LABORATORY AND EXPERIMENTAL STUDIES

OF PROSTHESIS RELATED ORAL CANDIDIASIS

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T H E S I S

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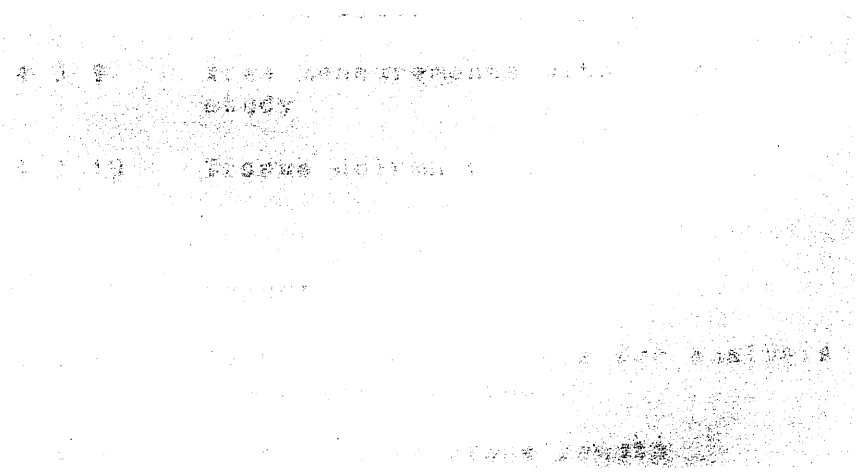

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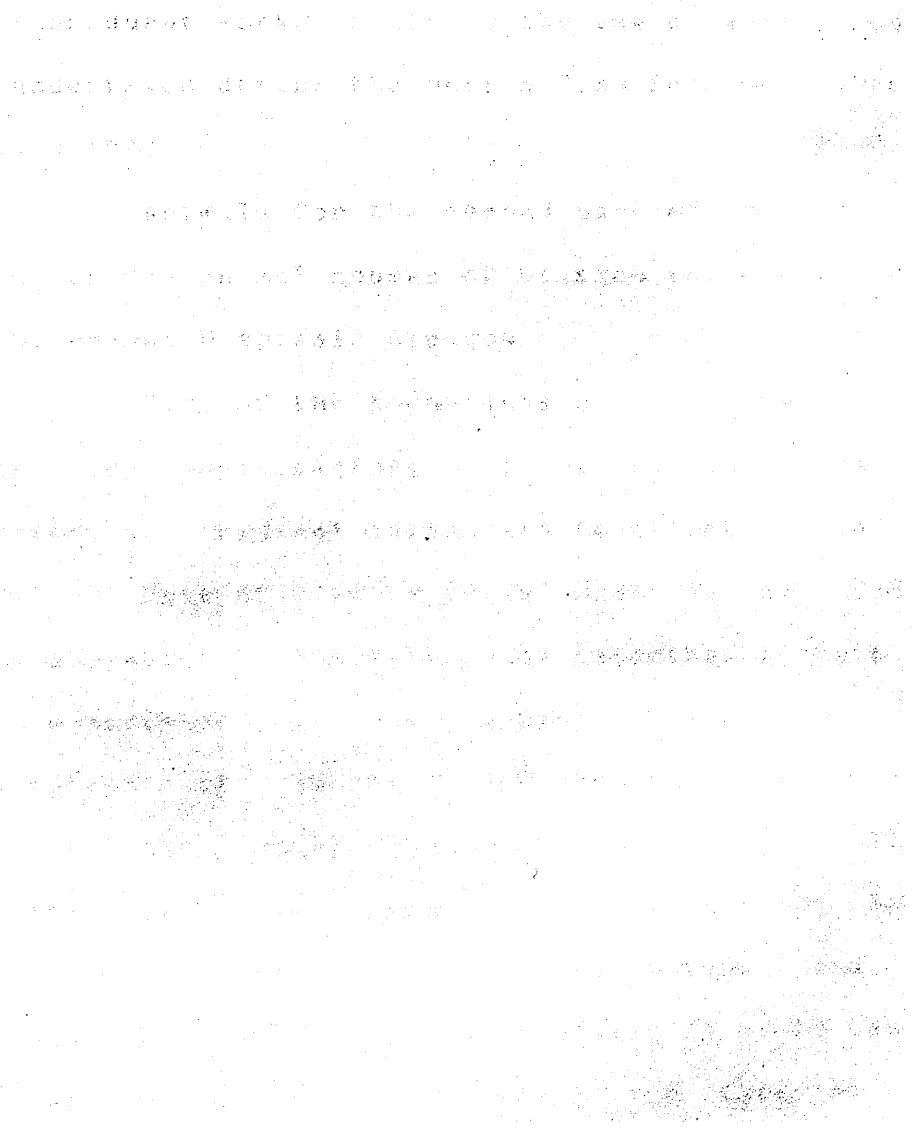


PREFACE

This work was undertaken in the Department of Prosthodontics and the Department of Oral Medicine and Pathology in the University of Glasgow Dental School.

The first part of the work, relating to the study of denture-induced stomatitis in humans, was undertaken in the period from August 1981 to April 1982. The subsequent work, involving the use of animal models, was undertaken during the period from September 1986 to January 1988.

Animals for the second part of the work were caged in the animal houses of Glasgow Western Infirmary and Gartnavel Hospital, Glasgow.

Some of the techniques used in this thesis are modifications of previously published work and some are original techniques developed by the Oral Pathology Unit of Glasgow Dental Hospital and School. The application of the techniques described in this work was undertaken by the author personally. The preparation of acrylic appliances, histological sections, and fungal inoculum was carried out by technical staff of Glasgow Dental Hospital and School under direct supervision of the author and his supervisors. Haematological analysis of blood samples was carried out by staff within the Department of Haematology in Glasgow Western Infirmary.

Part of the work of this study has been

presented in April 1982 at the annual conference of the British Society for the Study of Prosthetic Dentistry in Edinburgh in a presentation entitled "Quantitative histological changes in denture-induced stomatitis".

SUMMARY

The nature of the histological appearance of clinically inflamed epithelium found under dentures in the condition of denture-induced stomatitis has been recorded in qualitative terms in the literature over many years. Information of a quantitative nature relating to changes in palatal epithelium in the condition is scarce. It was the aim of this work to make observations based on quantitative data describing the epithelial changes in denture-induced stomatitis.

In the first part of the project, samples of palatal mucosa were collected from patients exhibiting the signs of denture-induced stomatitis. Quantitative stereological techniques were used in the analysis of tissue. Changes occurring were assessed by comparison of the findings with results from healthy oral epithelium similarly analysed by other workers. The importance of haematological and microbiological factors in the pathogensis of denture-induced stomatitis was examined.

There was found to be a varied histological presentation within this single clinical entity. There also appeared to be a link between the haematological and microbiological findings.

Further study of the cellular changes induced in the condition was continued by investigations using an animal model. The Wistar rat was the animal chosen

for the model and further investigation of palatal epithelium within the animal model was in three parts.

In the initial animal study Candida albicans was inoculated under intra-oral acrylic appliances, and epithelial changes occurring were analysed. Differences between experimental and control animals were found, but problems of quantification of the epithelium under consideration were experienced. Problems arose due to the presence of undulations, or rugae, covering all of the palatal epithelium of the Wistar rat.

The second part of the animal study involved analysis of the histological structure of normal Wistar rat palatal epithelium and consideration of how such tissue could best be sampled to allow quantitative assessment. Tissue sections suitable for analysis on a quantitative basis were produced.

The animal study concluded with a more extensive experimental investigation involving the Wistar rat animal model. Epithelial changes induced under experimental conditions were measured. Experimental groups consisted of animals wearing acrylic appliances which were of two differing designs. In some of the animals, Candida albicans was innoculated underneath appliances, whilst in the others, appliances were worn without additional experimental procedure. Tissue was sampled in a manner to allow quantitative methods of analysis.

It was observed that changes were induced in

the experimental animals and that these were primarily related to the presence of an acrylic appliance rather than to the inoculation of Candida albicans. The location of the tissue analysed was found to play an important part in determining the nature of the tissue changes induced.

Histopathological change specifically related to the presence of Candida albicans were rare and appeared to occur only where ulceration of the epithelium was induced by the intra-oral appliance.

ABBREVIATIONS

ESL	Epithelial surface length (μm)
IL	Interface length (μm) (interface between the keratin and the cells of the epithelium)
BML	Basement membrane length (μm)
Area K	Area of keratin (sq μm) .
Area C	Area of cellular epithelium (sq μm)
TK	Thickness of keratin (μm)
TC	Thickness of cellular epithelium (μm)
TT	Total epithelial thickness (μm)

CHAPTER 1

ORAL MUCOSA AND THE EFFECTS OF WEARING DENTURES

1.1 INTRODUCTION

Denture-induced stomatitis is the condition characterised by chronic erythema and oedema of the oral mucosa of the residual alveolar ridge and hard palate in contact with the fitting surface of a maxillary denture. The changes which occur in the oral mucosa covering the palate in denture-induced stomatitis have been described in the literature over many years. The nature of these changes, whether described in the original or in more recent papers, has been considered mainly in terms of the qualitative changes within the oral mucosa.

1.2 AIMS OF RESEARCH PROJECT

In view of the lack of quantitative information relating to epithelial changes within this condition, it was proposed in this study to examine palatal epithelium from patients exhibiting the signs of denture-induced stomatitis, subject the tissue to quantitative analysis and assess if changes occurring followed a consistent pattern.

It was also proposed to assess any relationship between the changes present and the quantity of Candida albicans cultured from within the

oral cavity. It was further proposed to assess if the histological features found within the epithelium were influenced by the haematological status of the individual.

The limited quantity of tissue available for analysis from biopsies restricted the amount of quantitative information available from the study of human subjects. The purpose of the second part of this study was to examine the use of an animal model as an alternative in the quantitative analysis of the histological changes induced in prosthesis related inflammation of palatal mucosa.

1.3 NORMAL ORAL MUCOSA

1.3.1 Functions of oral mucosa

Before consideration of oral mucosa in the condition of denture-induced stomatitis, discussion of normal oral mucosa is appropriate.

Oral mucosa, the moist lining of the oral cavity serves several functions. Its main function is to protect the deeper tissues of the oral cavity, but it also acts as a sensory organ and is the medium through which saliva is secreted. Prehension and mastication of food expose the oral tissues to mechanical forces which the oral mucosa is designed to withstand. The oral epithelium also acts as a barrier to the

penetration of the many micro-organisms found within the oral cavity and their toxic products.

Receptors responding to temperature, touch and pain provide perception of events occurring within the oral cavity. In addition the oral mucosa contains taste buds, specialised sensory receptors not found elsewhere in the body. There are also many minor salivary glands closely associated with the oral mucosa which contribute to the maintenance of its moist surface.

1.3.2 Structural organisation and variations

Oral mucosa is comprised of two main tissue components : a superficial stratified squamous epithelium and an underlying connective tissue layer, the lamina propria. Whilst the interface between the epithelium and the connective tissue is a distinct layer known as the basement membrane, the junction between the oral mucosa itself and the underlying tissue is often less easily identifiable. In regions such as the cheeks, lips and parts of the hard palate a layer of fatty or glandular tissue separates the oral mucosa from underlying bone or muscle. This is the submucosa. In contrast, in other parts of the hard palate and in the gingivae, there is no submucosa and the oral mucosa is directly attached to the periosteum of the underlying bone.

There are variations found throughout the

oral cavity in the structure of the oral mucosa which can be categorised into three main types : masticatory mucosa, lining mucosa and specialised mucosa.

Masticatory mucosa is found on the hard palate and the gingivae which are the areas most exposed to the physical stresses of mastication. The epithelium is moderately thick and keratinised. The lamina propria contains large closely packed bundles of collagen fibres and the junction between the epithelium and the lamina propria is formed by elongated rete ridges.

The oral mucosa found on the lips, cheeks, underside of tongue, floor of mouth, alveolar processes and soft palate is lining mucosa. The epithelium is thicker than that of masticatory mucosa and is nonkeratinised. The lamina propria is also thicker than masticatory mucosa and contains fewer collagen fibres. The interface between epithelium and connective tissue is relatively smooth. These factors give lining mucosa the quality of flexibility and the ability to stretch.

The specialised mucosa is to be found on the dorsal surface of the tongue and although it functions as a masticatory mucosa it contains papillae and taste buds which have specialised functions. The mucosa covering the posterior third of the tongue contains extensive nodules of lymphoid tissue.

1.3.3 Development of oral mucosa

The mucosa which lines the palate, cheek and gingivae is derived from the embryonic stomatodeum and is ectodermal in origin. The tongue which develops from the first and third branchial arches is covered with mucosa which is endodermal in origin.

1.3.4 The structure of oral epithelium

The oral epithelium is comprised of cells tightly attached to one another and arranged in a number of distinct layers. It is a stratified squamous epithelium. The epithelium maintains its structural integrity by a process of cell renewal such that cells produced by mitotic divisions in the deepest layers migrate to the surface to replace those that have been shed.

1.3.5 Cell proliferation and maturation

The cells of the epithelium consist of two separate populations of viable keratinocytes (Lavker and Sun, 1983). There is the group of progenitor cells whose function is to divide and proliferate. Another group of maturing cells migrates from the basal layer to form the protective surface layer and are ultimately shed.

The progenitor compartment containing the progenitor cells is confined to the deeper cell layers,

up to two or three cells in thickness and consists of two subpopulations of cells. A small proportion consists of stem cells which divide to produce basal cells and maintain the proliferative nature of the basal cell layer. The larger part of the progenitor compartment is made up of cells which divide to produce cells which will subsequently migrate to the surface. In different regions of the oral cavity the maturation of cells which accompanies migration follows one of two main patterns dependent upon whether the epithelium is the surface layer of masticatory mucosa or lining mucosa.

In masticatory mucosa, such as is found on the hard palate or the gingivae a surface layer of keratin is formed and the surface tissue is referred to as keratinised epithelium. In lining mucosa, such as is found on the lips, cheeks, alveolar mucosa, soft palate, floor or mouth and underside of the tongue, the epithelium is nonkeratinised.

1.3.6 Keratinised epithelium

Adjacent to the basement membrane there is a basal layer of columnar or cuboidal cells. Above the basal layer is the prickle cell layer. This layer consists of cells which are larger than those of the basal layer which have a characteristic appearance because they shrink away from each other on histological

processing, retaining contacts only in the areas of desmosomes.

Above the prickle cell layer is the granular layer which consists of larger flattened cells which contain a number of small, intensely basophilic, granules. These keratohyaline granules, which are synthesised by ribosomes, are associated with tonofibrils and it is thought that they form the matrix in which the filaments of the keratinised layer are embedded. The protein of which the keratohyaline granule is formed is filaggrin. In the upper part of this layer the membrane coating granules fuse with the cell membrane and discharge their contents into the intercellular space. This may be associated with the formation of a barrier limiting movement of materials through the intercellular spaces.

There is a marked change in the appearance of the cells when they reach the surface keratinised layer. All the organelles disappear and the cell is filled with closely packed filaments surrounded by filaggrin. These eosinophilic cells are extremely flattened and dehydrated. This pattern of cell maturation is termed orthokeratinisation.

Masticatory mucosa may show a variation in this pattern of maturation known as parakeratinisation. Within the granular layer keratohyalin granules are fewer and there is incomplete removal of organelles from

the cells such that in the keratinised layer the nucleus remains as a pyknotic structure, and the remnants of other structures may be present. These surface cells are however, still filled with closely packed filaments and stain for keratin.

1.3.7 Nonkeratinised epithelium

The process of cell maturation proceeds somewhat differently in nonkeratinised epithelium. The basal and prickle cell layers have a similar appearance to the equivalent layers found in keratinised epithelium. However the granular layer is not present. The superficial layer which does not stain discretely with eosin consists of cells which contain nuclei, are not dehydrated and in which the filaments are loosely arranged.

