd, Daly and Nansen (1974), in a study of the response of soft tissues in the area of the tibia to mechanical stress used balls of different diameter as applicators. They showed that an increase in diameter produced a decrease in the degree of narrowing of the thickness of the tissue. They also stated that the thicker the soft tissue, the greater the compression when load was applied. It was also demonstrated that doubling the load produced only five per cent more change in thickness.

No studies appear to have been conducted on the histological changes which occur in mechanically stressed lining mucosa.

The purpose of this study was to determine, quantitatively, the effect of applying a standardized load to four different regions of the oral mucosa in the Vervet monkey.

6.2 Materials and Methods

6.2.1 Requirements of Apparatus and Instrumentation

As the study was designed to investigate the histological changes in the various tissues which comprise the oral cavity, it was necessary to design an instrument which would satisfy the following requirements:

1. It should apply a dynamic force which would, as closely as possible, simulate the forces produced during normal mastication of solids.
2. It should be able to be used on all four areas concerned in the study without substantial alteration to the basic design of the instruments.
3. It should be able to produce consistently reproducible results on all the areas mentioned.
4. It should be easy to manipulate and be portable, and
5. It should not corrode when immersed in 10 per cent formol saline.

Considering the above requirements and the preliminary study described in Section 3, the following apparatus was designed and constructed.

6.2.2 Instrumentation

The instrument consisted essentially of two parts.

6.2.2.1 Loading Stage (Figs. 6.1 and 6.2)

This consisted of a circular, perspex base, 90 mm in diameter perforated near the edge at 120^{0} intervals by three radiating slots 20 mm in length, 5 mm from the edge. Into these slots were mounted three stainless steel

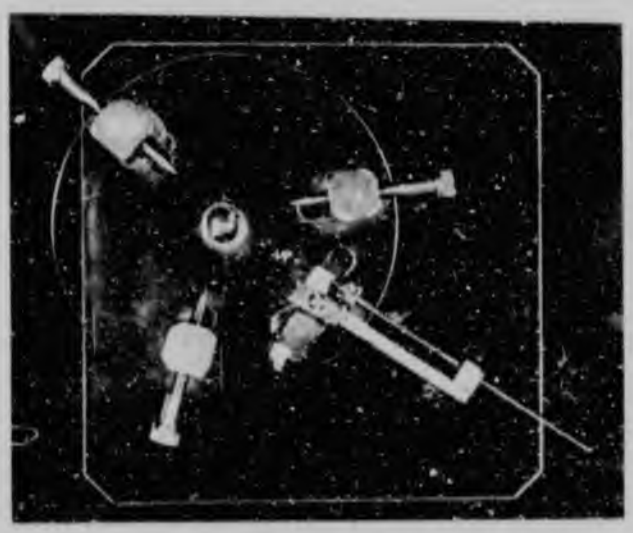

Fig. 6.1 Plan view of loading stage showing fixation screws and pressure loading instrument in position

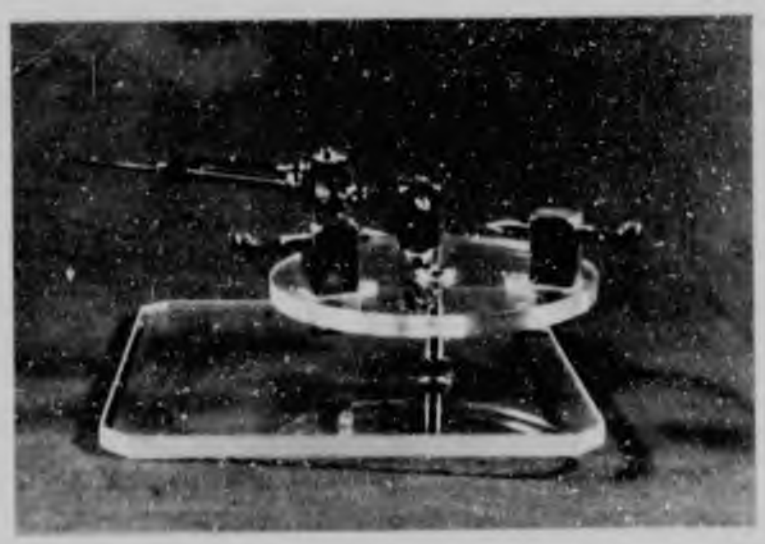

Fig. 6.2 Oblique view of loading apparatus

posts, 12 mm^2 in cross-section and 30 mm in height. Each post, which could be moved nearer to, or further away from, the centre of the base, as well as being able to rotate, was fitted with a pointed fixation screw. These screws were adjusted and screwed into the mandible or maxilla in the required position.

6.2.2.2 Pressure Loading Instrument (Fig. 6.3)

In another slot, 35 mm long, running at a tangent to the circumference of the loading stage, a fourth adjustable post, held a springloaded plunger at the end of which was affixed a 5 mm stainless steel ball. By means of vertical and horizontal adjustments, the plunger was positioned to apply the load at right angles to the surface of the tissue. The plunger was calibrated to deliver a load of 50 gm. This procedure was used when the load was applied to the alveolar and cheek mucosa (Figs. 6.4 and 6.5). When the palate was subjected to load, the mandible was removed and the maxilla fixed in a vertical position on a central pin in the centre of the loading stage, and held in position by only two fixation screws (Fig. 6.6). Similarly, when the load was applied to the tongue, the mandible was fixed in a vertical position on the central pin and two fixation screws (Fig. 6.7). The pressure loading instrument was adjusted, so that the plunger was at right angles to the tissues. The set screw was loosened, and the ball adjusted in contact with the tissues. The set screw was then retracted to the predetermined position and tightened and this exerted a continual force of 50 gm on the tissues.

Fig. 6.3 Pressure loading instrument graduated to deliver 50 gm load

Fig. 6.4 Loading stage and pressure loading instrument in position to apply load to the alveolar mucosa

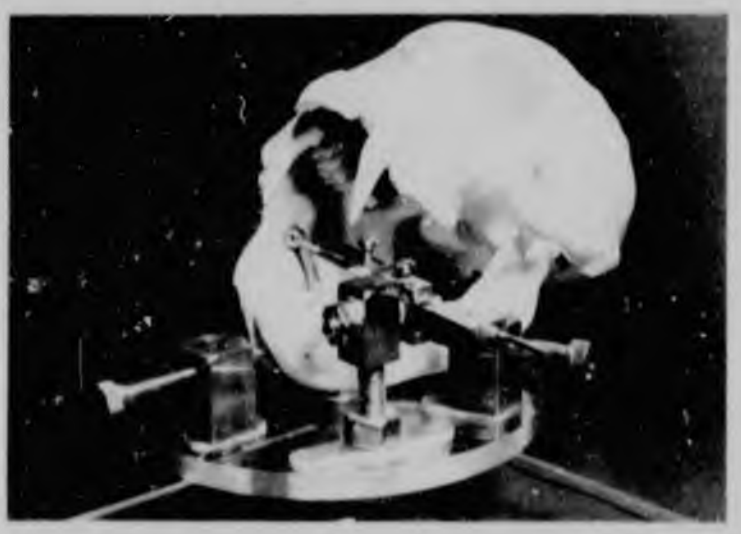

Fig. 6.5 Loading stage and pressure loading instrument in position to apply load to the cheek

Fig. 6.6 Loading stage and pressure loading instrument in position to apply load to the hard palate

Fig. 6.7 Loading stage and pressure loading instrument in position to apply load to the tongue

6.2.3 Selection of Loading Sites

It was considered that there were four different stress situations which could arise in the oral cavity.

1. Stress on keratinized tissue overlying bone, e.g. palate and attached gingiva.
2. Stress on non-keratinized tissue overlying bone, e.g. alveolar mucosa.
3. Stress on keratinized tissue not overlying bone, e.g. tongue, and
4. Stress on non-keratinized tissue not overlying bone, e.g. cheek.

The above sites were therefore selected in this study.

1. Palate

The area selected was approximately 10 mm medial to the second pre-molar.