Uppermost in nonkeratinised epithelium are the so-called intermediate and superficial cell layers. The intermediate layer lies above the prickle cell layer and it contains glycogen and loosely arranged tonofilaments. The superficial layer which lies on the surface, contains filaments which are dispersed rather than gathered into bundles. The cells of the superficial layer have a persisting pyknotic nucleus as well as a few other organelles present.

1.3.8 Nonkeratinocytes within the epithelium

The cells engaged in the process of maintaining the integrity of the oral epithelium are termed keratinocytes regardless of whether they are progenitor cells or cells involved in the maturation of keratinised or nonkeratinised epithelium. There are a number of cells within the oral epithelium which have other functions and these are grouped under the heading of nonkeratinocytes. Most of these cells lack desmosomal attachments to other cells and their cytoplasm shrinks during histological processing to produce a clear zone surrounding the nucleus. Within this category would be included melanocytes, Merkel cells, Langerhan's cells and lymphocytes.

Melanocytes synthesize the pigment melanin which is injected into the cytoplasm of adjacent keratinocytes. The regions of the oral mucosa where melanin pigmentation is most commonly seen are the gingivae, buccal mucosa, hard palate and tongue. Colour differences between individuals are due not to differences in numbers of melanocytes, but differences in melanocyte activity. Melanocytes are found in the basal layer of oral epithelium.

Merkel cells are also found in the basal layer of oral epithelium. They contain tonofilaments. Having some desmosomal attachments to adjacent cells they may appear somewhat different from other clear cells in histological sections. Fortman and Winkelman (1977) suggest that the Merkel cell is a neural receptor

probably originating from the neural crest.

The Langerhan's cells are considered to fulfil a function within the immunological response, and are usually found in the upper part of the epithelium.

A small number of inflammatory cells, usually lymphocytes, is commonly found in the suprabasal layer of the oral epithelium. These cells are regarded as being a normal component of oral epithelium.

1.3.9 The structure of the lamina propria

The epithelium of the oral mucosa is supported by a connective tissue layer, the lamina propria, which separates it from the underlying bone or submucosa. The lamina propria consists of fibres embedded in a ground substance. It can be considered as consisting of two layers contrasted by the arrangement and density of the fibres. The superificial papillary layer is associated with the epithelial ridges and in it the fibres are thin and loosely arranged. In the deeper reticular layer the fibres are arranged in dense bundles which lie parallel to the surface plane.

1.3.10 Fibres and ground substance

The fibroblast is responsible for the formation of the fibres and ground substance. The major types of fibres found are elastin and collagen. Elastin is the major component of the elastic fibres

which are most commonly found in lining mucosa where they restore tissue form after stretching. The collagen fibres are loosely arranged in the papillary layer and form thick bundles in the reticular layer. The collagen fibres in masticatory mucosa are thick and anchor the mucosa firmly to the underlying bone. In lining mucosa there are fewer collagen fibres which follow an irregular course allowing the tissues to stretch to a certain extent. The collagen cells are assembled from long polypeptide chains that include in particular the amino acids proline and lysine. The ground substance consists of heterogeneous protein-carbohydrate complexes permeated by tissue fluid. It appears amorphous in histological sections.

1.3.11 Cells of the lamina propria

In addition to the collagen and elastic fibres the lamina propria contains a variety of cells. The actively synthesising fibroblast plays the main role in maintaining the integrity of the lamina propria. The fibroblasts appear as stellate cells and are associated with collagen fibres. Other than at times of wound healing, fibroblasts have a low rate of proliferation.

Macrophages are also present and unless these are actively phagocytosing extracellular debris they may be difficult to differentiate from fibroblasts with the light microscope. The large mononucleated mast cell is

frequently found in association with small blood vessels and it plays a role in the initiation of the inflammatory process and vascular homeostasis. Other than in the inflammatory process, lymphocytes and plasma cells are present only in small numbers scattered throughout the lamina propria.

1.3.12 The basement membrane

The undulating interface where the connective tissue papillae interdigitate with the epithelial ridges is the basement membrane. The arrangement gives a much larger surface area to the basement membrane than if it were a simple flat junction. This provides a better attachment between the two layers and enables forces applied to the epithelial surface to be dispersed over a greater area of connective tissue. Papillae are more numerous in masticatory mucosa than lining mucosa. As there are no blood vessels within the epithelium this junction represents the major route for metabolic exchange.

Although the basement membrane appears structureless in light microscope sections, electron microscope analysis has shown it to consist of two distinct layers known as the lamina densa and the lamina lucida (Stern 1965). Fibrils of collagen from the connective tissue anchor the deeply placed lamina densa. There are condensations of material in the superiorly

placed lamina lucida opposite the hemidesmosomes of the epithelial cells.

1.3.13 Blood supply to the oral mucosa

The blood supply to the oral mucosa arises in arteries which run in the submucosa. When the submucosa is absent and the lamine propria is bound to underlying periostium, arteries run within the deep part of the reticular layer of the lamina propria. These vessels give off progressively smaller branches which form a capillary network within the papillary layer of the lamina propria deep to the basal cells of the epithelial layer.

1.3.14 Nerve supply to the oral mucosa

The sensations perceived within the oral cavity are temperature, touch, pain and taste. The sensation of taste is limited to the oral cavity and pharynx. It is perceived by specialised receptors, the taste buds, which are found mainly within the epithelium of the papillae on the dorsum of the tongue. Some taste buds occur in the epithelium of the soft palate and pharynx.

In addition to taste buds the sensory nerve endings within the oral mucosa occur as free nerve endings or specialised nerve endings. The specialised nerve endings consist of coiled fibres surrounded by a connective tissue capsule. Such specialised receptors

include Meissener's corpuscles, bulbs of Krause and mucocutaneous end organs. Within the epithelium only free nerve endings occur and these are frequently associated with Merkel cells. Other free nerve endings within the epithelium may pass between the keratinocytes and terminate in the middle or upper layers of the epithelium. Within the lamina propria free nerve endings and specialised nerve endings occur. The organised nerve endings are found mainly in the papillary layer. The sensory nerve networks are more developed in the oral mucosa of the anterior part of the mouth and this results in a greater sensory perception in this part of the oral cavity.

1.3.15 Age changes in the oral mucosa

Among the physiological changes which occur in oral mucosa with age are a thinning of the epithelium and a flattening of the interface between the epithelium and the lamina propria. In a study of autopsy material Nedelman and Bernick (1978) observed changes in the connective tissue of oral mucosa. They noted that in specimens from the older age group, collagen was arranged into regular compact bundles. In contrast, in specimens from young adults collagen was irregularly arranged. They also noted a change in the constituents and a loss of water content from the ground substance with advancing age. Vascular changes were also noted

within oral mucosa. and there was found to be arteriosclerosis of the larger blood vessels and hyalinisation of smaller vessels.

Shklar (1966) gave a descriptive account of atrophy affecting the structure of oral mucosa in elderly persons. He noted differences between the elderly and the very young but observed no distinctive characteristics evident in the intermediate age group between 25 years and 50 years.

Scott et al. (1983) studied human lingual epithelium in autopsy material from 86 cases. They used morphometric techniques to make quantitative assessment of this material and observed a thinning of the epithelium with age. A 30% reduction in epithelial thickness was seen over the age range within the study. The range was from 16 to 98 years. This reduction in thickness was attributable to the maturation compartment of the epithelium. There was no significant change in the thickness of the progenitor compartment. A flattening of the contours of the basement membrane with age was noted.

1.4 NORMAL ORAL MUCOSA AND DENTURES

1.4.1 Methods of investigation

Knowledge of the structure of healthy oral mucosa and of the mechanisms which maintain normal function is important in order that the changes which

occur in disease can be interpreted. Likewise the response of oral mucosa to the wearing of dentures is important and a knowledge of the structure of healthy oral mucosa under dentures will aid the understanding of any denture-related disorder such as denture-induced stomatitis. There is no consensus of opinion as to the effect of the wearing of dentures on oral mucosa, although it has been the subject of much investigation. The main methods of investigation have been in the use of human autopsy and biopsy material.

1.4.2 Autopsy investigation

The tissue changes induced by the wearing of dentures were first investigated by examination of autopsy material by Pendleton (1934). He noted that up to that time investigation in this field had been limited to clinical observation. This study on the effects of denture wearing on the oral mucosa included examination of autopsy material from two maxillary specimens and the epithelium covering the palate was described simply as being of a stratified squamous type.

Pendleton (1940) also described subjectively the structural changes occurring following the extraction of teeth and insertion of dentures as observed in an autopsy study. The mandible and maxilla of only one specimen were examined. The discussion dealt largely with the bone changes found underlying

dentures but mention was made of subjective changes found within the soft tissues. It was noted that the tissues had a surprisingly healthy appearance and that the connective tissue was free from inflammation. The epithelium covering the hard palate and the alveolar ridge was found to have a cornified surface which in some areas showed evidence of parakeratosis.

Van Scotter and Boucher (1965) in a study of eighty postmortem specimens specifically examined the effect of denture base materials on the surface epithelium of maxillary mucosa. Using an optical micrometer, measurements of stratum corneum thickness were made in the maxillary premolar region. Four categories of specimen were examined and the following values for the mean thickness of the stratum corneum layer were found :

a) dentate specimens 14.25 μm

b) edentulous (not wearing a denture) 20.24 μm

c) edenulous (wearing acrylic denture) 17.8 μm

d) edentulous (wearing vulcanite denture) 5.8 μm

Groups (a) (b) and (c) are of interest in relation to work undertaken by the present author. In those groups keratinisation was evenly divided in type between parakeratosis and orthokeratosis. It would appear from the above figures that loss of all teeth without prosthetic replacement encourages thickening of the stratum corneum, and that the use of an acrylic replacement complete denture also stimulates thickening

of the cornified layer. Although restricted in the amount of analysis carried out, the quantitiative assessment of stratum corneum thickness in the postmortem specimens gave a clearer understanding of the degree of change observed within the tissues than subjective assessment would have done.

Watson and MacDonald (1980) investigated the value of autopsy material in the quantitative assessement of palatal mucosa. They examined 3 mm diameter tissue sections obtained from the alveolar ridge in the maxillary first molar region. However, in 3 out of 9 cases they examined, some autolytic changes were evident and they cite this occurrence, along with the absence of a detailed dental history as being the potential disadvantages of this mode of investigation. Using the technique of stereological analysis they found the mean epithelial thickness at the crest of the ridge in the first molar region in the maxilla to be 247.9 μm. The stratum corneum thickness in the maxillary specimens had a mean thickness value of 11.45 μm. The type of keratinisation was equally divided between parakeratosis and orthokeratosis in this small sample.

Watson & MacDonald (1983) in a further study of eight autopsy specimens assessed the regional variations in the structure of human palatal mucosa. Sagittal sections of intact decalcified palates were examined. All specimens were from edentulous

individuals who had been wearing complete dentures. Stereological methods of quantitative analysis were used, and it was found that although there was a wide range of epithelial thickness among the sample specimens there was a pattern of epithelial thickness dependant upon the anatomical site examined. The pattern of thickness was similar in all the specimens examined, being greatest at the crest of the ridge and least on either side close to the midline. A regular pattern of variation throughout the cross section of the palatal epithelium highlights the need for standardisation of sample sites, and the problems of comparison of results of workers using variable sampling procedures.

Krajicek et al. (1984) studied autopsy material from thirty-seven edentulous subjects, fourteen of whom had worn dentures and twenty three of whom had not. The type of epithelial surface specialisation was noted. There was also a subjective assessment of epithelial thickness and form. Specimens were taken from the first molar region of the mandible. The specimens contained epithelium, connective tissue and bone. No significant difference between the two groups was noted in terms of epithelial thickness or form. However the denture wearing group showed more nonkeratinised epithelium, and the non denture wearing group showed more parakeratosis.

The examination of palatal epithelium is of particular relevance to the present study. From the

descriptive findings of the initial work by Pendleton (1934) to the quantitative assessments made by recent workers (Watson & MacDonald, 1983), much data has been gathered on the effect of complete dentures on oral mucosa by autopsy investigation. The study by Watson and MacDonald (1983) highlights the importance of site selection in any comparative study of oral mucosa.

1.4.3 Biopsy investigation

Pendleton (1951) on examination of biopsy material found areas of orthokeratinisation and parakeratinisation as well as areas which did not exhibit keratinisation. However the predominant form of surface specialisation was parakeratinisation. The biopsy material from 126 edentulous subjects, was taken from the maxillary tuberosity region.

Ostlund (1958) examined biopsy material from immediately anterior to the junction of soft and hard palate adjacent to the midline. He observed a reduction in thickness of the cornified layer induced by the wearing of dentures.

Kapur and Shklar (1963) examined biopsy material from 9 dentate subjects with no experience of denture wearing, and compared it with that found in the same subjects following extraction of teeth and a 12 week period of wearing of complete dentures. The observation was made that the thickness of the surface

layer, the stratum corneum, was found to increase following a period of denture wearing.

Nedelman et al. (1970) examined biopsy material from the alveolar ridge mucosa of 62 patients. 42 of the patients in this study had not worn dentures previously and 20 had experience of wearing partial dentures. The findings indicated that the stratum corneum layer was thinner in those patients who had been wearing partial dentures.

Van Mens et al. (1975a) biopsied the palatal mucosa of 40 patients halfway between the alveolar ridge and the midline at the level of the first permanent molar. Half the biopsy specimens were from dentate patients with no experience of the wearing of dentures and the other half were from patients who had worn complete dentures for a minimum of four years and whose oral mucosa was of healthy appearance. By examination of photomicrographs of palatal tissue, and by weighing tracings of the photomicrographs, observations were made on the effect of the wearing of complete dentures on the basement membrane morphology and epithelial thickness. The findings were :

1) the basement membrane is significantly less irregular in denture wearers than in non denture wearers.

2) no significant changes in epithelial thickness was noted between the two groups.

No changes in the surface cornified layer were noted.

Jani and Bhargava (1976) studied 40 edentulous patients by means of biopsy investigation before the insertion of dentures and again after three months of wearing of complete dentures. It was found that the wearing of dentures induced a thickening of the palatal epithelium which was most pronounced in the areas of the rete pegs and that there was an increase in the thickness of the cornified layer. Direct measurements showed the epithelial thickness to be 222μm in the areas of the rete ridges and 129μm between the rete ridges.

Watson (1978) provided quantitative information on the structure of oral mucosa by stereological analysis of biopsy tissue. He compared two groups with 12 subjects in each group. In one group the subjects were wearing dentures and in the control group no dentures were worn. The basement membrane was found to be more regular within the group wearing dentures, but there was found to be no significant difference in the epithelial thickness between the two groups. There was found to be no difference in the thickness of the stratum corneum between the two groups. The biopsy site in each case was at the level of the first molar region, midway between the alveolar crest and the palatine blood vessels.

Biopsied palatal mucosa from the same site

was examined in a further study of 48 male patients by Watson and MacDonald (1982). They reported that a thinner stratum corneum layer with a reduced degree of keratinisation was observed in 27 subjects wearing dentures when compared with a control group of 21 subjects. The mean stratum corneum thickness in the respective groups was 13.8μm in the denture group and 20.4μm in the control group. Data obtained from this study showed there to be considerable variation in the values for epithelial thickness in both groups of patients although the mean values for epithelial thickness did not differ significantly between the groups. The mean values of epithelial thickness were 240.4μm in the denture group and 268.9μm in the control group. These workers used the term epithelial morphology to describe the degree of regularity of the basement membrane in the investigation of oral mucosa. They defined epithelial morphology to represent the ratio of basement membrane length to surface length. Consequently the higher the epithelial morphology value the more irregular is the basement membrane. In this study it was reported that the epithelial morphology of the denture wearing group was significantly less than the control group. The mean values for epithelial morphology were 2.22 in the denture group and 2.68 in the control group.