2. Tongue

Approximately 20 mm from the tip of the tongue in the midline.

3. Cheek

Fifteen millimetres posterior to the commissure of the lips.

4. Alveolar mucosa

Two to three millimetres below the muco-gingival junction beneath the second mandibular pre-molar.

The attached gingiva was not used as the tissue is similar to the palatal mucosa and the results described in section 3.3.2 were too varied to obtain reproducible results.

Forty adult Vervet monkeys, with clinically healthy oral tissues, were selected post-surgically from nephrectomised animals obtained from the National Institute of Virology.

Ten animals were used for each of the four sites. The animals were decapitated and the heads with the apparatus attached were completely immersed in 10 per cent buffered neutral formol saline (Fig. 6.8). All procedures were completed within two minutes. The solution was changed after 24 hours.

After five days the apparatus was removed and the tissues processed as described in Section 2.2.1.

Determination of epithelial thickness and cell densities were carried out as described in Sections 2.2.3 and 2.2.4 respectively and the results computed as described in Sections 2.2.3.2 and 2.2.4.

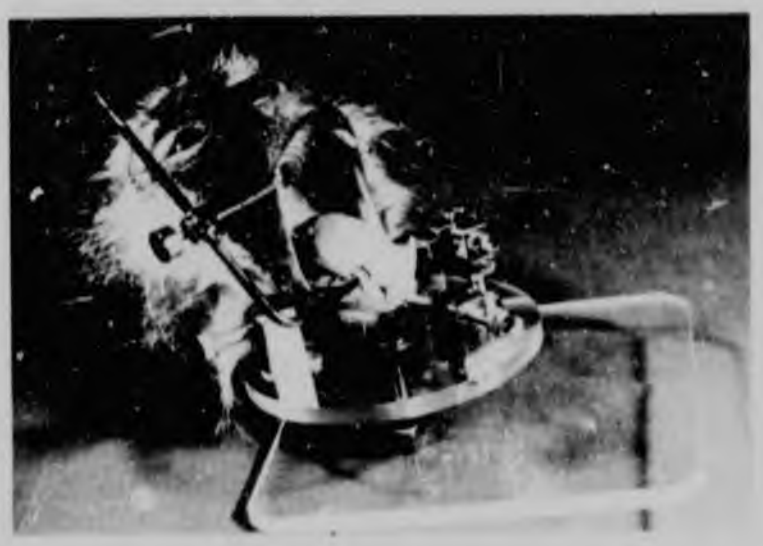

Fig. 6.8 Monkey head in position on loading stage prior to immersion into formol saline

6.3 Results

6.3.1 Alveolar Mucosa

The soft tissues overlying the alveolar bone was only slightly indented in the loaded area (Fig. 6.9). The normal undulating or "saw tooth" surface of the alveolar mucosa appeared smooth and flattened. The rete pegs and associated connective tissue papillae were completely obliterated or considerably reduced in size. The cells in the basal and spinous layer had flattened nuclei and the cells themselves showed considerable angularity (Fig. 6.10). However, both the nuclei and cytoplasm of the clear cells in the basal and spinous cell layers exhibited practically no change in shape (Fig 6.11). The connective tissue in the lamina propria showed some degree of reorientation to lie parallel to the epithelial surface. However, the loose areolar appearanc was still present (Fig. 6.11). The randomly orientated loose elastic fibre network seen in the unloaded tissue was reorientated to run parallel to the epithelial surface (Fig.6.12).

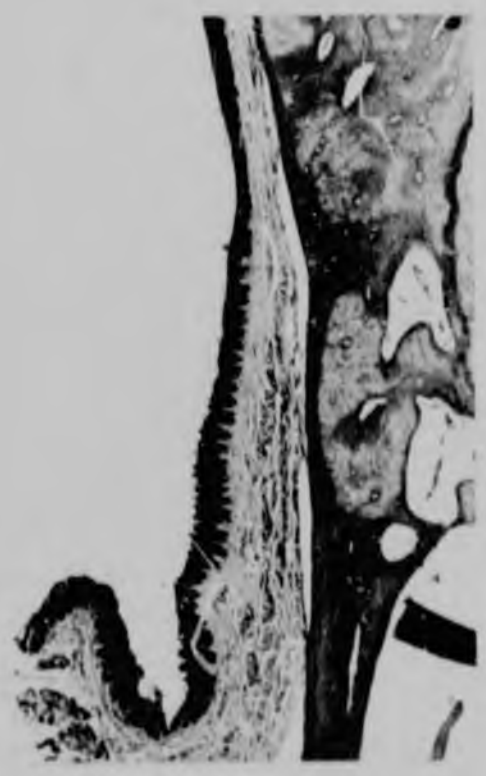

Fig. 6.9 Section of alveolar mucosa showing slight indentation. H and E. x 15

Fig. 6.10 Basal and prickle cell layers of alveolar mucosa showing flattening of the cells. H and E. x 360

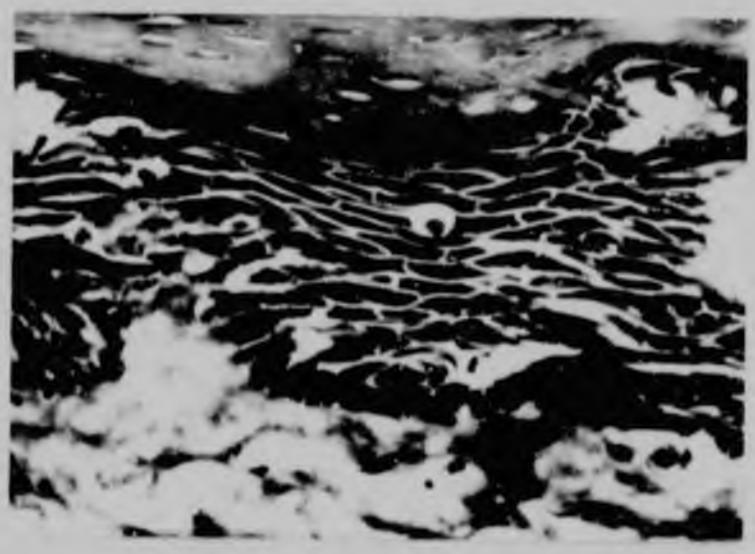

Fig. 6.11 Clear cells in loaded alveolar mucosa showing no change in morphology. Picromallory. x 600

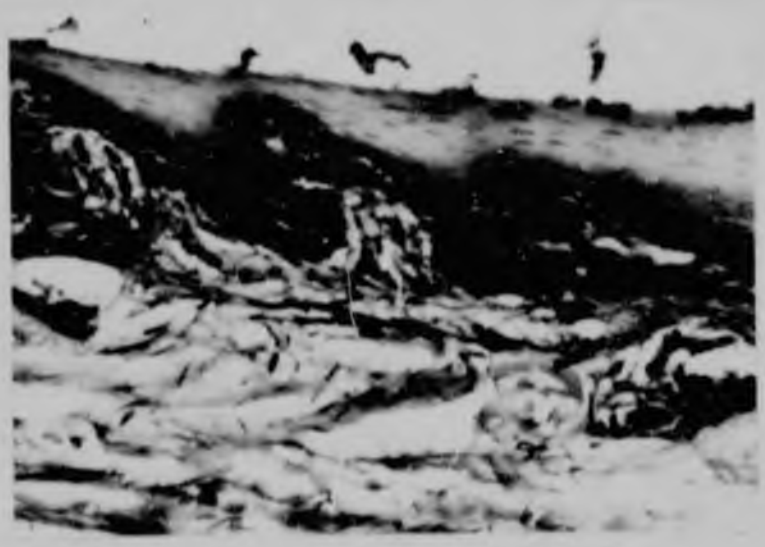

Fig. 6.12 Elastic fibres in loaded alveolar mucosa showing parallel orientation to epithelial surface (arrowed). 25 µm section Orcein. x 240

6.3.2 Cheek

Loading of this area produced a deep indentation of the tissues (Fig. 6.13). The rete pegs and connective tissue papillae were usually still discernible but considerably flattened and elongated (Fig. 6.14). The basal cell layer showed only slight flattening compared to the other cell layers which showed considerable flattening and elongation.