Overall analysis of the findings of the investigations described in which biopsy tissue was

examined, is complicated by the variety of sites and methods of analysis used by different workers. There are contradictory findings on the effect that denture wearing has on the thickness of the stratum corneum. No significant difference in epithelial thickness between denture wearing and non denture wearing groups was found by Van Mens et al. (1975), Watson (1978) and Watson and MacDonald (1982), but Jani and Bhargava (1976) found that denture wearing resulted in thickening of the palatal epithelium. Van Mens et al. (1975) and Watson and MacDonald (1982) found the basement membrane to be more regular in subjects wearing dentures when compared with subjects not wearing dentures.

1.4.4 Techniques of analysis of oral mucosa

Oral mucosa is an irregular tissue which exhibits variations in structure which are dependent upon the location of the sample site. Despite autopsy and biopsy investigation over a period of many years there is still no clear picture of the histological structure of the oral mucosa supporting complete dentures.

The subjective nature of reporting by many investigators has been unsatisfactory. Problems of autolytic tissue changes and unknown dental history in autopsy investigation have been apparent. The availability of a limited amount of tissue for

examination is the major disadvantage of the use of biopsy material. The inconsistent location of biopsy sites by various workers has contributed to the lack of clarity apparent on examination of the literature in relation to biopsied tissue.

The use of quantitative analysis of biopsy tissue and careful site selection should be important considerations in any further investigation of oral mucosa under dentures. From review of the literature it is apparent that more meaningful interpretation of results would be possible if quantitative objective analysis were reported and if careful selection of methods allowed comparison of the work of different individuals or groups.

1.5 ORAL DISEASE AND THE WEARING OF DENTURES

1.5.1 The incidence of edentulousness

The significance of dentures in the occurrence of oral disease in patients wearing dentures has to be assessed with consideration of factors such as the presence of systemic disorders, the incidence of oral disease in non denture wearing subjects and the effects of age on the oral tissues. A large proportion of the adult population of this country is edentulous, and the wearing of complete dentures is a relatively common occurrence. This is particularly the case

amongst the elderly population, the group most likely to present with oral disease.

The Office of Population Censuses and Surveys (1985, 1987) has published data gathered from the General Household Survey which indicates that in England and Wales the incidence of edentulousness in the population aged between sixty-five and seventy-four years of age is diminishing. Within this age group 64% were found to be edentulous in 1983 and 61% in 1985. Earlier studies by Todd and Walker (1980) had shown that in 1978 and 1968 the incidence of edentulousness for the same population age group was 74% and 79% respectively. There is an indication that the incidence of edentulousness in the elderly is diminishing, but it is still prevalent.

As the majority of the elderly are edentulous it follows that oral disease in the elderly will often occur in individuals wearing dentures. In most instances of oral disease in edentulous patients, denture wearing will not be the primary aetiological factor.

Budtz-Jorgensen (1981) states that in those disorders directly associated with denture wearing three broad categories exist. These are disorders caused by mechanical mucosal injury, microbial denture plaque and allergy to or chemical irritation by constituents of the denture base.

1.5.2 Allergy and chemical irritation

Allergy corresponding to a type IV hypersensitivity reaction is believed to occur as a result of wearing dentures. Pure polymers such as polymethylmethacrylate are inert materials and are unlikely to initiate a hypersensitivity reaction. It is more likely that other constituents of the denture base act as sensitising agents in the development of an allergic contact stomatitis.

Austin and Basker (1982) have reported that shortening of the processing time for polymethylmethacrylate, or failure to adhere to recommended processing temperatures can result in high levels of residual methylmethacrylate monomer being present in denture bases. It is recognised that residual monomer may give rise to an inflammatory reponse in the denture-bearing tissues, but evidence is lacking that the monomer acts as a hapten. It is likely that this adverse reaction is due to chemical irritation.

The release of formaldehyde from denture base materials, particularly those that have been chemically cured, has been shown by Ruyter (1980). Formaldehyde has been shown to produce allergic reactions, and may play a role in true denture allergy.

Benzoyl peroxide is used to initiate polymerisation of methylmethacrylate. Poole et al.

(1970) have reported on contact sensitisation with benzoyl peroxide. In sufficient concentration with repeated exposures this chemical could be regarded as a hapten in some cases of acrylic allergy.

1.5.3 Mechanical mucosal injury

The overextension or movement of a rigid denture base can cause irritation or ulceration of the mucosal lining of the mouth. Poorly fitting dentures or surface irregularities of a denture base can also produce traumatic injury. If ulceration due to trauma is allowed to persist, bacterial invasion and infection of the deeper tissues may occur.

Constant trauma from the flange of a denture can give rise to hyperplasia of the oral mucosa. Denture-induced hyperplasia is described as consisting of a raised lesion, which may be sessile or pedunculated, composed of dense fibrous tissue (Ralph and Stenhouse 1972). Nordenram and Landt (1969) and Cutright (1974) analysed the occurrence of this disorder in separate investigations involving 430 and 583 cases respectively. In both studies, the incidence and site of hyperplasia was recorded. It was found that the anterior area of the mouth was the most common site of occurrence. In both studies there was found to be a higher incidence amongst females than males and hyperplasia was most prevalent in patients in the age range between fifty and sixty years. The disorder is

associated with badly fitting dentures.

Although Vogler et al. (1962) could not relate oral cancer to the presence or fit of dentures, they have cited that chronic mechanical injury due to the wearing of dentures is a possible aetiological factor in the pathogenesis of oral malignancy. Wynder et al. (1957) noted that twice as many oral cancer patients were edentulous than an age matched control series. In a retrospective study of 194 cases of oral cancer Langdon et al. (1977) cite mechanical irritation as a predisposing factor in 7.2% of the sample. The nature of the mechanical irritation is not discussed.

Opinions vary greatly as to the significance of trauma in oral cancer and whilst it is believed by some that oral cancer is related to chronic irritation within the oral cavity, it has not been possible to prove this postulation (Graham et al. 1977). Injuries caused by badly fitting dentures have been considered with other causes of oral trauma, but as the occurrence of chronic mechanical intra-oral trauma is common, the occurrence of superimposed oral cancer may be coincidental and a relation between denture wearing and oral cancer has not been proven on a statistical basis.

Mechanical mucosal injury from dentures has been implicated as an aetiological factor in denture induced stomatitis, by Nyquist (1952). He examined 1090 subjects and stated that different types of trauma

played a significant part in the occurrence of denture-induced stomatitis. Factors of importance in avoiding trauma from dentures were that dentures be stable, with correct centric occlusion and balanced articulation.

1.5.4 Microbial denture plaque

Budtz-Jorgensen and Loe (1972) demonstrated that the use of chlorhexidine as a denture disinfectant can resolve inflammatory lesions related to the wearing of dentures. By the removal of denture plaque using chemical means and the modification of the bacterial environment under dentures, the signs of denture-induced stomatitis were resolved in a significant number of cases in a trial involving 59 subjects. This study highlights the role of denture plaque in the pathogenesis of some oral disease related to the wearing of dentures.

1.6 DENTURE-INDUCED STOMATITIS

1.6.1 General characteristics

Denture -induced stomatitis is the term used to describe inflammatory changes confined to the oral mucosa under a complete or a partial denture. The condition is usually painless and is characterised by erythema of the affected area. Inflammation of the denture-bearing tissues as a direct result of denture

wearing occurs more commonly under a maxillary denture than a mandibular denture.

Three stages in the pathogenesis of denture-induced induced stomatitis, dependent upon the clinical signs, have been described by Newton (1962). Stage one is described as exhibiting pinpoint hyperaemia, where small areas of inflammation are found around the orifices of the ducts of the palatal mucous glands. In the second stage of diffuse hyperaemia there is generalised inflammation of the entire denture-bearing area. The third, or granular, stage is described as exhibiting a nodular papillary hyperplasia over all or part of the denture-bearing surface. Newton suggested that the disorder proceeds from the first stage, through the second to the third.

Nyquist (1952) concluded that denture trauma is of considerable importance in the aetiology of denture-induced stomatitis, and Turrell (1966) suggested that at least in some cases, denture-induced stomatitis occurs as a result of denture trauma.

Cahn (1936) noted the possible role of the Candida organism in denture-induced stomatitis. The involvement of Candida albicans in the disorder was more generally accepted following the work of Lyon and Chick (1957). They demonstrated that denture-induced stomatitis can develop in the absence of trauma and can be eliminated by antifungal treatment alone without modification of dentures.

These findings were confirmed by Cawson (1963) in a study of 35 patients with denture-induced stomatitis. He pointed out that in 33 patients in this series there was positive indication of colonisation by Candida albicans. Successful treatment with antifungal therapy occurred in all cases, including those where no candidal organisms had been isolated. In those cases which exhibited the signs of denture-induced stomatitis, but where Candida albicans was not isolated, Cawson suggested that it was likely that fungus was present, but sampling methods were defective.

Cawson (1965) in a study of 59 patients with denture-induced stomatitis, found Candida albicans to be present in the hyphal form in 90% of the sample. In view of the preponderance of the fungus in the hyphal form in association with thrush and chronic candidiasis he stated that the presence of fungus in this form gives an indication that the organism is present as a pathogen within the oral cavity. This observation is of importance, as Candida albicans is present as a commensal in a sizeable proportion of the population. Bartles and Bletchman (1962) found that 40% of a sample of 160 apparently normal individuals aged between twenty and thirty years of age were carriers of oral Candida albicans, but showed no signs of oral disease. Berdicevsky et al. (1977), using salivary culture, found a carrier rate of 48% in clinically healthy individuals.

According to Budtz-Jorgensen (1974) either local or systemic factors may allow Candida albicans to assume a pathogenic role in the mouth. Included among local factors would be the wearing of ill-fitting or unhygienic oral appliances. Also included are conditions which result in a reduced salivary flow, for example, head and neck irradiation or certain drug regimes. Topical use of antibiotics, in lozenge or mouthwash form, can alter the intra-oral environment and may result in the proliferation of candidal organisms.

Systemic factors can result in a reduction in the efficiency of body defence mechanisms and allow proliferation of candidal organisms intra-orally. Such factors would include pregnancy, diabetes mellitus, immune deficiency or suppression and haematological disorders such as iron deficiency or leukaemia.

Oral candidal infection can be the starting point for systemic spread. Systemic candidiasis is most likely to occur in those patients on prolonged antibiotic or corticosteroid therapy, or those whose resistance is reduced by systemic disease (Hart et al. 1969). Candidal endocarditis is a small but potentially serious risk in patients with valvular heart disease who are receiving prophylactic antibiotic therapy (Andriole et al. 1962).

Two surveys have been undertaken in Scandinavia to assess the incidence of denture-induced stomatitis in the population in general. In 1975

Budtz-Jorgensen et al. assessed a random sample of the elderly population of Denmark and found that amongst the 465 individuals who were wearing dentures the prevalence of clinically inflamed palatal mucosa was 65%. An incidence of 39% was found in a random sample of Norwegian elderly denture wearers in a study published in 1985 by Ambjornsen. In the studies of Nyquist (1952), and Neil (1961) and Cawson (1965) there has been reported a preponderance of female patients presenting with the disorder.

Davenport (1970) in an investigation of 50 patients with denture-induced stomatitis, studied the intra-oral pattern of distribution of the candida organisms, by means of smears and a replica imprint culture technique. He concluded that the disorder is associated with a proliferation of the organism in denture plaque rather than on or in the palatal mucosa. He also found a higher incidence in females.

Methods of assessing levels of Candida albicans within the oral flora as a means of determining whether the organism is being harboured as a normal commensal or as an infective pathogen have been suggested. Renner et al. (1979) used a smear sample and by a method of serial dilutions were able to assess the concentration of Candida albicans in the original sample.

Comparison of techniques to assess microbial

populations under dentures was undertaken by Arendorf and Walker in 1980. In 54 healthy adult dentate patients they assessed the prevalence of oral carriage of Candida albicans using an imprint culture technique, salivary samples and impression culture methods. The use of an imprint culture technique proved to be most effective in revealing the presence of Candida albicans. A carrier rate of 44.4 per cent was found. The technique was useful in identifying which areas of the mouth harboured the largest quantity of organisms. The dorsum of the tongue was found to be the primary oral reservoir of Candida albicans.

Arendorf and Walker (1979) reported on a study which determined the changes in oral density and distribution of Candida albicans which occurred in denture-induced stomatitis. They used an imprint culture technique. The findings in patients with denture-induced stomatitis were assessed along with those from healthy dentate patients not wearing dentures and healthy patients wearing dentures and exhibiting no signs of denture-induced stomatitis. Colony counts were found to be highest in those patients with denture-induced stomatitis and least in the dentate patients. It was suggested that enumeration of candidal colonies could help to distinguish between the carrier state and oral candidosis. According to these workers colony counts in excess of 49 per square centimeter were an indication of oral candidosis. It has been suggested

by Wain et al. (1976) that large numbers of yeasts are required to produce sufficient enzymes to penetrate oral mucosa.

The concept that the quantity of yeasts found under a complete denture is significant in the pathogenesis of denture-induced stomatitis is supported by Budtz-Jorgensen et al. (1983). They found that significantly higher numbers of yeasts and bacteria were cultured in patients with the signs of denture-induced stomatitis than in controls. These authors suggested that bacteria as well as yeasts have a pathogenic role in the condition and that the primary therapeutic measure which should be undertaken is denture hygiene to reduce denture plaque rather than antibiotic therapy.

Little is known about the mechanisms whereby Candida albicans adheres to acrylic surfaces. Samaranayake & MacFarlane (1980) have shown that Candida albicans will adhere to acrylic strips in vitro. Samaranayake et al. (1980) suggest that the effectiveness of the attachment can be enhanced or diminished by a variety of factors. Amongst the factors found to increase adhesion was incubation of the yeasts in glucose or sucrose. Reduced adhesion was achieved by coating the acrylic strips with mixed saliva or 2% aqueous chlorhexidine.

1.6.2 Histological investigations

In his study to examine the effects of complete dentures on oral mucosa, Ostlund (1958) included a group of patients wearing dentures who exhibited the diffuse erythema of the palatal denture-bearing mucosa typical of denture-induced stomatitis. A combination of macroscopic measurement and projection of histological specimens suitable for measurement gave an imprecise measure of the thickness of oral mucosa and epithelium. The biopsy specimens were taken from an area immediately anterior to the vibrating line lateral to the midline. Among the findings was the observation that in denture-induced stomatitis the thickness of the oral epithelium showed a marked increase in comparison with that of patients not wearing dentures. No quantitative comparison with normal oral mucosa under dentures was made.

Budtz-Jorgensen (1970) carried out an extensive subjective examination of the histological changes found in denture-induced stomatits. Biopsy specimens, 5 mm in diameter, were taken 1.5 mm anterior to the vibrating line and 1.5 mm lateral to the midline in 24 subjects. Areas of alternating epithelial atrophy and hyperplasia were found. Also noted were chronic subepithelial inflammation and intra-epithelial infiltrations of leucocytes. There was acanthosis of the epithelium and the surface layer exhibited either parakeratosis or complete absence of keratin. No intra-epithelial hyphal infestation was identified.

Van Mens et al. (1975b) took biopsy material from the first molar region of the maxilla halfway between the alveolar ridge and the midline in 9 subjects. The specimens were 1.5 mm in diameter. Following preparation and staining, the sections were examined in the form of photomicrographs to allow quantitative assessment to be made. No significant difference in epithelial thickness was noted in the subjects exhibiting the signs of denture-induced stomatitis in comparison with control subjects.