The nuclei and cytoplasm of the clear cells showed no apparent changes (Fig. 6.15). The collagen fibres in the lamina propria were reorientated to lie parallel to the overlying epithelium. The elastic fibres were generally unaltered (Fig. 6.16).

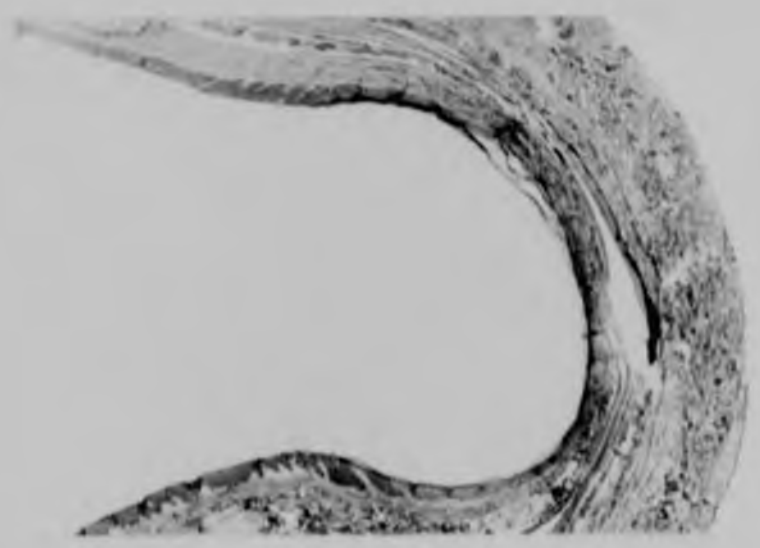

Fig. 6.13 Section of cheek showing deep indentation after loading.
P.T.A.H. x 15

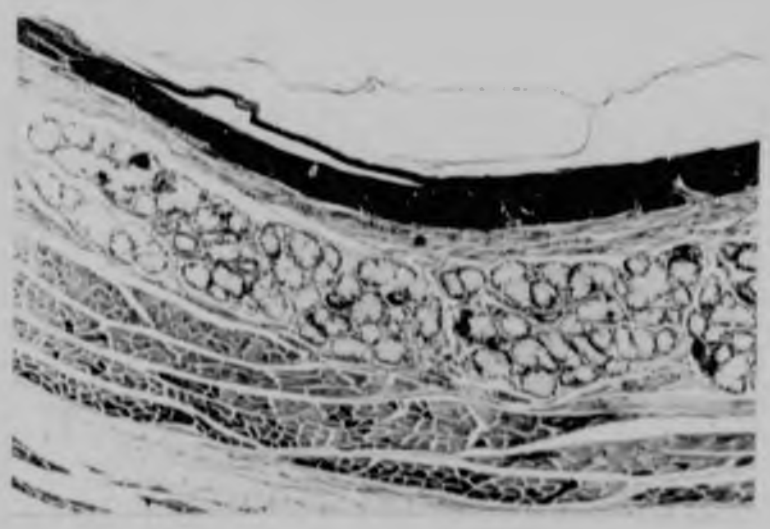

Fig. 6.14 Section of loaded cheek showing flattening of the rete pegs. Note the mucous glands are unaltered. H and E. x 60

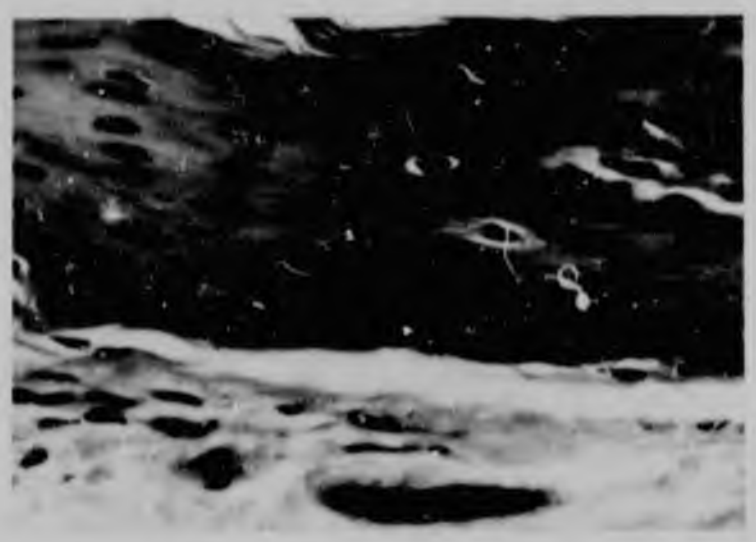

Fig. 6.15 Clear cells in loaded cheek epithelium showing no apparent change in nuclei and cytoplasm. H and E. x 600

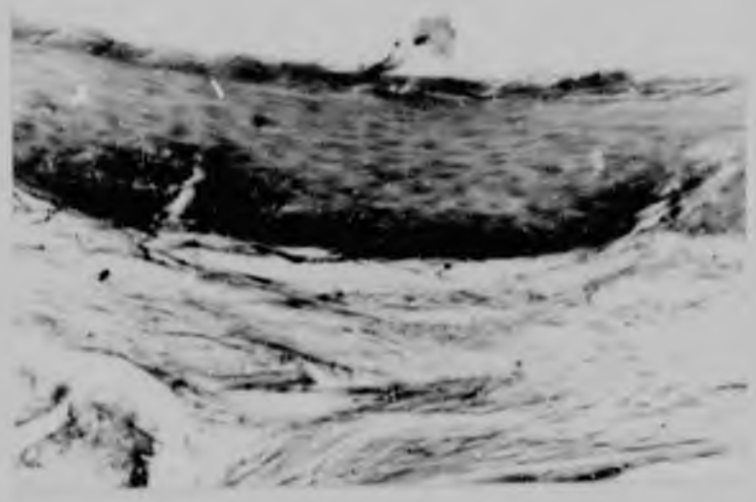

Fig. 6.16 Section of loaded cheek lamina propria showing little change in elastic fibre arrangement. 25 μm section Orcein. x 240

6.3.3 Tongue

Marked indentation of the mucosa and underlying tissues occurred in the loaded area (Fig. 6.17). The filliform papillae had become flattened although the individual connective tissue papillae were still discernible (Fig. 6.18). Similarly the rete pegs and connective tissue papillae had become flattened slightly.

The epithelial cells were flattened but the basal cells showed little or no alteration. The clear cells were unaltered (Fig. 6.19). The elastic fibres conformed to the altered shape of the connective tissue papillae. The longitudinal collagen fibres in the lamina propria were reorientated by loading and ran parallel to the overlying epithelium (Fig. 6.20).

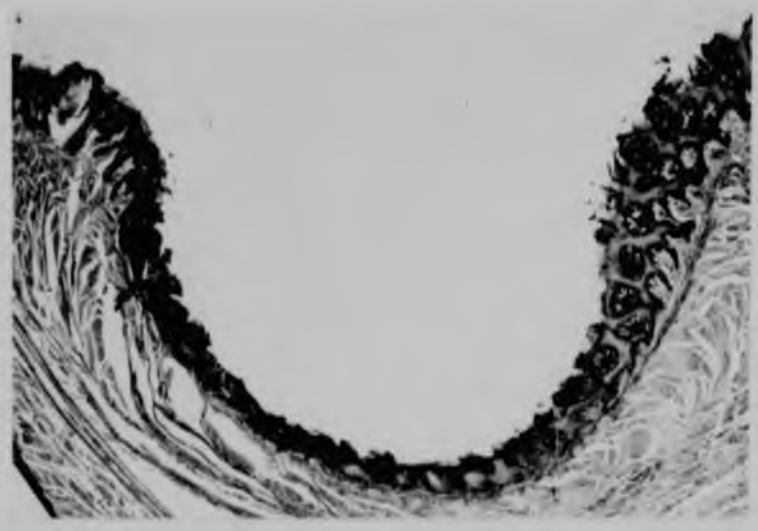

Fig. 6.17 Section of tongue showing marked indentation after loading.
P.T.A.H. x 15

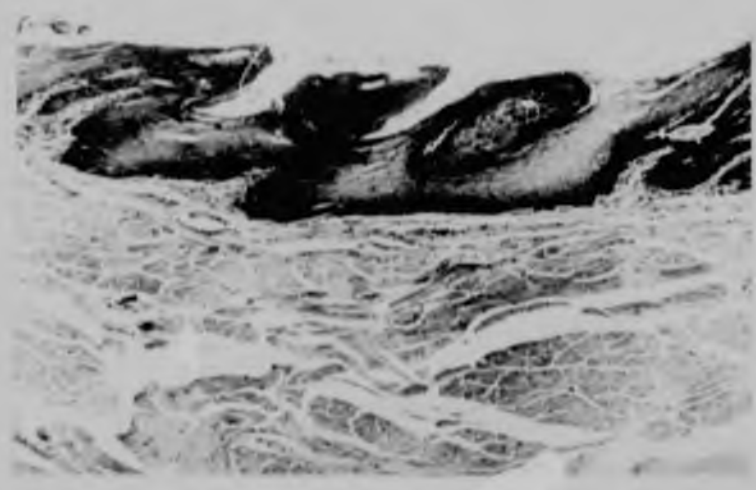

Fig. 6.18 Section of loaded tongue showing flattening of filliform papillae. Connective tissue papillae still discernable.
H and E. x 60

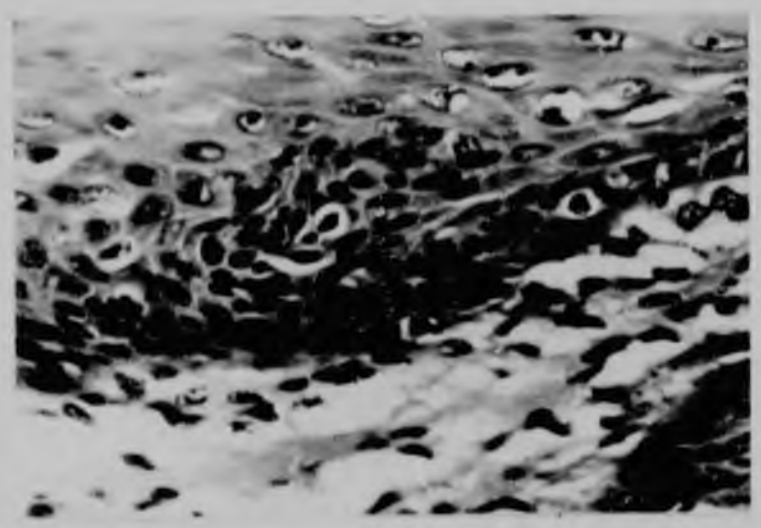

Fig. 6.19 Clear cells in loaded tongue epithelium showing no apparent alteration in nuclei and cytoplasm.
H and E. x 360

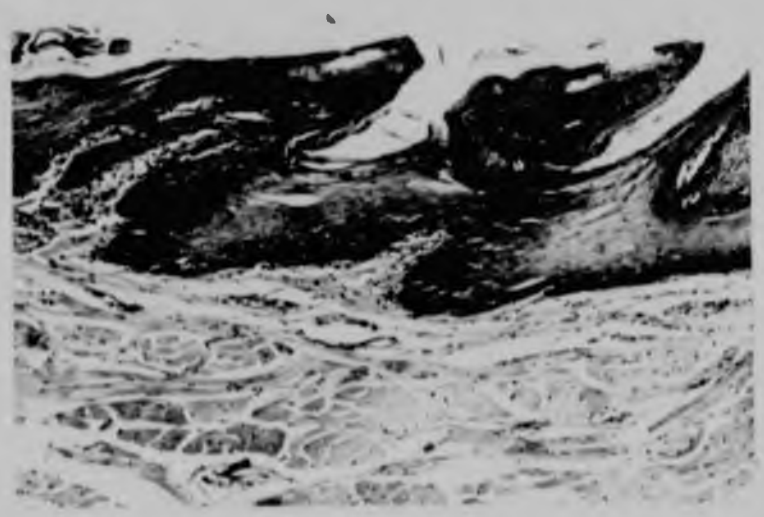

Fig. 6.20 Section of lamina propria of loaded tongue showing reorientation of collagen fibres to run parallel to the surface.
H and E. x 90

6.3.4 Palate

The load was applied to mucosa lying in a trough between the rugae and a slight indentation of the mucosa was apparent (Fig. 6.21). There was no change in the keratin layer. The rete pegs appeared shorter, broader and rounder and the associated connective tissue papillae narrower (Figs. 6.21 and 6.22). In areas adjacent to the point of maximum load, the tips of the rete pegs were pointed towards the centre of the depression. The basal and spinous cell layers showed normal angularity and elongation. The clear cells were apparently unaltered (Fig. 6.23). The collagen fibres in the lamina propria as well as the mucous glands and fat cells in the submucosa showed little change.

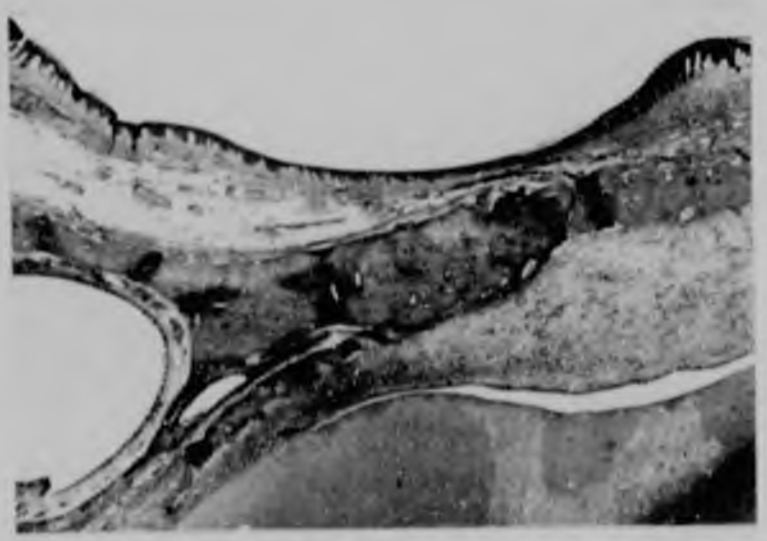

Fig. 6.21 Section of hard palate showing slight indentation after loading. H and E. x 15

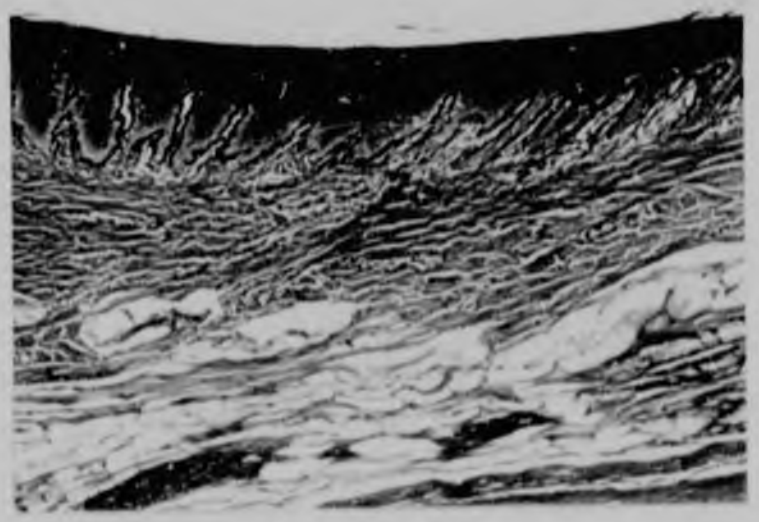

Fig. 6.22 Loaded palatal epithelium showing shortened, broader rete pegs. Masson. x 60