Anneroth and Wictorin (1975) carried out histological analysis of biopsy tissue in a group of 12 subjects exhibiting the signs of denture-induced stomatitis. The tissue was taken from the buccal aspect of the alveolar ridge in the second premolar area of the maxilla. Wictorin et al. (1975) re-examined 10 of the same group of patients, 9 of whom had persisting signs of denture-induced stomatitis despite intervening prosthodontic therapy. The subjective histological assessment of the affected oral mucosa suggested that the main mucosal changes found in denture-induced stomatitis were a reduction in epithelial thickness and either incomplete keratinisation or the complete absence of a keratinised layer. A marked inflammatory reaction within the epithelium as well as in the connective tissue was also noted.

Bergendal et al. (1979) studied biopsy tissue

from an unspecified palatal site in patients with denture-induced stomatitis and noted that there was no evidence of fungal hyphae invading the epithelial tissues.

Bergendal & Isaacsson (1983) sampled what they described as a representative part of palatal mucosa affected by generalised diffuse denture -induced stomatitis. In 19 subjects punch biopsies, 3.9 mm in diameter, were analysed. The subjective observations describing the histopathological appearance of the mucosa were that a nonkeratinised epithelium was present which showed alternating areas of atrophy and deep rete ridge formation. Leucocytes were found within the epithelium and a subepithelial inflammatory response was present with lymphocytes and plasma cells in evidence.

From the evidence available in the studies reviewed, the histopathological changes associated with denture-induced stomatitis appear to be non specific. An absence of keratin or parakeratosis, atrophy, hyperplasia and acanthosis have been the epithelial changes noted. A chronic inflammatory reaction within the lamina propria has been characteristic. Tissue invasion by hyphae has not been apparent. Attempts at quantifying the extent of the morphological changes within the epithelium have been restricted in number and have produced no clear picture of the changes occurring.

1.6.3 Animal investigations

The use of animal models for experimental investigation has been widespread in many fields of medical and dental research. Review of the literature shows that this mode of investigation has been undertaken by several workers studying the histopathological changes induced in palatal mucosa following insertion of an intra-oral appliance.

1.6.4 Experimental models - monkeys

An experimental animal model for the investigation of denture-induced stomatitis was first described by Budtz-Jorgensen (1971). He utilised the Macaca irus species of monkey in this innovative series of experiments. Acrylic oral appliances were used in conjunction with inoculated Candida albicans. The appliances were retained by extension into saddle areas created by dental extraction. The effects on palatal mucosa, of wearing an appliance for an extended period of time, with or without inoculation of Candida organisms, were examined. The effects of the topical application of tetracycline under these conditions, and the reinfection with Candida albicans following initial healing were also examined.

It was noted in the findings that the lesions produced in the animals were clinically similar to the appearance of the generalised palatal candidiases seen in human denture-induced stomatitis. It was also

described that certain histological features of the lesions were similar to those described in acquired human candidiasis, notably that epithelial atrophy and intra-epithelial leucocyte infiltration were present. It was found that prolonged topical application of tetracycline resulted in a more intense and sustained inflammatory response to candidal inoculation than did exposure to candida alone, and that reinfection with Candida albicans following healing produced a more intense sub-epithelial inflammatory response than that produced by the primary infection. Epithelial invasion by candidal hyphae was not detected.

According to Olsen & Haanaes (1977), the work of Budtz-Jorgensen, using Macaca monkeys, demonstrated the suitability of the animal model, and they used a similar animal model for investigation in this field. There were differences in the technique of Olsen & Haanaes who used the Cercopithecus aethiops species of monkey. This was a different species to that used by Budtz-Jorgensen, and in addition these were young animals whose immune defence system may have been incompletely developed. The use of a different strain of Candida albicans introduced variations of virulence and pathogenicity, and the variation in the method of fixation of the palatal appliances may have produced a closer fitting prosthesis in the experiments of Olsen & Haanaes. They used direct ligature fixation, which

would have produced a difference in environment underneath the appliance.

As opposed to the palatal lesions produced in the work of Budtz-Jorgensen (1971) who observed a generalised atrophic candidiasis, Olsen & Haanaes (1977) found an acute pseudomembranous candidosis in all inoculated monkeys. The discrepancy in response to the induced fungal infection may be related to the differences in technique.

The use of the monkey animal model by differing groups of investigators, examining the pathogenesis of palatal candidal infection provided useful information for subsequent work, as it showed that candidal inoculation under an acylic prosthesis in the monkey could produce lesions which were clinically and histologically similar to those acquired in the human. The results produced in an animal model are dependent upon many different and unrelated factors. Standardisation of technique and cautious interpretation of results are appropriate.

1.6.5 Experimental models - rats.

The use of Wistar rats in the investigation of the pathogenesis of oral thrush was recorded by Jones and Adams in 1970.

The use of the Wistar rat in an animal model for the investigation of palatal candidiasis was first described by Olsen and Bondevik in 1978. This model

has formed the basis of several subsequent studies by various authors, and is the basis of the animal investigation undertaken by the present author.

The original work by Olsen and Bondevik (1978) involved two series of experiments using a total of 38 rats. The experimental period was two weeks. In this investigation the animals were considered in four groups. These groups consisted of, control animals, animals wearing acrylic appliances, animals inoculated with Candida albicans, and animals inoculated with Candida albicans under an acrylic appliance. The appliances in question were small plates constructed in self cured acrylic, which covered the palatal mucosa and which were secured to the incisor teeth. Examinations of clinical appearance, microbial cultures, smears and histological sections of epithelium were undertaken.

It was found that a generalised simple inflammation developed in most of the experimental animals. This occurred most frequently, was most intense, and persisted longest, in the group inoculated with Candia albicans under an appliance. Histological changes within the palatal epithelium were evident in the experimental animals when compared with the control animals, and were most marked in the group inoculated with Candida albicans under an appliance. The histological changes were characterised by

parakeratosis, epithelial hyperplasia, epithelial atrophy, and intra-epithelial or sub-epithelial leucocyte infiltration. Epithelial penetration by hyphae was not seen.

All appliances in this study were constructed from a single master cast and consequently adaptation to the underlying mucosa may not have been accurate. There is irregularity of the Wistar rat palate due to coverage of all of the surface by rugae. The traumatising effect of a poorly fitting prosthesis may have played an important role in the histopathological results reported, a point not raised by the authors. Olsen and Bondevik concluded that the Wistar rat provided a suitable animal model for the study of experimental denture-induced stomatits. No further study in this field by either author was apparent in a search of the literature.

Shakir et al. (1981) used the Wistar rat animal model in a study of induced palatal candidiasis. Using an animal model of similar design to that developed by Olsen and Bondevik (1978), they extended the experimental period to six weeks and used a defined strain of Candida albicans from the National Mycological Reference Laboratory, London. The strain used in this study was Candida albicans 3091 (Serotype A), and it was chosen because it was readily available from the reference laboratory and had been shown to produce

hyphal penetration in rat tongue explants (Howlett 1976).

These workers noted marked histological changes within the epithelium induced in animals fitted with appliances and inoculated with Candida albicans. There was noted to be profound intra-epithelial acute inflammatory cell infiltration, micro-abscess formation and hyperplasia of much of the epithelium. Areas of epithelial atrophy were also noted. Penetration of candidal hyphae to the granular layer of the epithelium was observed. A chronic inflammatory cell infiltrate was present within the connective tissue underlying the palatal epithelium. All changes, which were described in subjective terms, were established after four weeks of the experimental period. The epithelium of those animals which were inoculated with Candida albicans only, and in those animals fitted with acrylic plates alone, was found to be histologically indistinguishable from untreated animals.

No mention is made in this study of the irregularity of contour of the Wistar rat palate. As all appliances were constructed from a single master cast, they may not have been well fitting and may have induced mechanical injury to the palatal surface. Brief mention was made of food impaction under the appliances, which were in place for up to six weeks, but the importance of this occurrence was not highlighted. Prior to tissue processing and preparation for

histological examination the palatal mucosa was stripped from the underlying bone. The mucosa was described as being divided into strips, but no account was given of the orientation of tissue for division.

These investigators were of the opinion that the presence of the fungus alone was insufficient to induce candidiasis, and that the presence of the acrylic appliance was necessary for the role of the organism to change from commensal to pathogen. The significance of trauma caused by the appliances was not considered.

1.6.6 Experimental models - therapy

In vitro studies by Addy (1981) investigated the concept of a denture base material or a lining material as a carrier for long-standing locally released medication within the oral cavity. Lamb and Martin (1983) using the Wistar rat model studied the effect of chlorhexidine incorporation into auto-polymerising acrylic plates upon the growth of Candida albicans. They found that chlorhexidine acetate could diffuse intraorally in fungicidal quantities for a period of at least three weeks. Inoculated candidal organisms were eliminated from the fitting surface of the plates and no histological changes in the epithelium were found in those animals fitted with acrylic appliances incorporating chlorhexidine. Control animals showed proliferation of candidal organisms and epithelial

changes including hyperplasia, inflammatory response and hyphal penetration, under unmodified acrylic plates, following Candida albicans inoculation.

1.6.7 Experimental models - iron deficiency

The regression of denture-induced stomatitis with iron therapy alone has been recorded in iron deficient patients by Cawson (1963) and a high incidence of candidal infection has been recorded in iron deficient patients by Fletcher et al. (1975). Due to the association of iron deficiency and persisting fungal infection it has been suggested by Jenkins et al. (1977) that patients with chronic oral candidal infections should routinely be screened for iron deficiency.

A study by Rennie et al. (1983) using Sprague Dawley rats treated with Candida albicans, but without the use of acrylic plates has also shown that iron deficient rats, particularly when given tetracycline, are less able than normal rats to eliminate Candida albicans from their tissues. There was no indication that under these circumstances iron deficiency predisposes to oral candidosis. No iron deficiency studies using the Wistar rat animal model with intra-oral appliances were found in the literature.

1.6.8 The role of Candida albicans

Winner (1969) stated that for candidal infection to occur it is essential for the organisms to

assume hyphal form, and Goldstein et al. (1965) described the rate of hyphal formation as being the criterion by which virulence of the candida organism should be determined. The exhaustion of metabolites and low oxygen tension found in the occlusive conditions under a denture may induce hyphal production and epithelial penetration according to Kemp and Solotorovsky (1962). Although hyphal invasion of palatal epithelium has been observed in a study of human biopsy material by Cawson (1966) most investigations have revealed no evidence of hyphal invasion of the superficial epithelial tissues.

In the Wistar rat model of Olsen and Bondevik (1978) no evidence of hyphal invasion was found after a two week period of wearing an oral appliance under which Candida albicans had been inoculated. However, Shakir et al. (1981) found hyphal invasion of eithelium occurring in the Wistar rat on extending the experimental period.

There has been speculation on differences in pathogenicity of the two forms of candida occurring. Salterelli et al. (1975) investigated the comparative pathogenicity of the yeast and mycelial forms of Candida albicans by intraperitoneal injection in mice. They found the mycelial form to be more lethal. However, Evans and Mardon (1977) in an animal model also involving mice reported that the yeast form of the

organism was the more likely to initiate disseminating systemic infection. Mardon et al. (1975) comparing the virulence of the yeast forms in mice suggested that variations in virulence may be due in part to the preferential localisation of each form of the microorganism in specific host organs.

On assessment of the evidence available Odds (1979) stated that there was an unquestionable association between hyphal production and an increase in the virulence of Candida albicans within the oral environment.

The Wistar rat animal model has been utilised in the investigation of the various yeasts present on the palatal tissues in human candidiasis. Shakir et al. (1983) found that in the animal model, Candida albicans (serotype A) consistently produced the histological picture commonly associated with palatal candidiasis when inoculated under an acrylic plate. Other candida species which are commonly found in debture-induced stomatitis, Candida tropicalis and Candida torulopsis, produced no such histological appearance in the animal model.

Although it would appear that formation of hyphae is necessary for the induction of oral candidiasis in the rat, the exact role is not clear. Localised conditions found under an acrylic plate appear to be essential for the organism to adopt a pathological role and decreased oxygen tension, exhaustion of

metabolites, mechanical trauma from appliances and exclusion of protective elements of saliva may be important in this regard.

The production of pathological changes in the epithelium of hamster cheek pouches in response to inoculation of candidal organisms may be significant in this context. The hamster cheek pouch is an area of low oxygen tension, has no salivary glands and is generally unaffected by saliva. The histological changes in candida treated hamster cheek pouch and human denture induced stomatitis are similar according to McMillan & Cowell (1985). This may indicate that lack of salivary protection and low oxygen tension are important.

The apparent difference in virulence between strains of Candida albicans requires further investigation. Identification of the factor or factors responsible for the increased virulence for a specific strain could be of help in the further understanding and treatment of candidiasis. The use of a suitable animal model would be of value in this respect, and further investigation and development of the Wistar rat model seems appropriate.

1.7 DISCUSSION

In this literature review the microscopic appearance of healthy oral mucosa, the changes induced

by wearing dentures and the structure of clinically inflamed mucosa under dentures have been considered. The importance of site selection and the advantages of quantitative techniques in analysis of palatal mucosa have been emphasised. In Chapter 2 of this research project human palatal epithelium, exhibiting the signs of denture-induced stomatitis, was investigated. Careful site selection and quantitative techniques of analysis were considered critical in procedures used. Results showed a wide range of epithelial change in this condition and further investigation of these changes involved the use of an animal model.

Descriptions within the literature of the use of an animal model in the study of palatal epithelium have been considered. In particular the Wistar rat animal model and its use in investigation of palatal candidiasis has been of interest. Chapters 3, 4 and 5 of this project were concerned with the use of the Wistar rat animal model in the investigation of palatal epithelium and the development of quantitative methods of tissue analysis for use in this context.

CHAPTER 2

HISTOLOGICAL, MICROBIOLOGICAL AND HAEMATOLOGICAL INVESTIGATIONS IN HUMAN ORAL CANDIDIASIS

2.1 INTRODUCTION

This study was undertaken to investigate the structure of epithelium found under dentures in patients exhibiting the signs of denture-induced stomatitis. The investigation undertaken involved ten patients who attended Glasgow Dental Hospital for the provision of replacement complete dentures and who exhibited signs of Type II stomatitis (Newton 1962).

2.2 AIMS OF STUDY

There were three aims of this study. These were to quantify the histological structure of inflamed mucosa under dentures, to assess quantitatively the growth of Candida albicans associated with the epithelial changes and to examine if the haematological status of the subjects appeared to have any influence on the findings.

2.3 MATERIALS AND METHODS

2.3.1 Selection of patients

Ten female patients exhibiting the signs of

Type II denture-induced stomatitis were selected. All the patients exhibited diffuse erythema covering all of the upper denture bearing mucosa, but were otherwise in good health. None had complaints relating to discomfort of the palatal epithelium. In all cases the dentures were being replaced because of inadequate tissue contact of the fitting surface or defects in the occlusal relationship of the dentures. No detailed assessment of the dentures was carried out. Each patient was informed of the nature of the project and written permission was obtained.

2.3.2 Selection of biopsy site

To allow comparison with quantitative data relating to clinically normal mucosa under complete dentures reported by Watson and MacDonald in 1982, the biopsy site chosen lay in the palate approximately midway between the alveolar ridge and the mucosa covering the palatal blood vessels at the level of the first molar (Fig. 2:1). This corresponded to the site used by Watson and MacDonald.

2.3.3. Specimen collection

Prior to the first clinical stage in complete denture construction, tissue was collected for analysis. Biopsies were carried out following injection of adrenaline-free local anaesthetic around the biopsy site

at a distance of approximately 1 cm from it. Using a trephine type biopsy punch, 3 mm in diameter, a circular cut was made through the full thickness of the palatal mucosa. Using a scalpel and fine tissue forceps this column of tissue was then carefully dissected away from the periosteum. Care was taken not to contact the epithelial surface of the biopsy specimen during cutting or removal of the specimen (Fig. 2: 2). Following removal of biopsied tissue each upper denture was replaced. This was sufficient to promote haemostasis and in all cases healing proceeded uneventfully.