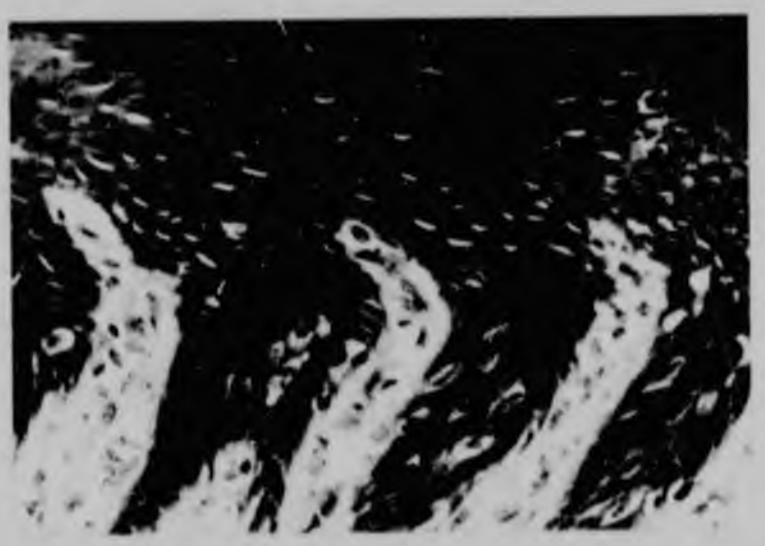

Fig. 6.23 Clear cells in loaded palatal epithelium showing no apparent change in nuclei and cytoplasm. Masson. x 360

6.3.5 Cell Density (See Table III)

The cell density of 7,4 cells/700 μm^2 in the cheek indicated a 54,2% increase in density after loading. However, in the palate the cell density of 6,0 cells/700 μm^2 was only an increase of 41,7% after loading. The cell densities in the alveolar mucosa and tongue showed increases of 50,8% and 42,5% respectively.

6.3.6 Epithelial Width (Tables IV, V, VI, VII)

The greatest reduction in the length of the rete pegs was in the alveolar mucosa, 67,02% and the cheek, 60,04% and the least was in the palate, 47,42% and in the tongue, 45,25%. However, the largest reduction in supra-papillary width was in the cheek, 55,3% and alveolar mucosa, 48,46% as compared to the tongue, 6,61% and palate 16,19%.

6.4 Discussion

The histological changes produced by loading the various areas of the oral mucosa have shown that in each area studied the tissues responded in a characteristic and consistent manner to the applied load. The results can be directly related to the histological structure of lining and masticatory mucosa in each area studied and to the functional demands which these tissues are required to meet. The presence of a keratin layer as in the palate and tongue appears to resist the forces applied to them as compared to the non-keratinized epithelium of the cheek and alveolar mucosa. However, it is well to consider at this point, the observations of Osmanski and Meyer (1967) who studied the differences in the alveolar mucosa of mouth, cheek and palate, both tissues being orthokeratinized. They state that "differences in the functional properties of different ortho-keratinising epithelium may be as marked

Table III - Comparison of Cell Densities of Normal and Loaded Tissues

		Mean Cell Densities/700 µm² Epithelium						
		Normal		Loaded			Student's t Test	
	n	$\bar{x}$	± SD	x	± SD	% Difference	t value	p value
Tongue	50	4,1	± 1,0	7,1	± 2,0	42,5	10,48	< 0,0001
Cheek	50	3,4	± 0,9	7,4	± 2,0	54,2	14,55	< 0,0001
Palate	50	3,5	± 0,8	6,0	± 0,9	41,7	13,12	< 0,0001
Alveolar Mucosa	50	5,6	± 1,2	11,4	± 2,7	50,8	13,22	< 0,0001

Table IV - Comparison of Epithelial Widths in µm of Loaded Tissues

		Rete peg length (A)	Suprapapillary width (B)	Epithelial width $\frac{(A+B)}{2}$
	n	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Tongue	500	592,10 ± 95,79	283,86 ± 59,12	437,95 ± 77,46
Cheek	500	116,82 ± 26,32	59,08 ± 17,91	88,15 ± 22,12
Palate	500	224,38 ± 55,82	113,37 ± 31,50	168,91 ± 43,67
Alveolar Mucosa	500	91,73 ± 24,81	48,32 ± 18,47	70,02 ± 21,64

Table V - Comparison of Rete Peg Lengths in µm Between Normal and Loaded Tissues

		Normal	Loaded	% Difference	Student's t Test	
	n	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD		t value	p value
Tongue	500	1081,42 ± 147,26	592,10 ± 95,79	45,25	61,90	< 0,0001
Cheek	500	389,97 ± 87,31	116,82 ± 26,32	70,04	66,91	< 0,0001
Palate	500	426,77 ± 74,42	224,38 ± 58,82	47,42	48,36	< 0,0001
Alveolar Mucosa	500	278,13 ± 48,84	91,73 ± 24,81	67,02	75,87	< 0,0001

Table VI - Comparison of Suprapapillary Widths in µm Between Normal and Loaded Tissues

		Normal	Loaded	% Difference	Student's t Test	
	n	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD		t value	p value
Tongue	500	303,97 ± 94,61	283,86 ± 59,12	6,61	4,02	< 0,0001
Cheek	500	132,10 ± 56,57	59,08 ± 17,91	55,30	27,31	< 0,0001
Palate	500	135,27 ± 34,59	113,37 ± 31,50	16,19	10,46	< 0,0001
Alveolar Mucosa	500	93,78 ± 27,90	48,32 ± 18,47	48,46	30,40	< 0,0001

Table VII - Comparison of Epithelial Widths in µm Between Normal and Loaded Tissues

		Normal			Loaded				Student's t Test	
	n	$\bar{x}$	±	SD	n	$\bar{x}$	±	% Difference	t value	p value
Tongue	500	692,16	±	121,01	500	437,95	± 77,46	36,73	46,01	< 0,0001
Cheek	500	261,04	±	71,87	500	88,15	± 22,12	66,23	56,15	< 0,0001
Palate	500	281,03	±	54,51	500	168,91	± 43,67	39,90	40,28	< 0,0001
Alveolar Mucosa	500	185,99	±	38,40	500	70,02	± 21,64	62,35	69,29	< 0,0001

as those between non-keratinized and keratinized regions." We therefore, cannot conclude that keratin in the palate and tongue is the only factor which resists the forces applied to them. The presence of a high concentration of tonofibrils and of keratinohyalin granules in the granular layer is a consistent feature in keratinized epithelium. The heavy, dense tonofibrils orientated in a three-dimensional pattern may also contribute to the rigidity of the cells and resist displacement when forces are applied to them (Scapino, 1971).

In unstressed lining mucosa the rete pegs of the epithelium viewed in in cross-section are broad and blunt and ended an interdigitate with proportionately narrow connective tissue papillae. In unstressed masticatory mucosa the rete pegs and connective tissue papillae are long and slender. This structural arrangement provides for a greater area of attachment between the epithelium and the connective tissue of masticatory mucosa than of lining mucosa, and the connective tissue network has also been shown to be far better developed in masticatory mucosa than in lining mucosa (Smith, 1969).

The angularity and irregularly polygonal shape of the cells of palatal and tongue and epithelium also contribute to the stability of the epithelium, as compared to the cells of the cheek and alveolar mucosa which appear to be more rounded and according to Osmanski and Meyer (1967), have a highly convoluted cell border that aids in the cells of non-keratinized epithelium to be able to move over each other. The above observations are substantiated by a smaller percentage difference between the cell densities of normal and loaded tissues seen in the palate and tongue, 41,7% and 42,5% respectively as compared with that of cheek

and alveolar mucosa - 54,2% and 50,8% respectively (see Table III). Although the tissues were subjected to the same load of 50 gm, the resultant thickness of the epithelium (Table IV) still varied considerably and no conclusion can be drawn from these figures when comparing one tissue with another. It is only the degree of reduction of epithelial thickness within the same tissue which is significant.