2.3.4 Tissue preparation and section cutting

Each biopsy specimen was divided and one half fixed in Bouin's fluid, the other in 10 per cent buffered formalin. The tissue blocks were then processed in a Histokine automatic tissue processor and embedded in paraffin wax. Sections, 5μm in thickness, were cut on a rotary microtome. The tissue blocks were aligned so that the epithelial surface met the microtome knife first with the direction of the cut perpendicular to the plane of the epithelial surface.

2.3.5 Staining of sections

The sections were stained for general analysis with haematoxylin and eosin. This widely used stain shows cellular elements and connective tissue effectively. Other stains demonstrate keratin more

clearly and the Crooke-Russell modification of Mallory's stain (Shum & Hon 1969) was used to show the presence of keratin and also to demonstrate the degree to which the epithelial surface was keratinised.

2.3.6 Methods of analysis

In this study the methods of analysis of the microscopic features of the epithelium were both subjective and quantitative. The stratum corneum layer was described in relation to the type and degree of keratinisation present, in subjective descriptive analysis. The type of keratinisation was examined and classified on haematoxylin and eosin stained sections (Fig. 2:3). The degree of keratinisation was examined and classified on sections stained with the Crooke-Russell modification of Mallory's stain (Fig. 2:4). Epithelial thickness and epithelial morphology (Watson & MacDonald, 1982) were described objectively. Quantitative methods of analysis were used in the examination of these parameters. The use of quantitative data to describe structural features in tissue allows a relatively sensitive form of assessment.

2.3.7 Principles of stereology

Quantifying the structural parameters of tissue comes under the heading of morphometry. Weibel (1969) has described a form of morphometry known as

stereology where, by geometrico-statistical reasoning, examination of two-dimensional sections allows derivation of the three-dimensional properties of a structure. The surface length or basement membrane length in a stratified, squamous epithelium can be calculated stereologically by intercept point counting using a grid of parallel lines. The surface length is a function of the number of intercept points counted and the distance between the grid lines (Weibel 1969). The length is represented by the formula

$$L = \frac{\pi}{2} \times d \times i$$

L = length being measured

d = distance between lines on grid

i = number of intersections

Area can be estimated by superimposition of a lattice or grid upon the tissue to be examined and counting the number of points falling on each area to be quantified (Fig. 2: 5). The total area of an irregular piece of tissue can be calculated with reference to the known area of each of the squares on the grid and the number of intercept points falling upon the tissue. The smaller the squares of the grid, the more precise will be this method of area estimation (Glagoleff 1933).

2.3.8 Selection of tissue for analysis

For each patient in this study, a section of tissue from each half of the biopsy specimen was selected for examination. Within each selected section, the field of tissue lying in the middle was located. The fields of tissue chosen for analysis were those lying immediately adjacent to the middle field on either side. For each patient two sections were chosen for analysis and within each of these sections, two fields were analysed. This gave a total of four fields analysed for each of the patients in the study.

Measurements of length and area were undertaken by examination of the projected image of the appropriate fields of the chosen epithelial section. A Leitz Ortholux microscope with a back projecting teaching head was used for this purpose. (Fig. 2:6). The width of tissue examined was restricted to that lying between two vertical lines marked on the projection screen. The column of tissue under examination was therefore of a standardised width which was dependent upon the magnification used on the microscope.

2.3.9 Lengths measured in this study

Epithelial surface and basement membrane lengths were measured by superimposing a grid of parallel lines upon the projected image and counting the intercept points. Lengths were calculated by the

principles of stereology using the formula described in section 2.3.7. Each length was measured three times. For each successive measurement the grid of parallel lines was rotated through 60 degrees. There was, therefore, a systematic modification in the angulation of the grid of parallel lines for each of the three estimations of the length in question. The mean of the three values was calculated to give the value of each length measured. Watson & MacDonald (1982) defined the term epithelial morphology as describing the ratio of basement membrane to surface length of a column of epithelium. When the epithelial surface is flat and regular an indication of the irregularity of the basememnt membrane is given by the value of the epithelial morphology. In this study the surface and basement membrane lengths were calculated and the comparative values expressed as the epithelial morphology. As the epithelial surfaces were found to be relatively flat, the value of this parameter gave an indication of irregularity of the basement membrane in the oral mucosa in the condition of denture-induced stomatitis.

2.3.10 Areas measured in the study

The area of each field of epithelium under examination was calculated by the method of point counting stereology as previously described (2.3.7). A

grid of squares of 1 cm size, was superimposed upon the projected image. The number of squares contained within the area of epithelium was estimated by counting the number of intersections of the grid falling within the tissue. For each field three counts were made, the grid being rotated through 60 degrees between each estimation. The final area estimation was the mean of the three individual values found.

From knowledge of the total area of the field the average thickness of the section was calculated as each column of epithelium under examination was of known width. This parameter of epithelial thickness was estimated in all ten cases.

2.3.11 Method of microbiological sampling

An imprint culture technique was used in microbiological sampling (Arendorf and Walker 1979). A sterile square pad of plastic foam was dipped into Sabouraud's broth and placed firmly against the sample site. Each foam square had an area of 6.25 square centimetres. Each pad was held in place for a period of one minute before removal, and placement on a Sabouraud's plate (Fig. 2:7).

2.3.12 Selection of sample sites

Three sites were sampled. The first of these was the surface of the middle area of the hard palate. The foam square was centrally placed along the

midline of the palate with its anterior edge placed 1.5cm posterior to the posterior edge of the incisive papilla. The central part of the dorsal surface of the tongue was the second sample site. The third area to be sampled was the central area of the fitting surface of the upper denture. The foam square was centrally placed against the fitting surface of the denture immediately following its removal from the mouth, prior to rinsing or immersion of the denture.

2.3.13 Incubation

Immediately on removal from the sample site the foam square was placed firmly on a Sabouraud's agar plate. Each Sabouraud's agar plate was incubated aerobically at 37°C for 48 hours. The foam squares were removed from the plates after the first eight hours of incubation.

2.3.14 Counting and identification of colonies

Following incubation the number of colony types present on each Sabouraud's agar plate (Fig. 2:8) was noted and enumerated by means of a manual laboratory counter. The yeasts present were identified by the germ tube test and sugar assimilation reactions. Staphylococcus aureus was identified by the slide coagulation test (Lennette et al. 1980).

2.3.15 Haematological assessment

A 25 ml sample of venous blood was withdrawn from each patient and dispatched for analysis. Full blood count, erythrocyte sedimentation rate estimation and measurement of serum levels of vitamin B12, iron and folate were undertaken.

2.4 RESULTS

2.4.1 Clinical appearance of palatal mucosa

As patients had been selected with reference to the clinical appearance of the palatal mucosa, in all cases there was erythema of the upper denture bearing tissues, typical of chronic atrophic candidiasis.

2.4.2 Age and sex of sample

The ages of the patients investigated in this study are shown in Table 2:1. The mean age of the sample was 68 years. Most of the patients who presented to Glasgow Dental Hospital exhibiting the signs of denture-induced stomatitis were female. As it was considered desirable to restrict the sample analysed to a single sex, the ten patients included in the study were female.

2.4.3 Haematology results

The results of investigations undertaken showed that in 4 out of the 10 patients involved in this study, haematological values outwith the range of normal were evident. The values outwith the range of normal are shown in Table 2:2.

2.4.4 Microbiology results

Culture produced a profuse growth of Candida albicans in nine out of the ten cases investigated. In one of these cases there was found to be a background growth of Staphylococcus aureus and in another there was also a marked growth of Candida glabrata. The remaining case produced a large number of colonies of Staphylococcus aureus but no candida was evident.

In comparison between patients there was no indication from the results that the pattern of colonisation favoured any of the three sites sampled. It is noteworthy that the number of colonies found in the four cases with abnormal haematology values was in general lower than in those patients with a blood picture within the range of normal. The numbers and types of organism found in each of the sample sites are shown in Table 2:3

2.4.5 Histology results

The histological parameters assessed were epithelial thickness, epithelial morphology and the presence and degree of keratinisation. The values for

epithelial thickness and epithelial morphology were estimated quantitiatively from examination of four fields taken from two sections in each patient as previously described (2.3.8). The results are shown in Table 2 : 4.

Only one of the ten cases showed no trace of keratin on the epithelial surface. Three came into the category of equivocal keratinisation where it was questionable if there was mild parakeratinisation or complete lack of keratin. In six of the cases unequivocal parakeratinisation was found although in no case was this evident over the whole surface of each section. Examination of sections stained with Mallory's stain showed the type of keratinisation to be incomplete parakeratinisation.

2.5 DISCUSSION

2.5.1 Haematology

A high incidence of haematological abnormality was found in this relatively small sample. In the four cases where such abnormal results were found, comparatively low numbers of organisms were cultured although inflammation of the denture bearing tissues was evident. This may indicate that the presence of fewer organisms is required to produce palatal inflammation in the compromised individual.

Further investigation of this proposal is merited.

Only in the two cases exhibiting a low value for corrected whole blood folate was the organism Staphylococcus aureus cultured. In the case where the folate value was found to be lowest no evidence of Candida albicans was found but there were numerous colonies of Staphyloccus aureus. The possibility of a relationship between a low value of corrected whole blood folate, Staphylococcus aureus and denture-induced stomatitis would merit further investigation.

2.5.2 Microbiology

The finding of numerous colonies of Candida albicans in 9 out of the 10 cases studied is in accordance with the view that Candida albicans plays a major causative role in the pathogenesis of denture-induced stomatitis. (Cawson 1963, Budtz-Jorgensen 1974). Comparison of the numbers of colonies found in the three sites sampled in this study did not confirm Davenport's view (1970) that the fitting surface of the denture provides the primary oral reservoir for Candida albicans. There was no regular pattern of distribution within the sample.

The imprint culture technique used in the present study was described by Arendorf and Walker (1979) who stated that colony counts in excess of 49 per square centimetre indicated that Candida albicans was occurring as a pathogen rather than in a commensal form.

Examination of the colony counts in those patients with abnormal haematology values revealed that in none of the four cases was the count of candida colonies in excess of this critical value in any of the three sites sampled. In contrast, in five out of the six cases with a normal haematological profile, the results showed a colony count in excess of 49 per square centimetre in at least one of the sites sampled (Table 2:3). It appeared that the density of candida colonies produced did give an indication of the role of the organism but the haematological status of the patient had a bearing on the interpretation of these findings.

2.5.3 Histology

Examination of the results of the histological analysis of the palatal epithelium showed there to be a considerable range of values for both the parameters of epithelial thickness and morphology in the sample.

The epithelium showed a marked variation in thickness within the sample. The range was from 236μm to 537μm. The variation in thickness is highlighted in the comparison of the two histological sections in Fig. 2:9. These specimens are from two cases within the sample and the photographs are of identical magnification. The average value for epithelial thickness in the sample was 373μm. Watson and

MacDonald (1982) found the epithelial thickness in healthy oral mucosa under dentures from the same site and measured by similar techniques to have an average value of 240 µm. Comparison of these average values would indicate that a slight thickening of the palatal epithelium was produced in denture-induced stomatitis. However the size of the present sample is small and further investigation of changes of epithelial thickness induced in this condition is merited. Perhaps of more interest was the large range of values found within the sample. This suggests that despite a uniform clinical appearance within the sample there was a marked variation in the tissue reaction at a cellular level. This is in agreement with the findings of Budtz-Jorgensen (1970) who reported alternating areas of epithelial atrophy and hyperplasia in biopsy specimens from patients with denture-induced stomatitis.

The configuration of the basement membrane, recorded as the epithelial morphology, showed a similar variation in structure. The range of values for epithelial morphology was from 1.21 to 6.52 with a mean value of 3.84. In its most regular form the basement membrane length was only fractionally longer than the epithelial surface. However at the other extreme within the sample the basement membrane was found to be irregular and undulating. This increased value of the epithelial morphology indicated an increase in rete ridge formation, which is associated with a hyperplastic

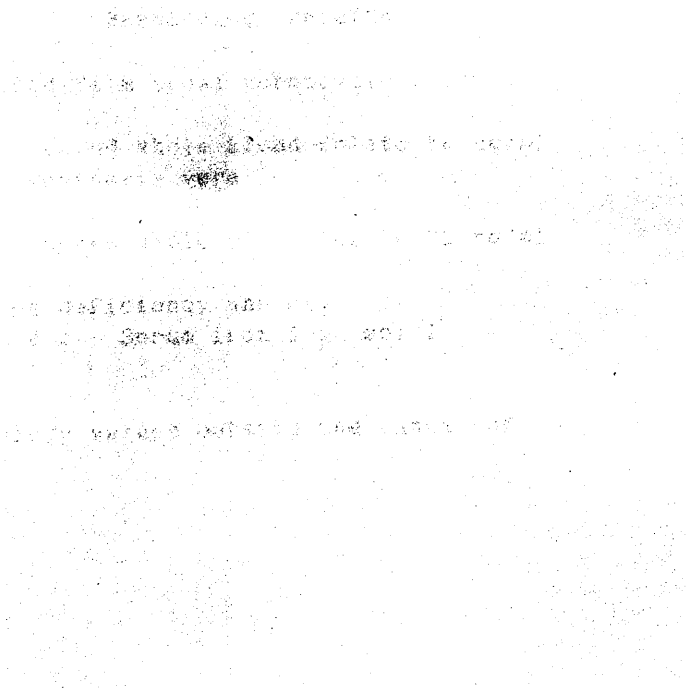
reaction. The values of epithelial morphology found were spread widely throughout the sample indicating a range in the response of the epithelium in denture-induced stomatitis from atrophy to hyperplasia. Because of the limited amount of tissue available for analysis it was not possible to tell whether in each case in the sample this variation in epithelial structure was present. It is possible that in each case the reaction of the epithelium was consistent with the variation being exhibited between the individuals in the sample.

The presence of incomplete parakeratinisation or the lack of a keratin layer on the surface of the epithelium is in agreement with the findings of Budtz-Jorgensen (1970) and Wictorin et al (1975). The literature in general supports the concept of a lack of keratinisation in denture-induced stomatitis.

2.6 CONCLUSIONS

This study produced interesting results in relation to the haematological, microbiological and histological features of denture-induced stomatitis. The relationship between the findings of the haematology investigation and the microbiological investigation offers scope for further examination. The variety in epithelial thickness and basement membrane structure between the cases which all exhibited a uniform clinical

appearance requires further clarification. The use of a greatly increased area of human palatal biopsy tissue would be of considerable use in this respect. The use of an animal investigation provides another approach producing ample tissue for investigation. If successfully established an animal model could provide a medium for further investigation of the haematological and microbiological factors involved in this disorder. The next stage in this study involved the development of an experimental animal model.