All the loaded tissues showed p values of 0,001, indicating a high degree of significance between normal and loaded tissues. If we consider all the tissues combined, the rete pegs have been reduced in length by 57,43% as compared to a reduction of 31,64% in the thickness of the supra-papillary area. This would indicate that the greatest degree of change appears to be in the prickle cell layer as the basal cell layers generally appear to be little affected by the loading of the tissues. In comparing the changes in epithelial thickness of the four tissues after loading, (Table VII) the greatest reduction in thickness occurred in the cheek, 66,23% and the least in the tongue, 36,73%. It is interesting to note the consistent pattern of reduction of thickness. The palate and tongue have shown the least change in rete peg length, supra-papillary width and cell density and the cheek and alveolar mucosa the most. We can therefore conclude that there is a direct ratio between the reduction of the epithelial thickness and increase in cell density when the tissues are subjected to load.

A consistent finding in all the four tissues studied was that the clear or dendritic cells showed a remarkably consistent resistance to deformation or flattening. They appear to retain their original shape whereas the cells surrounding them show considerable change. A possible explanation of this phenomenon is that these cells have long

protoplasmic processes which inter-digitate between the other cells of the epithelium (Hutchens et al., 1971). When the cells are compressed, the cytoplasm within these processes is forced into the main body of the cell, producing an increased hydrostatic pressure within the cell which resists distortion. The patency of the clear cells under loading negates the suggestion of Squier, Johnson and Hopps (1976), who state that the "clear" effect is an artefact produced by the shrinkage of the cytoplasm around the nucleus due to the lack of desmosomes in these cells. If this were true these cells would be even more flattened than the surrounding epithelial cells.

The collagen fibres of the lamina propria of the four areas studied, all responded in a similar manner to loading, by reorientation of the fibre network parallel to the overlying epithelium. The fibres in the alveolar mucosa however, still appear to retain the loose areolar character as seen in the tissues at rest. The "bent" appearance of the rete pegs of the palatal epithelium is probably due to the firm attachment of the collagen fibres to the tip of the rete peg. When the load is applied, the surface layers of the epithelium tend to elongate laterally away from the area of load. The collagen fibres, particularly those attached to the rete pets however, do not stretch to the same degree and prevent further lateral movement of the epithelium. This produces the effect as seen in Fig. 6.23.

In the submucosa of the palate and cheek, the mucous glands show remarkably little if any, change in shape. They retain their normal acini and the ducts remain patent.

It is suggested that the mucous glands have a higher intercellular hydrostatic pressure in order to produce a positive flow of mucous. This in turn prevents the compression of these cells. The elastic fibres of the alveolar mucosa show considerable reorientation to lie parallel to the epithelial surface when the tissue is stressed. This may be explained by the fact that the cheek and tongue have a well developed muscle fibre system to return the tissues to their rest position after the load is removed. This is not the case in the alveolar mucosa which has little or no muscle fibres. The elastic fibres therefore assume the role of the muscle fibres in the other tissues, i.e. to restore the tissue to its resting state (Treagar, 1971). Another important feature influencing the response of oral mucosae to loading, is the nature of the tissues to which they are attached. When the collagen fibres of the lamina propria are attached to bone as in the alveolar mucosa and hard palate, the epithelium is firmly tethered to a rigid structure. This will enable the collagen fibres in the lamina propria to support greater tensile forces and give the mucosa greater load-bearing properties. Where the mucosa is attached to muscle as in the cheek and tongue, localised loads will be distributed over a much wider area by indentation of the soft tissue, and produce the wider pattern of tension in the collagen fibres of the lamina propria as seen in the cheek and tongue mucosa.

The responses of masticatory and lining mucosae to standardised loads applied in this study appear to be directly related to the mechanical properties imparted to the tissues by epithelial keritinization, the nature of the epithelial rete pegs and connective tissue papillae and the attachment of the mucosa to underlying bone or soft tissue.

CHAPTER 7

SCANNING ELECTRON MICROSCOPY OF LOADED ORAL MUCOSA

7.1 Introduction

The histological differences in keratinization of the oral mucosa have been demonstrated by Meyer and Gerson (1964), Alvares and Meyer (1971) and Weinman and Meyer (1959). These findings have been described in Chapter 2. Cleaton-Jones (1973), Wilding (1973) and Cleaton-Jones and Fleisch (1973) have described the surface appearance of keratinized and non-keratinized epithelium of the oral cavity as viewed with the scanning electron microscope. They showed that keratinized epithelium had a pitted appearance whereas non-keratinized epithelium had linear microplications. Fleisch, Cleaton-Jones and Austin (1976) described folds in the non-keratinized epithelium of the oral mucosa.

This study was undertaken to demonstrate the effect of mechanical loading on the surface characteristics of the epithelium of the oral cavity.

7.2 Materials and Methods

Sixteen adult Vervet monkeys were obtained post-surgically from the National Institute of Virology. The mucosa loading procedure as described in Section 6.2.2, was carried out with four animals used for each area giving a total of sixteen specimens. After fixation for five days, the required tissues were dissected off the bone in the case of palatal and alveolar mucosal tissues. The specimens were dehydrated in

graded ethanol series followed by amyl acetate. The specimens were then placed in a Polaron Critical Point Drying Apparatus E2000(Polaron Equipment Limited, Waterford, Herts., England) using CO_2 at 1,200 psi pressure at a temperature of 42°C. The specimens were then coated with a thin layer of gold palladium in an Edwards vacuum-coating unit, Model E12E4 (Edwards Limited, Crawley, Kent, England). The coated specimens were examined in a Cambridge S4 stereoscan operated at 20 Kv with the beam-specimen angle varied to produce the best results.

7.3 Results

7.3.1 Tongue

Under low magnification the normal filliform papillae which had an irregular thorny appearance (Fig. 7.1) appeared to be flattened in the stressed area (Fig. 7.2).

Under higher magnification there was no difference in the surface characteristics of normal and loaded tongue epithelium.

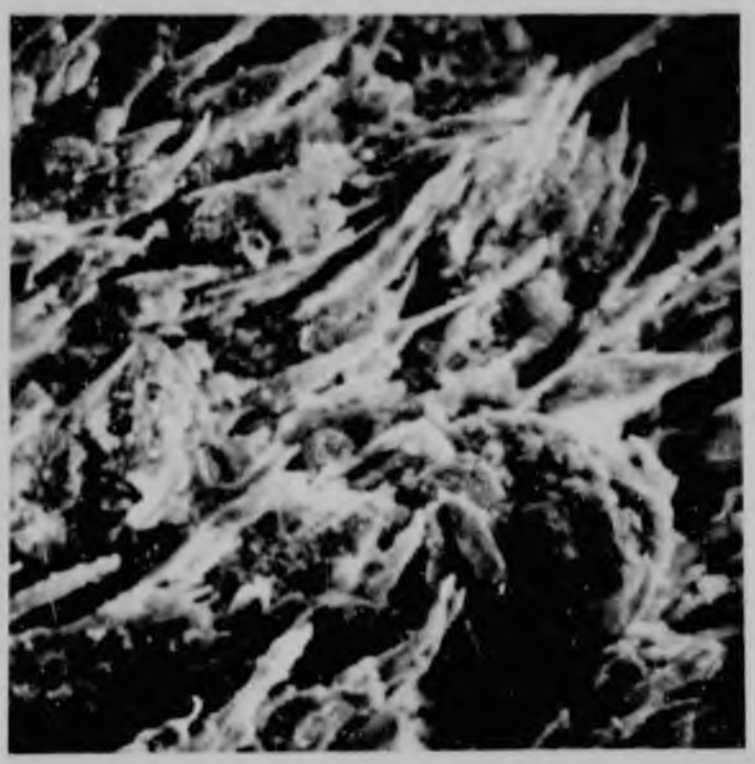

Fig. 7.1 Scanning electron micrograph of normal tongue epithelium showing filliform papillae. x 130

Fig. 7.2 Scanning electron micrograph of filliform papillae after loading showing flattened appearance. x 100

7.3.2 Cheek

Under low magnification a definite indentation of the mucosa was seen which appeared smoother than the surrounding area (Fig. 7.3).

Under higher magnification the microplications of normal, cheek epithelium (Fig. 7.4) were flattened and the intervals between the microplications were widened (Fig. 7.5).

Fig. 7.3 Low magnification of surface of cheek specimen showing indentation following loading. x 22

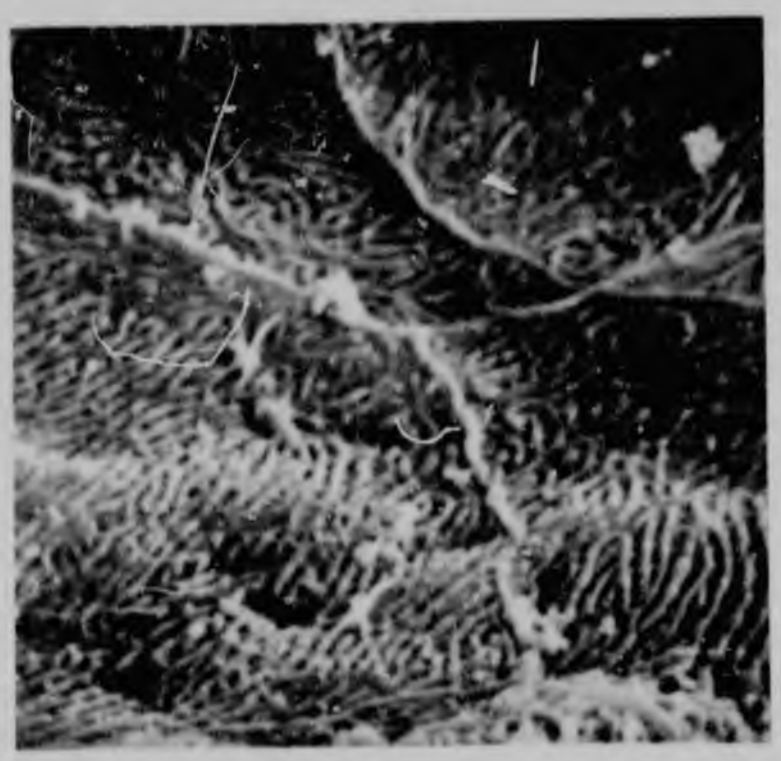

Fig. 7.4 Non-keratinized epithelium of cheek showing interconnecting microplications. x 5400

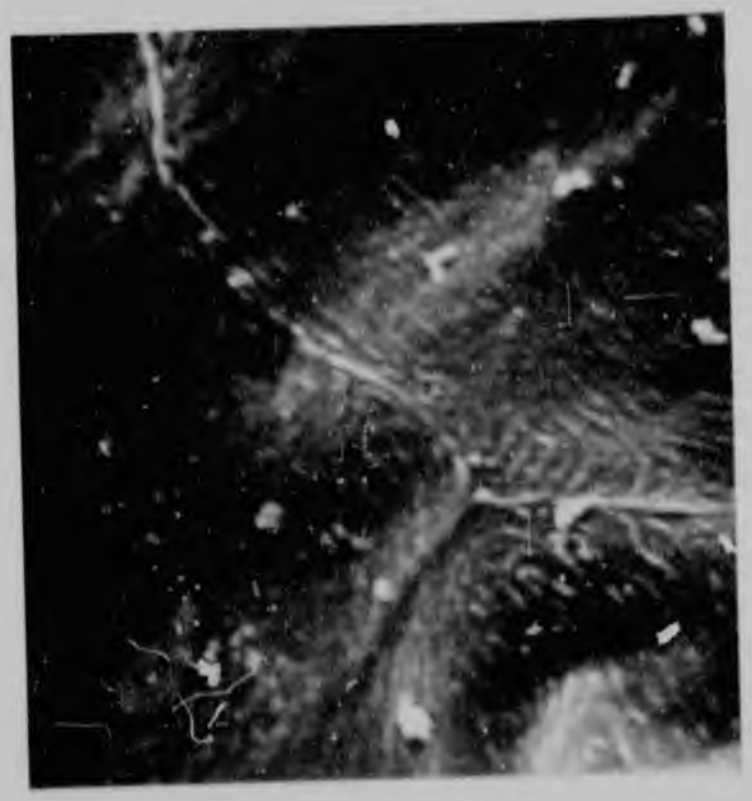

Fig. 7.5 Non-keratinized epithelium of the cheek after loading. The microplications are less prominent than in Fig. 7.4. x 5400

7.3.3 Palate

Under low magnification the stressed area was indented in a similar manner to that seen in the cheek (Fig. 7.6).

Under higher magnification the normal pitted appearance (Fig. 7.7) was unchanged in the stressed area (Fig. 7.8).

Fig. 7.6 Low magnification of surface of loaded area of palatal specimen.
x 20

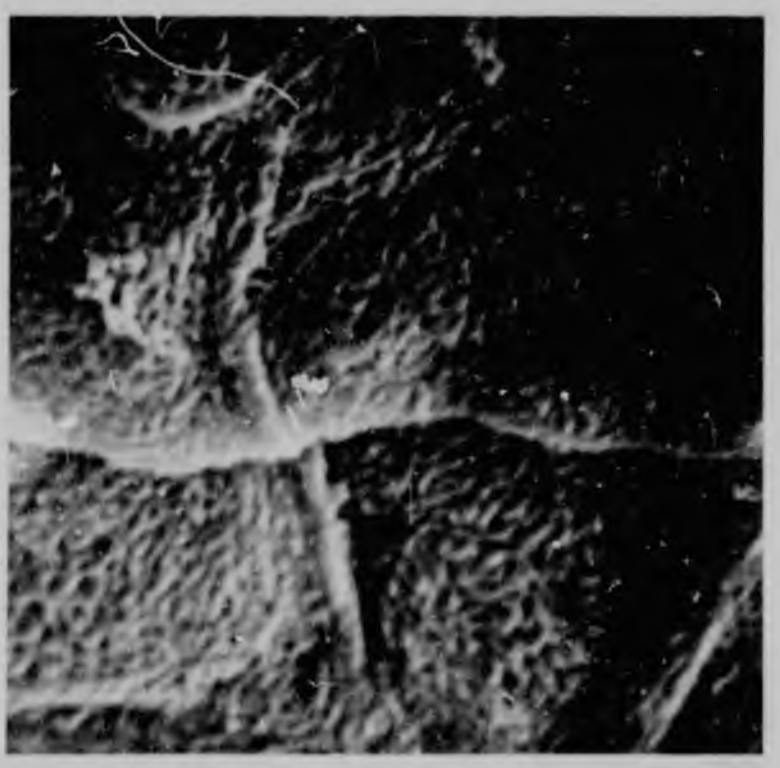

Fig. 7.7 Normal pitted appearance of keratinized epithelium of palate.
x 5500

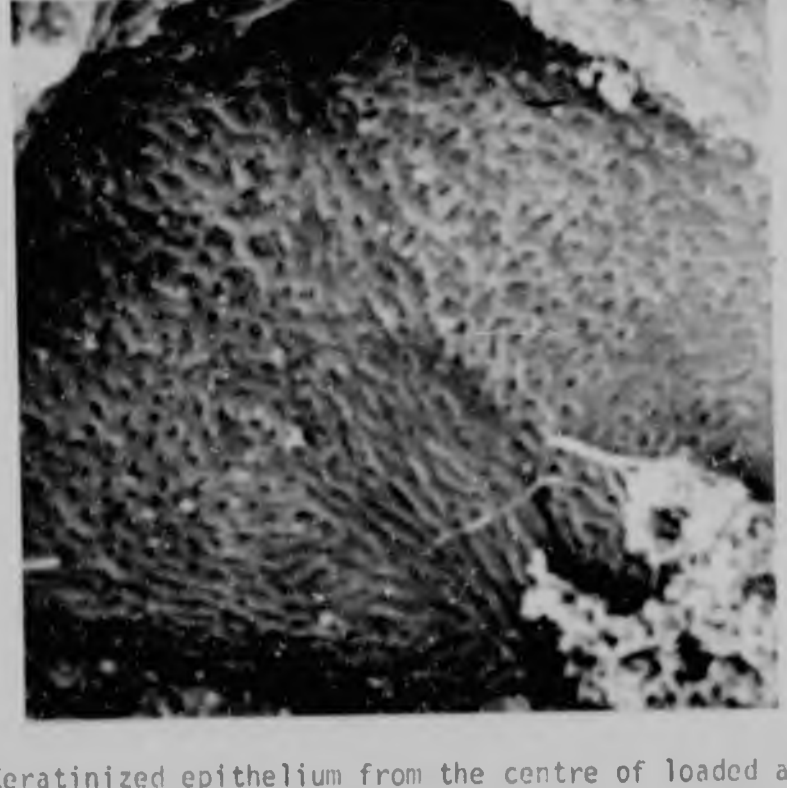

Fig. 7.8 Keratinized epithelium from the centre of loaded area showing no change in pitted appearance. x 5500