Patient	Age (years)
1	76
2	63
3	84
4	44
5	57
6	72
7	74
8	74
9	63
10	74
Mean age :	68

TABLE 2 : 1 Age range of sample

Case Number	Haematology Results
1	Blood film shows normocytic anaemia
6	Corrected whole blood folate 56 ng/ml Polycythemia vera
9	Corrected whole blood folate 72 ng/ml
10	Iron deficiency anaemia Hb 8.2 Serum iron 4 μ mol/l

TABLE 2 : 2 Haematology values outwith the range of normal

Patient	Total number of colonies (cm^2)			Organism
	Denture	Palate	Tongue	
1	24	24	24	Candida albicans
2	36	44	52	" " *
3	44	8	6	" "
4	76	16	52	" "
5	32	60	52	" "
6	36	52	36	Staph. aureus
7	68	36	52	Candida albicans
8	48	28	52	" "
9	20	20	20	" " **
10	24	20	24	" "
Average	41	31	37	

TABLE 2 : 3 Microbiology results

* Showing a growth of Candida glabrata
** Includes background growth of Staphylococcus aureus (less than 20%)

Patient	Epithelial thickness(μm)	Epithelial morphology
1	337	5.07
2	236	1.21
3	537	3.09
4	474	5.26
5	407	4.24
6	350	2.24
7	394	6.52
8	273	2.00
9	336	2.61
10	384	6.15
Average	373	3.84

TABLE 2 : 4 Histology results

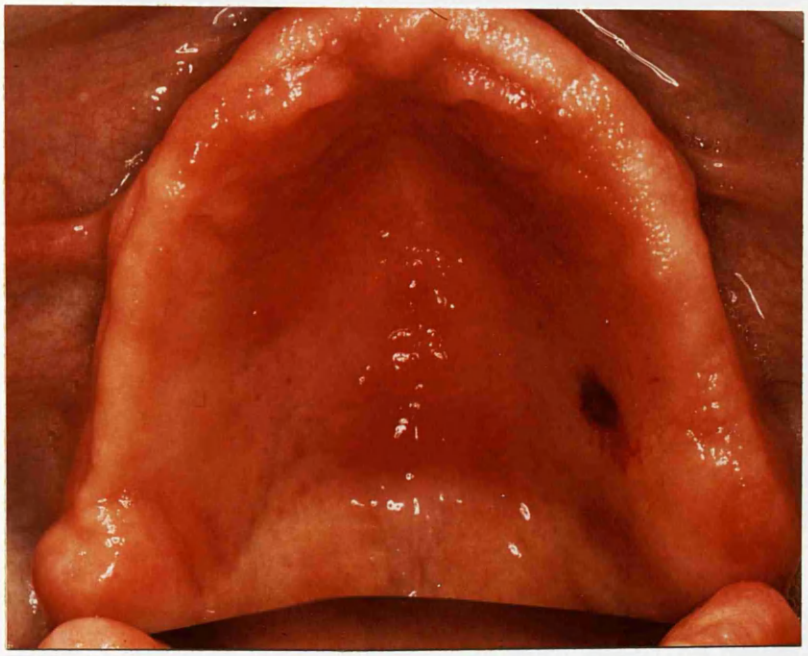


Fig 2:1 Biopsy site in human subjects

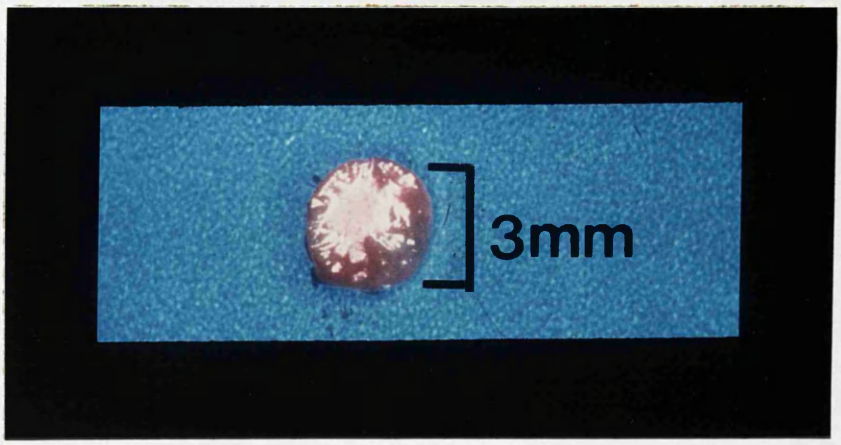


Fig 2:2 Epithelial surface of biopsy specimen

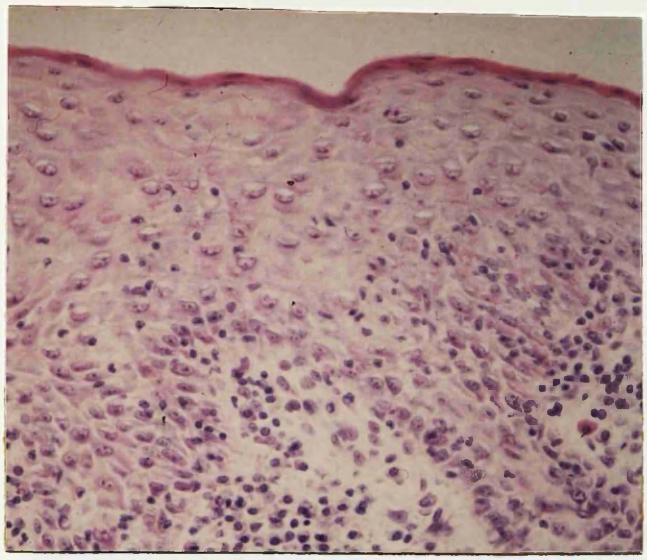


Fig 2: 3 Parakeratinisation of surface epithelium H & E x 175

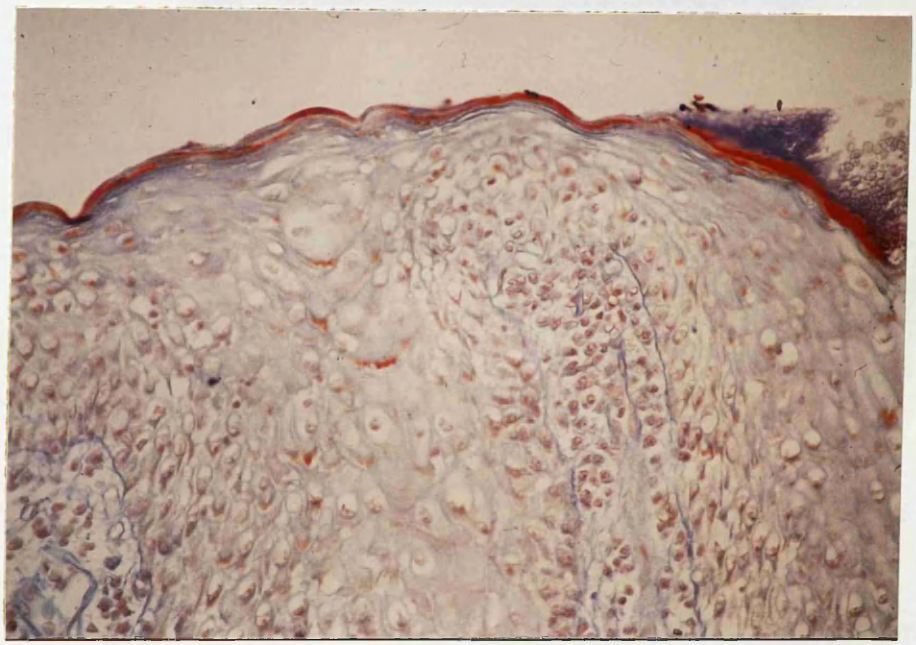


Fig 2: 4 Incomplete parakeratinisation of surface epithelium. Crooke-Russel modification of Mallory's stain x 175

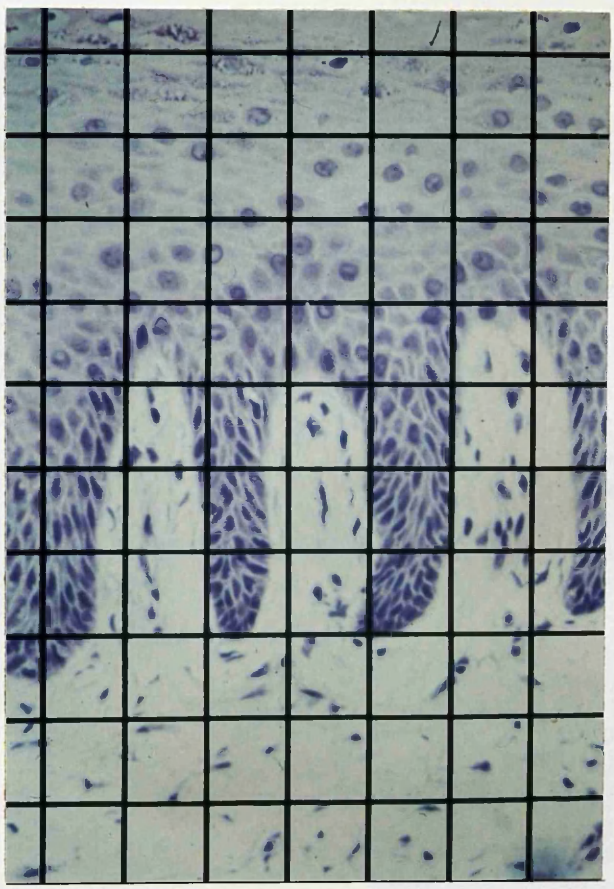


Fig 2:5 Superimposition of a grid of squares upon a histological section of epithelial tissue for area estimation of irregular tissue
H & E x 275

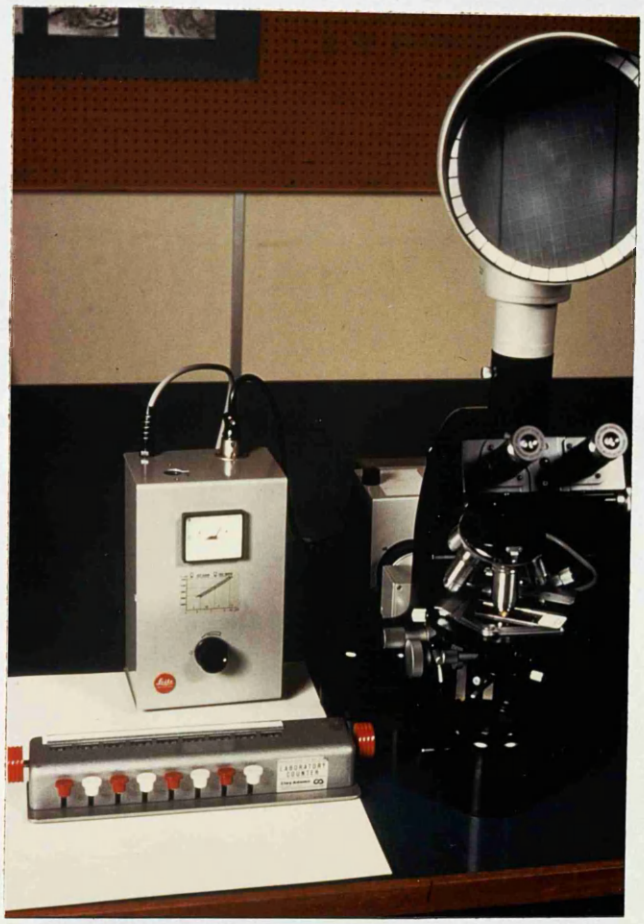


Fig 2:6 Leitz Ortholux microscope with back projecting teaching head, used in the analysis of human biopsy tissue.

Fig 2:7 Plastic foam square, bottle containing Sabouraud's broth and Sabouraud's agar plate used in microbiological sampling.

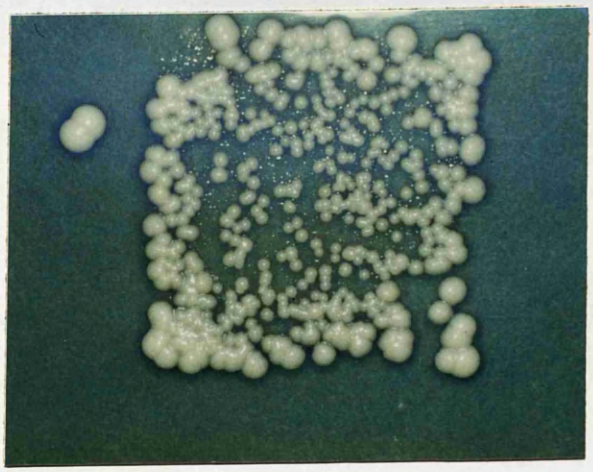


Fig 2:8 Microbial colonies on Sabouraud's agar plate following culture.

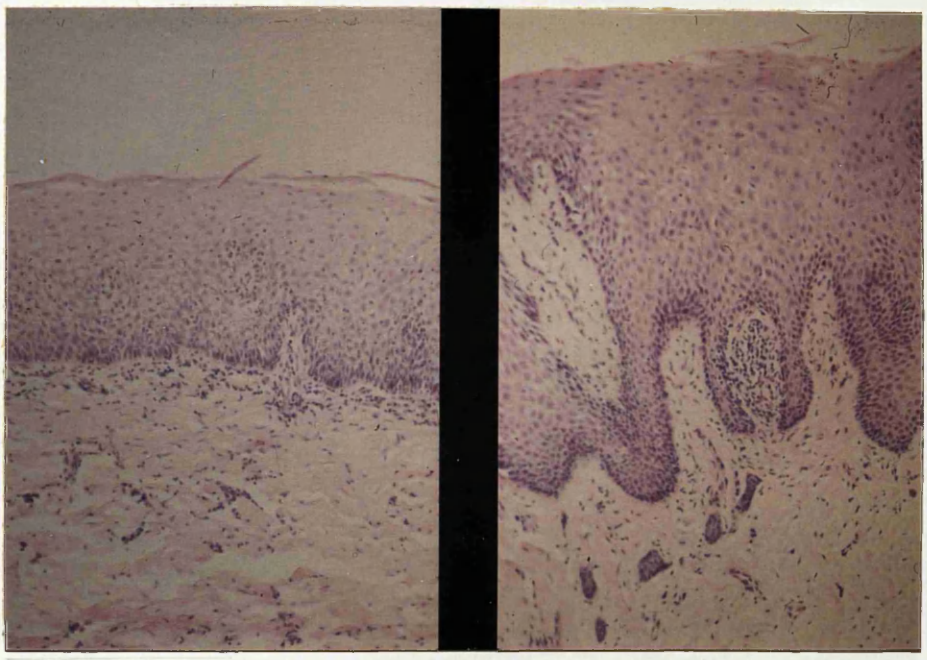


Fig 2:9 Histological sections showing extremes of epithelial thickness found in human subjects. H & E x 55

CHAPTER 3

PRELIMINARY ANIMAL MODEL STUDY

3.1 INTRODUCTION

The initial animal investigation served to introduce the author to the use of an animal experimental model, and to allow examination of the intra-oral characteristics of the Wistar rat, the animal used in this and subsequent animal studies.

3.2 AIMS OF STUDY

It was the main purpose of this study to examine the changes induced in the palatal epithelium of the Wistar rat following inoculation of Candida albicans under an acrylic appliance. The experimental techniques used and the suitability of these techniques for use in further investigations was also considered.

3.3 MATERIALS AND METHODS

3.3.1 Experimental animals

There were nine animals used in this study and for reasons of availability all the animals within the study were female. Animals were divded into three experimental groups of three animals. The animals of

the control group were sacrificed without being subject to experimental procedure. Group I and Group II were the experimental groups, for which there was a period of wearing an acrylic appliance. Within the experimental groups, two different strains of Candida albicans were inoculated under the appliances, one strain to each experimental group.

The animals were maintained in cages and fed commercial rat fodder and water ad libitum. Within each cage were animals from only one experimental grouping. The experimental animals were weighed prior to insertion of acrylic appliances and twice weekly during the experimental period.

3.3.2 Anaesthesia

The impression stages and the fitting of appliances were undertaken whilst each animal was under general anaesthetic. A single dose of the anaesthetic agent Hypnorm * was administered intraperitoneally for each procedure. 0.25 ml Hypnorm gave adequate working time and there was no anaesthetic related mortality.

3.3.3 Preliminary impression and fabrication of impression trays

It was intended that the acrylic appliances be as closely adapted to the palatal mucosa as possible.

* Janssen Pharmaceuticals Ltd., Grove, Oxford.

and therefore individual impressions in a suitably shaped impression tray were required. To enable fabrication of dental impression trays suitable in shape and size for use in the Wistar rat a preliminary impression was made in one animal from the experimental group, selected at random. The impression was made whilst the animal was under general anaesthesia and was recorded in silicone rubber material* using a putty and wash technique in which the material was supported by a spatula without the use of a tray. A stone cast was made from the preliminary impression (Fig. 3:1) and six impression trays were constructed by a pressure forming technique using a Drufomat-u** pressure forming apparatus. Plastic sheets were uniformly plasticised within the apparatus by infra-red rays, then suspended over the preliminary stone cast and pressure formed with compressed air. This produced trays of uniform thickness with adequate strength and minimal bulk which were suitable for impression taking in the Wistar rat.