7.3.4 Alveolar Mucosa

The linear folds which run parallel to the muco-gingival junction were seen at low magnification to disappear in the area of load which had a more flattened surface with many exfoliating cells (Fig. 7.9).

Under higher magnification the cell junctions in the loaded area appear to be more prominent than in normal tissue (Fig. 7.10) while the intervals between the microplications of the microplications were also wider (Fig. 7.11). The width of the microplications themselves seemed unaltered.

A quantitative analysis of possible changes in the surface morphology was not undertaken as it proved impossible to obtain sufficiently

constant results as a result of the variations in specimen angulation with the indentation of the tissue following loading.

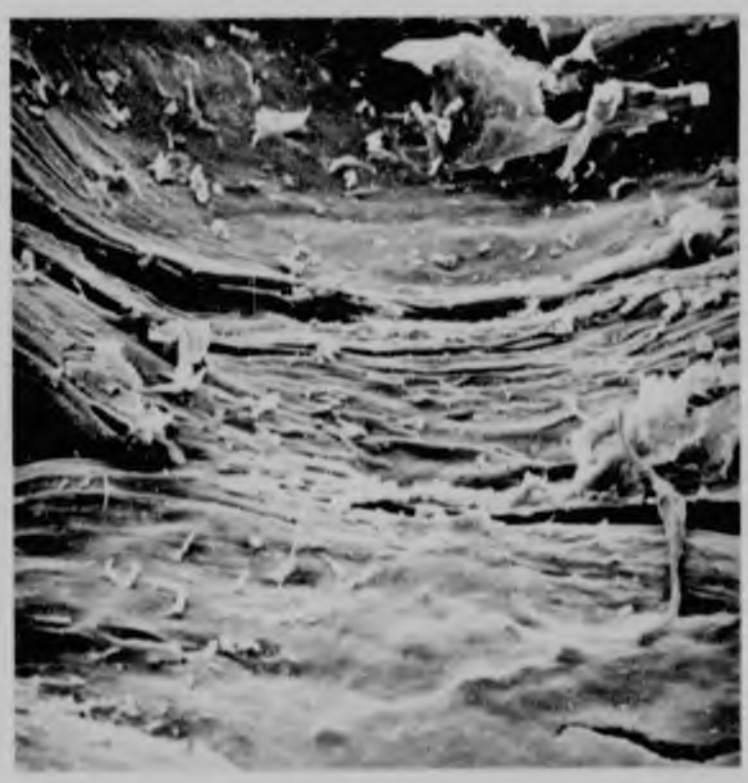

Fig. 7.9 Low magnification of edge of loaded area in alveolar mucosa showing linear folds (arrowed). Note numerous exfoliating cells. x 60

Fig. 7.10 Interconnecting microplications of non-keratinized epithelium of the alveolar mucosa. Intercellular ridge is arrowed. x 5000

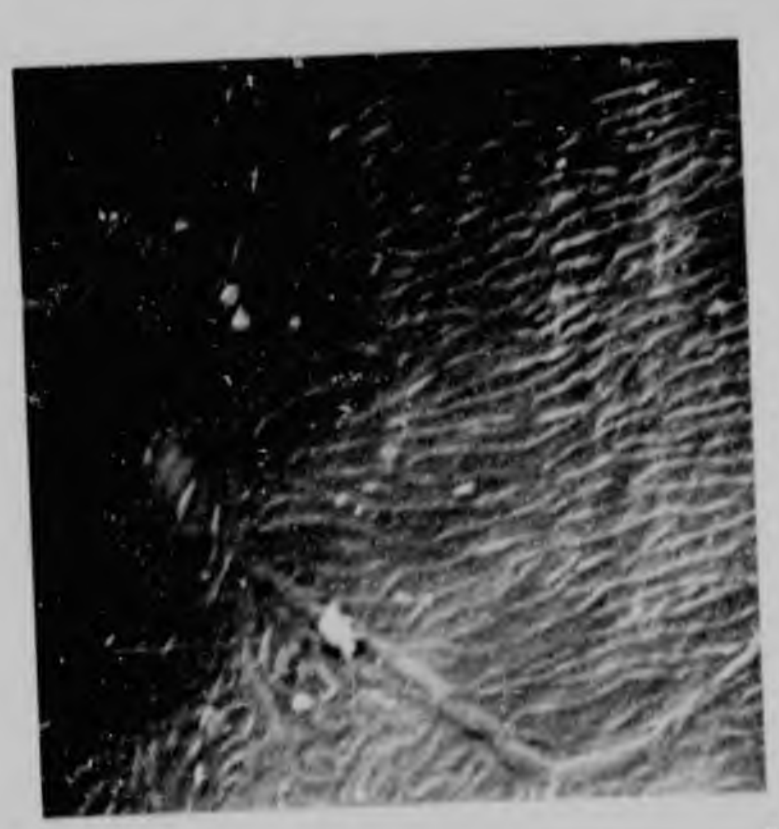

Fig. 7.11 Non-keratinized epithelium of alveolar mucosa showing apparent widening of the spaces between the microplications.
x 5500

7.4 Discussion

Whittaker and Adams (1971) described furrows in the cheek running in an anterior-posterior direction similar to the linear folds seen in the alveolar mucosa at low magnification. They suggested that these furrows were related to masticatory movement. The close correlation between these furrows and the linear folds in the alveolar mucosa and the observation that they disappear when the tissues are subjected to load, support the view that the orientation of the linear folds is directly related to the direction in which the tissues may be stressed during mastication, that is, the folds are orientated at right angles to the direction of stress.

It is difficult to come to a definite conclusion, as to whether the surface morphology of non-keratinized epithelium such as in the cheek and alveolar mucosa change under mechanical loading. However, the widening of the intervals between the linear microplications (Fig. 7.12) tend to substantiate the views of Osmanski and Meyer and Meyer (1967), and Gerson (1964), that the general configuration of non-keratinized epithelial cells, with a highly convoluted border, and the lack of tonofibrils permits the cells to move over or away from each other. This is in agreement with Cleaton-Jones (1972),who states that the microplications are the surface appearance of normal cell inter-digitations.

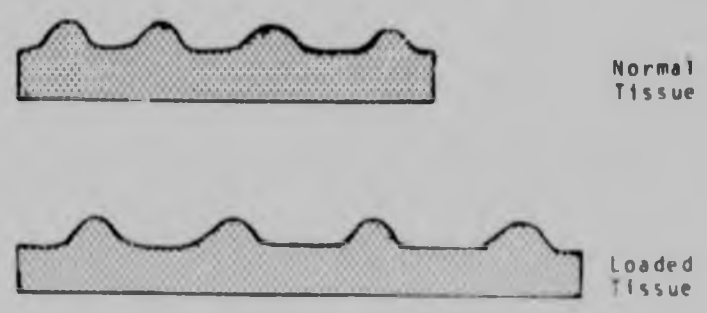

Fig. 7.12 Diagram showing widening of intervals between microplications.

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