* Provil, Bayer Dental
** Drufomat, Panadent Ltd., 15 Gt Dover St london SE14Y

3.3.4 Master impressions and construction of appliances

In each of the six experimental animals, following application of adhesive to the impression tray, a master impression was recorded using a regular viscosity polysulphide rubber impression material (Fig. 3:2).* A highly detailed master impression was produced in each case (Fig. 3:3). Following the pouring of master casts in improved dental stone,** a band encompassing the upper incisors was constructed in 2 mm wide stainless steel. A loop of stainless steel wire was soldered to the band using a low fusing solder. This distally placed loop formed a retentive element for acrylic which was processed against the band. A wax pattern laid down distal to the incisor teeth covered all of the palatal mucosa. The lateral margins of each appliance were finished against the palatal surfaces of the molar teeth, and distally the appliance extended behind the last molar teeth. Each appliance was then processed in clear heat cured polymethylmethacrylate *** (acrylic) (Fig. 3:4). Each appliance measured approximately 25 mm in length.

* Permlastic (Regular body) Kerr UK Ltd., Peterborough
** Velmix Stone, Kerr UK Ltd., Peterborough
*** Trevalon C., Detrey Division, Dentsply Ltd., Weybridge, Surrey.

3.3.5 Preparation of fungal inoculum

The strain of Candida albicans 3091 (serotype A) used by Shakir et al. (1981) was found to be no longer available on request from the National Mycological Reference Laboratory, London, and therefore alternative strains of the microorganism had to be considered. It was decided to use two strains of organism, the virulence of which was being studied in the Oral Microbiology Unit within Glasgow Dental Hospital. These yeasts were oral isolates from patients attending Glasgow Dental Hospital which had been cultured and stored under sterile distilled water.

The organisms were removed from storage and prepared for use in the animal model by aerobic incubation on Sabouraud's dextrose agar plates overnight at 37°C. After incubation, 30 mg aliquots of each yeast culture were harvested in sterile bijoux bottles by scraping the growth from the agar with a sterile wire loop. The Glasgow Dental Hospital reference numbers for the two yeasts used are RST A8 and RST A20.

3.3.6 Fitting of the acrylic appliance

The fitting of each acrylic appliance was undertaken whilst each animal was under general anaesthetic induced as previously described (3.3.2). In the six experimental animals the upper incisor teeth

were prepared using a no. 5 size fissure bur in a conventional speed dental handpiece to notch the distal surfaces. 60 mg of pure culture of Candida albicans was spread evenly over the fitting surface of each acrylic appliance. The animals of experimental Group I were inoculated with yeast RST A8, and Group II with yeast RST A20. The anteriorly placed orthodontic band was filled with self curing polymethylmethacrylate * and each appliance was placed firmly in position covering the palatal mucosa. This method of retention proved sufficient to keep all six appliances in position for the duration of the study.

3.3.7 Animal sacrifice and specimen preparation

The three control animals were sacrificed at the onset of the experimental period, and the six experimental animals were sacrificed on the 28th day after insertion of acrylic appliances. Animals were sacrificed by ether anaesthesia followed by fracture of the spinal cord. Each animal was decapitated and the mandible dissected free. Photographs of the palate were taken before and after removal of the acrylic appliance.

Following removal of the rat mandibles and the acrylic appliances, the heads were fixed in 10

* Simplex Rapid Self Curing Acrylic. Howmedica International Ltd. 662 Western Ave. London W3 0TF

percent buffered formalin for a minimum of three days. The tissues were then trimmed with a rotating saw parallel to the palate to remove the upper part of the nose and much of the skull. The palate and remaining maxilla were then decalcified in 20% formic acid. Following decalcification cross sectional blocks of the palate were prepared. The palatal tissue was trimmed into six blocks (A, B, C, D, E, F) for sampling. The location of these blocks is shown in Fig. 3:5.

The blocks were routinely processed in paraffin wax. 5 μm sections were cut on a rotary microtome from the posterior surface of each block. The tissue blocks were aligned such that the palatal mucosa met the knife first as this orientation gave least distortion of the epithelium. Sections were stained with haematoxylin and eosin.

3.3.8 Examination of sections

The prepared sections were examined by use of light microscopy. Epithelial thickness from the depth of the rete ridges to the surface of the epithelium was estimated along with the corresponding thickness of the cornified layer in each section analysed. At a standardised magnification, dimensions were estimated at sites considered to be representative of each section. A counting grid within the microscope eyepiece was used as a guide in the subjective estimation and comparison

of measurements. No detailed quantitative analysis of tissue was carried out in these sections. For reasons outlined in Section 3.4.5, in each of the nine animals within this part of the study tissue examined was restricted to that from sites A, B and C, so that, in total twenty seven sections were analysed.

3.3.9 Microbiological sampling

Debris taken from the fitting surface of each appliance for all three animals in experimental group II was removed for analysis. The presence of Candida albicans was assessed following culture of material on Sabouraud's agar at 37°C for 48 hours. Some debris was stained with haematoxylin and eosin and examined microscopically to assess its nature.

3.4 RESULTS

3.4.1 Animal weights during experimental period

The weights of individual animals during the experimental period are shown in Table 3:1. All six experimental animals lost weight appreciably during the first four days following insertion of appliances. This would seem to indicate that all the animals experienced difficulty with feeding immediately following plate insertion. Only two of the six animals lost any further weight between day 4 and day 7, and in

both cases this weight loss was small. From day 7 all animals showed a gradual increase in weight which would seem to indicate that the rats adjusted to the wearing of the appliance. In no case did the wellbeing of the animal appear to be affected by the wearing of an appliance. Within the twenty eight day period of the experiment only one animal regained and surpassed its original weight. However four out of the other five animals showed a continuing increase in weight up to day 28.

3.4.2 Macroscopic appearance following experimental period

Following sacrifice and removal of the mandibles in each of the six experimental animals an accumulation of debris was evident under each acrylic appliance (Fig. 3:6). The likely sources of this material were considered to be remnants of the diet, accumulation of shed epithelial cells or aggregates of bacteria. Removal of appliances showed that this debris contained a large amount of fibrous material (Fig. 3:7). Following careful removal of part or all of this debris the underlying mucosa of the sacrificed animals showed no signs of erythema.

3.4.3 Histological and microbiological investigation of debris

Culture of debris material on Sabouraud's

agar revealed evidence of candidal organisms from one animal only. Growth in the one positive specimen was found to be scanty. Staining of the debris material with haematoxylin and eosin followed by histological examination showed most of the material to be vegetable in origin.

3.4.4 Surface characteristics of palatal epithelium

Examination of palatal epithelium in the Wistar rat revealed problems of site selection which were not encountered in the study of human epithelium. (Chapter 2). In the study of human palatal epithelium the biopsy site chosen was level in a coronal plane with the first permanent molar. At this site on the human palate, distal to the palatal rugae, the epithelial surface was found to be free of undulation. Examination of the gross anatomy of the Wistar rat showed epithelial rugae covering all of the palate and extending distally to the last standing teeth (Fig. 3:8).

3.4.5 Preliminary histological examination of two animals

To assess the suitability of the palatal tissues for analysis six sites were examined from two animals. One animal from the control group had not worn an appliance and the other animal from experimental Group I had worn an appliance for 28 days with Candida

albicans (RST A8) inoculated underneath the plate. The six sites sampled (A, B, C, D, E, F) have previously been described (3.3.5). Differences in epithelial structure were evident between these two animals but it was observed that the changes were most prominent in the epithelium of the broad part of the hard palate lying between the molar teeth.

The structure of the epithelium found in the area between the molar teeth showed a contrast between the control and the experimental animals. Fig. 3:9 shows epithelium sampled from site A in both animals. Differences in epithelial structure of a similar nature were observed in sections from site B and site C.

Sites sampled anterior to the molar teeth showed less variation between the control and the appliance wearing animals. A similarity of epithelial structure is shown in sections taken from site F in both animals (Fig. 3:10). This relative uniformity of structural appearance was evident in epithelium sampled from site D and site E.

3.4.6 Further considerations in analysis of epithelium of hard palate

In view of the findings described in section 3.4.5, further investigation was restricted to sample sites A, B and C. Tissue was prepared from blocks obtained by making vertical cuts through the palate in four locations. The coronal planes of incision to obtain

the blocks were:

i) distal margin of the third molar teeth

ii) mid-point of second molar teeth

iii) mid-point of first molar teeth

iv) mesial margin of first molar teeth

Sections for analysis were cut from the posterior aspect of each block and prepared as previously described. (3.3.7).

Analysis of epithelial morphology and thickness is complicated in the Wistar rat by the presence of surface rugae. To illustrate this, the appearance of sections cut at corresponding sites in control rats is considered (Fig. 3:11). The characteristics of both sections of epithelium shown are similar, but quantitative analysis of the sections is difficult. These sections from site B in two of the control animals show that comparison of equivalent sites, for example, over the major palatal blood vessels, could not be valid on a quantitative basis because of differences introduced by the presence of surface rugae.

The material in the preliminary animal model was therefore considered subjectively. The degree of uniformity of the epithelium was noted along with estimation of rete ridge depth and prominence of the keratinised layer (3.3.8).

3.4.7 Uniformity of epithelial thickness and appearance

This parameter was divided into four categories. The epithelial thickness and appearance was categorised as being uniform, uniform other than variation introduced by the presence of surface rugae, moderately variable or irregular.

It is evident from the results of Table 3 : 2 that the epithelium in the experimental animals was more irregular than in the control animals. In site A there was almost no difference to be found between the separate groups, and in site B the differences were most marked.

3.4.8 Rete ridge depth

This parameter was divided into four categories. The rete ridge depth was categorised as minimal and uniform, moderate and uniform, prominent and uniform, or of variable appearance.

It was evident from the results of Table 3:3 that the rete ridges were more prominent in the experimental animals than the control animals. In common with results relating to the epithelial thickness and appearance, differences between the experimental and control groups were most marked in site B and least evident in site A. Rete ridges were found to be of minimal or moderate depth and uniform appearance in 89% of control sites sampled and in 33% of experimental

sites sampled. It is noteworthy that in none of the experimental sites sampled were the rete ridges of minimal depth.

3.4.9 Prominence of cornified layer

This parameter was divided into two categories. The cornified layer was classified as being either of moderate thickness or prominent (Table 3:4).

A keratinised layer was present in all sites sampled. In the control group all sites sampled exhibited a moderate depth of the keratinised layer, but in 66% of the sites sampled in the experimental group this layer was found to be prominent. There appeared to be a generalised increase in thickness of the keratinised layer within the experimental groups.

3.4.10 Epithelial thickness

Subjective estimation of epithelial thickness in what were considered to be representative areas of tissue suggested that an increase in epithelial thickness was evident in the animals from the experimental groups.

3.5 CONCLUSIONS

3.5.1 Material on epithelial surface

A potential problem in the design of this particular animal model was apparent on visual examination of the experimental animal palates. There was significant accumulation of debris on the surface of the palatal tissues. Visual, histological and microbiological examination lead to the conclusion that the material under the plate was largely food debris rather than a gross accumulation of microorganisms or shed epithelial cells. The absence of even rudimentary hygiene measures has implications for any comparison of the animal model with the human situation. This problem however has not been highlighted by other investigators, but it does suggest that some further investigation into the technique is in order.

3.5.2 Palatal surface characteristics

That the selection of sample sites may pose difficulties due to an irregular epithelial surface was evident on both macroscopic and microscopic examination. Rugae were observed to cover all of the hard palate, a factor which had not been highlighted in the literature. The tissue blocks for microscopic examination were selected so that uniformity of location could be guaranteed when comparing sections. It was apparent from this study that coronal sections cut across the

width of the palate at sites standardised by the location of teeth were not ideal. The rugal pattern does not conform to this method of site selection and irregularities introduced made quantitative assessment of the epithelium difficult.

3.5.3 Preliminary histological findings

Preliminary histological examination of palatal epithelium, in tissue sections from one control and one experimental animal, showed that epithelial changes had been induced in the experimental animals, and that these changes were most apparent in the epithelium of the tooth bearing part of the hard palate. It was therefore considered reasonable to confine further investigations to the oral mucosa lying on the tooth bearing portion of the hard palate in the Wistar rat animal model.

3.5.4 Histological assessment of preliminary animal model

Evidence was produced in this animal model to suggest that changes were induced in the structure of the palatal epithelium within the experimental groups. An increase in the total thickness of the epithelium was identified. In the experimental groups there was also found to be an increase in rete ridge depth which accompanied a more irregular appearance of the

epithelium. These changes were most marked in site B where the occlusive conditions found under the appliance might have been expected to produce maximum effect. Whether these changes resulted from the presence of the plate alone, the accumulation of material under the plate or the inoculation of Candida albicans under the plate was not examined in this animal model.

3.5.5 Experimental strains of Candida albicans

There was a marked epithelial response in the experimental animals which was similar in both groups and did not seem to be dependent upon the strain of Candida albicans inoculated. As the role of Candida albicans in producing epithelial changes was not determined, there was no indication of which strain was the more virulent and most suited for use in subsequent investigations.

	Day	0	4	7	11	14	18	21	25	28
Group I										
Animal	1	270	230	225	230	235	235	240	235	240
	2	224	180	212	202	220	217	220	215	220
	3	273	253	258	280	280	287	292	285	290
Group II										
Animal	1	308	270	275	285	285	290	285	280	285
	2	272	230	250	250	250	255	255	250	250
	3	302	260	255	255	250	255	255	255	260

TABLE 3:1 Animal weights during experimental period (grams)

		Site A	Site B	Site C
Control	Animal I	2	2	2
	Animal II	1	1	2
	Animal III	2	1	2

		Site A	Site B	Site C
Group I	Animal I	2	2	2
	Animal II	2	3	2
	Animal III	2	4	4

		Site A	Site B	Site C
Group II	Animal I	2	4	2
	Animal II	2	2	1
	Animal III	2	3	3

TABLE 3:2 Assessment of epithelial thickness and appearance

1 - Uniform

2 - Uniform other than variation introduced by presence of surface rugae.

3 - Moderately variable

4 - Irregular

		Site A	Site B	Site C
	Animal I	4	1	1
Control	Animal II	2	1	1
	Animal III	2	2	2

		Site A	Site B	Site C
	Animal I	2	3	2
Group I	Animal II	3	3	2
	Animal III	3	4	3

		Site A	Site B	Site C
	Animal I	3	4	2
Group II	Animal II	2	3	2
	Animal III	3	3	3

TABLE 3:3 Rete ridge depth

1 - Minimal depth, uniform appearnace

2 - Moderate depth, uniform appearance

3 - Prominent depth, uniform appearance

4 - Variable appearance

		Site A	Site B	Site C
	Animal I	1	1	1
Control	Animal II	1	1	1
	Animal III	1	1	1

		Site A	Site B	Site C
	Animal I	2	2	1
Group I	Animal II	2	2	1
	Animal III	2	1	2

		Site A	Site B	Site C
	Animal I	1	2	2
Group II	Animal II	2	2	1
	Animal III	2	2	1

TABLE 3:4 Prominence of cornified layer

1 - Moderate thickness of keratinised layer

2 - Prominent keratinised layer

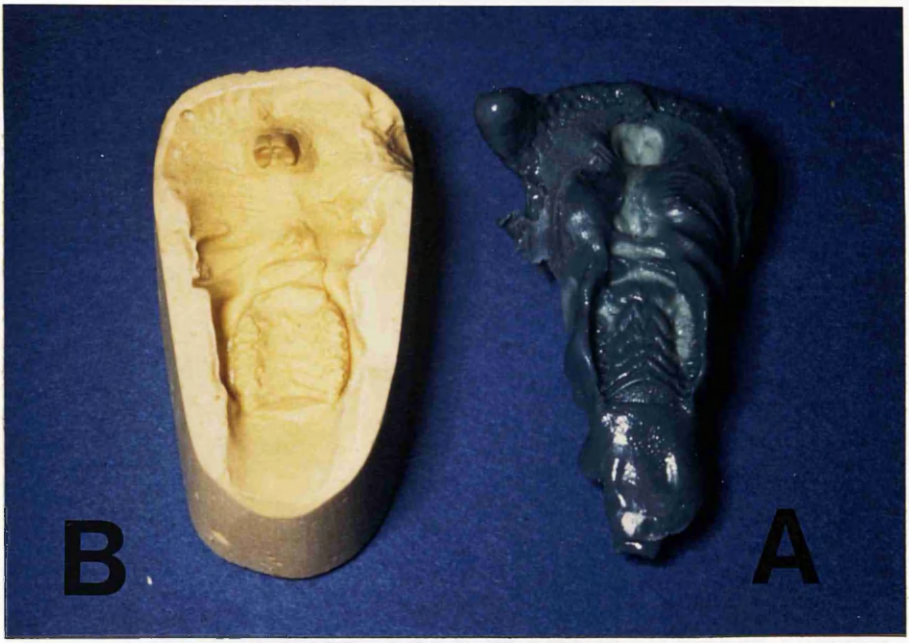


Fig 3: 1 A: Preliminary impression recorded in silicone rubber
B: Stone cast poured from preliminary impression

Fig. 3: 2 Master impression recorded in polysulphide rubber, supported by impression tray

Fig. 3: 3 Fine detail of polysulphide rubber impression

Fig. 3: 4 Acrylic intra-oral appliance

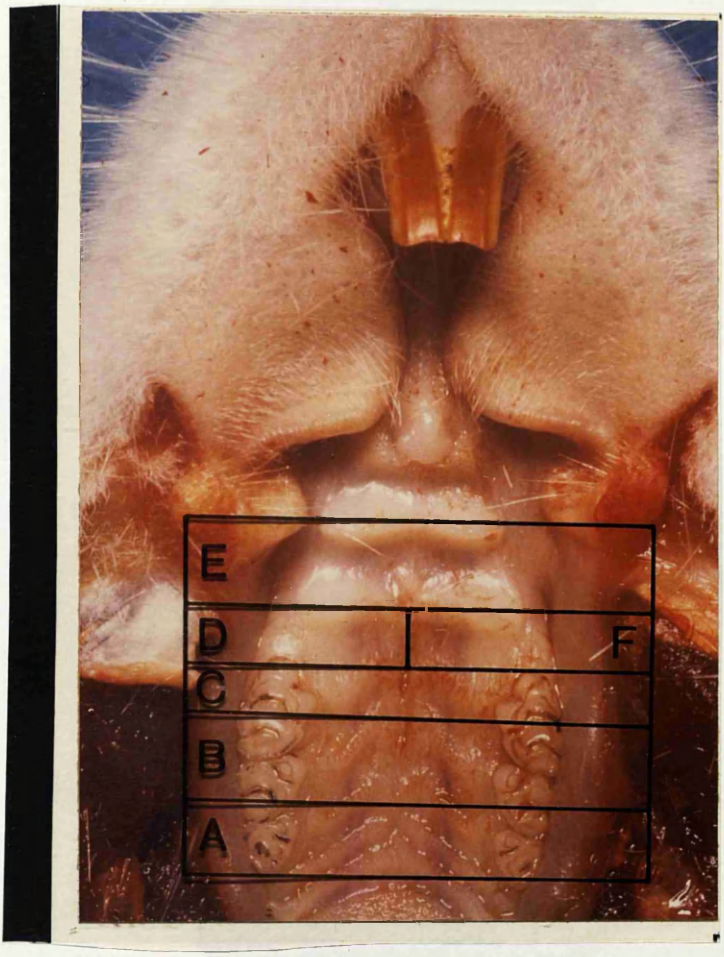


Fig. 3: 5 Location of tissue blocks prepared for analysis in the preliminary animal model

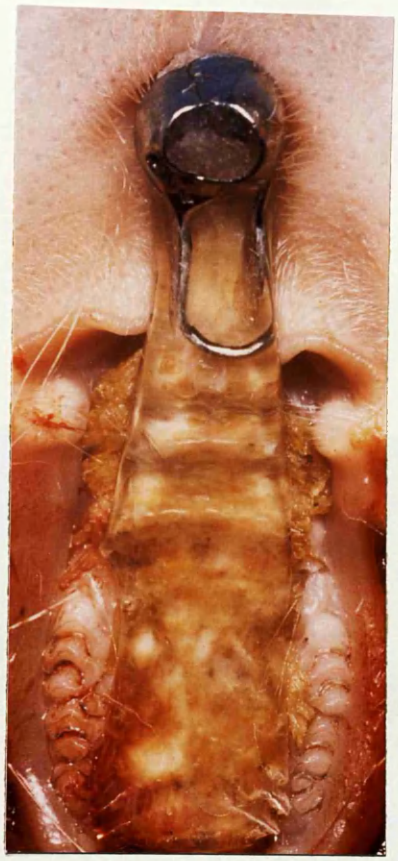


Fig. 3: 6 Acrylic appliance following experimental period

Fig. 3: 7 Debris on palatal surface

Fig. 3: 8 Position of rugae on Wistar rat palate

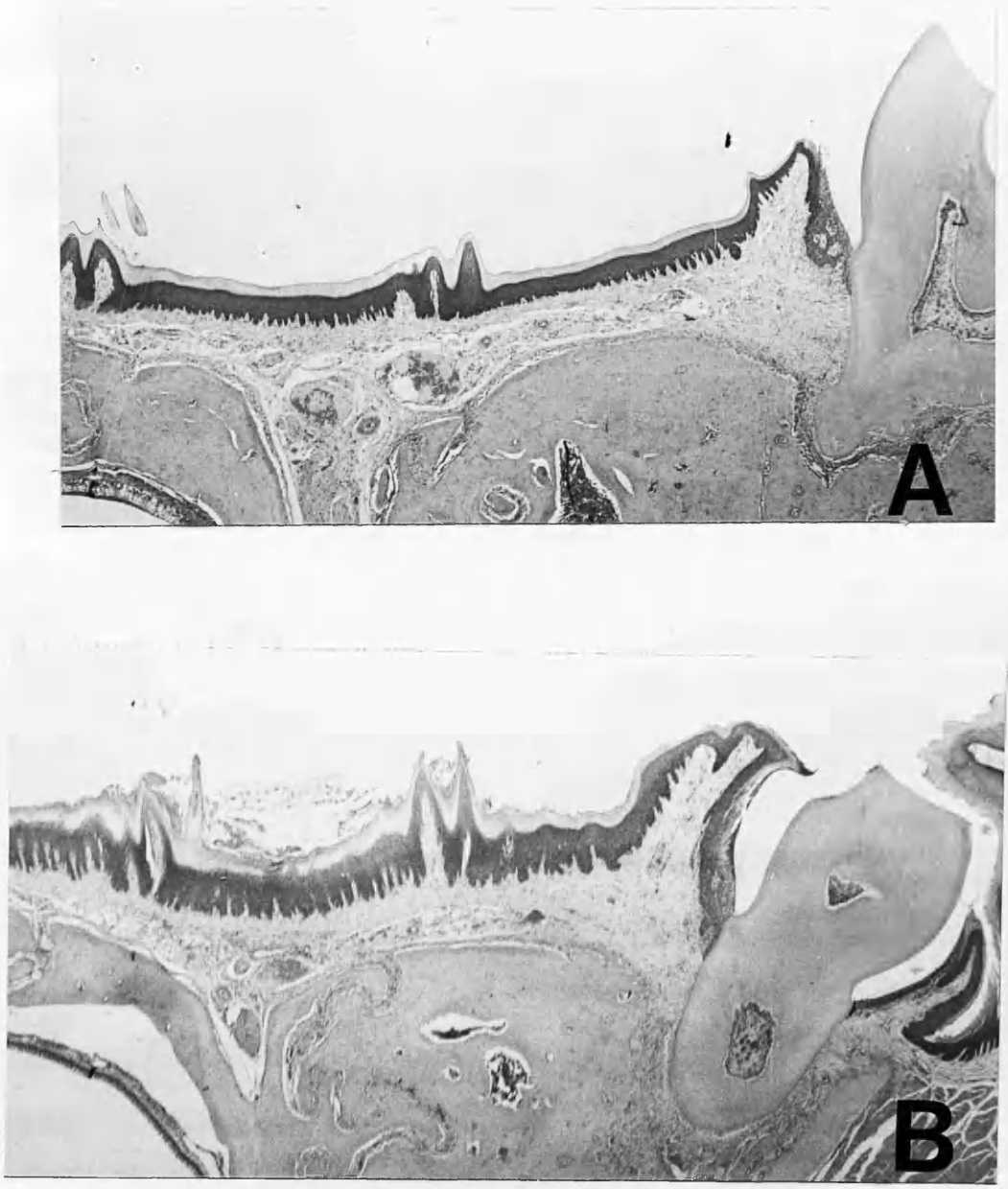


Fig. 3: 9 Preliminary animal model. Sections taken from tissue between the molar teeth.

A: Control animal
B: Experimental animal
H & E x 35

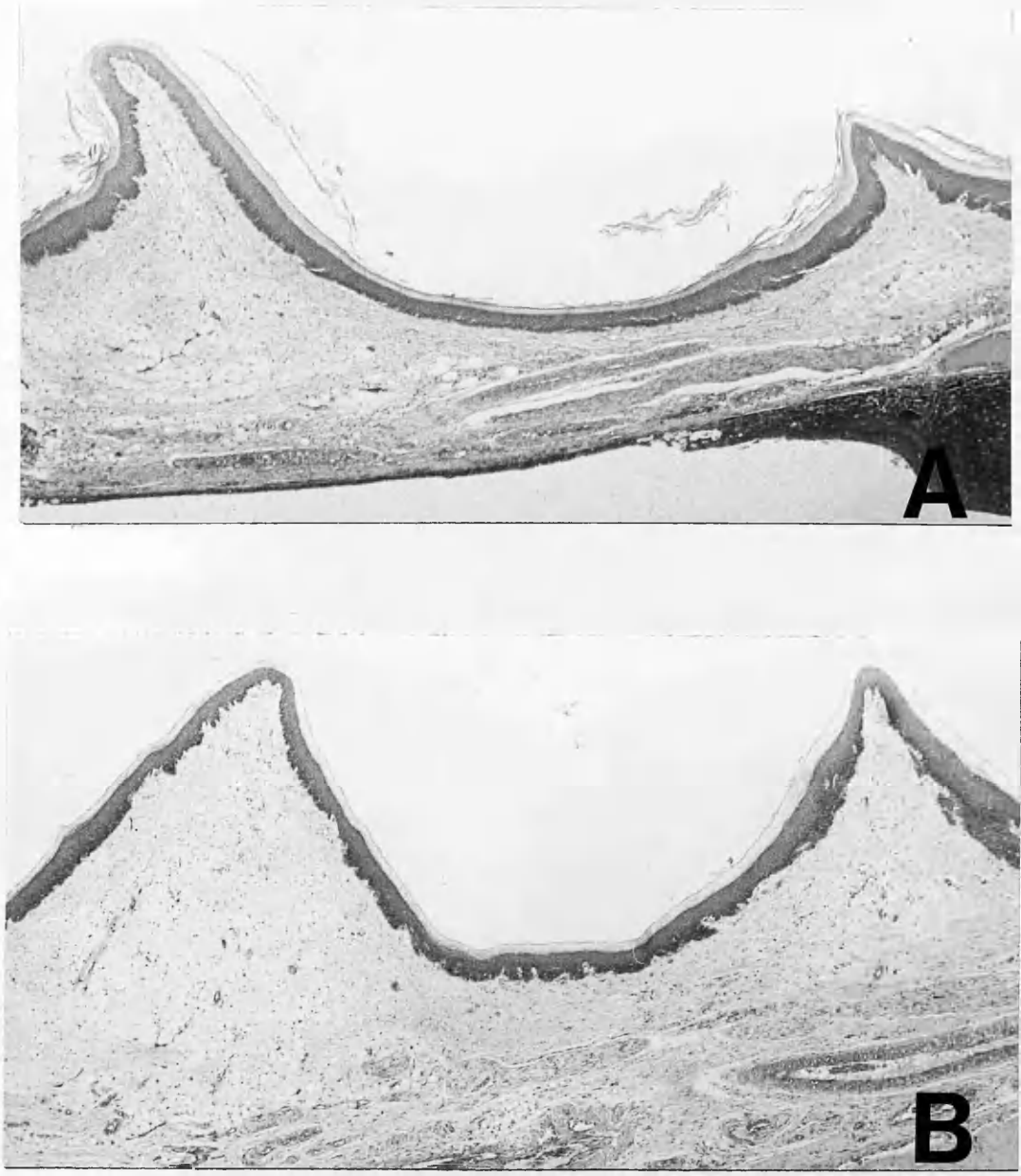


Fig. 3: 10 Preliminary animal model. Sections taken from tissues anterior to the molar teeth.

A: Control animal
B: Experimental animal
H & E x 40

Fig. 3: 11 Epithelium from control animals showing difficulties of comparison introduced by presence of surface rugae
H & E x 90

CHAPTER 4

INVESTIGATION OF THE MICROSCOPIC ANATOMY OF THE WISTAR RAT PALATE

4.1 INTRODUCTION

In the preliminary investigation which made use of the Wistar rat animal model, problems of quantitative analysis of tissue were encountered because of the anatomical structure of the epithelium overlying the hard palate. In the preliminary investigation coronal sections were cut across the palate at levels standardised with reference to the positions of the teeth. Although subjective anaylsis of material was possible this method of section preparation produced tissue which was unsuitable for quantitative analysis due to the unpredictable siting of surface rugae in individual histological sections. An investigation into an alternative method of orientating tissue sections was undertaken to assess the suitability of material so prepared for quantitative analysis.

4.2 AIMS OF STUDY

The first aim of this study was to assess if tissue sections could be accurately and predictably trimmed along the length of the palatal rugae, despite the small size of the Wistar rat palate. The plane of such sections runs obliquely across the palate in a forwards direction from the teeth to the midline. The

second aim was to produce sections of palatal epithelium cut in an anterposterior plane. The third aim of the study was to analyse the tissue produced by these two methods to assess the suitability of the sections for quantitative analysis in any further experimental model.

4.3 MATERIALS AND METHODS

4.3.1 Selection and sacrifice of animals

Adult male Wistar rats were used in this study. The use of male rats precluded the possiblity of variations in oral epithelial structure related to sex hormone variations. Examination of material from five animals was considered sufficient to assess the microscopic anatomical features of the palatal epithelium of the Wistar rat. The animals in this study were not exposed to any experimental procedures prior to sacrifice. Animals were sacrificed by ether anaesthesia followed by fracture of the spinal cord.

4.3.2 Specimen preparation

The animals were decapitated and in each case the mandible was dissected free. The remaining tissues of the upper part of the head were fixed in 10 per cent buffered formalin for three days. Following fixation the upper part of the skull was removed with a rotary saw. The palate and maxilla were decalcified in 20 per

cent formic acid. Following decalcification the aim was to prepare tissue blocks in such a way that the presence of the surface rugae did not result in such variability that the material was unsuitable for quantitative analysis as had occurred in the initial animal model described in Chapter 3.

The maxilla was split in half along the midline in the anteroposterior plane. The two halves of the maxilla were then trimmed with razor blades into blocks for processing, using two different experimental methods.

In the right half of the maxilla the tissue blocks were prepared in planes which corresponded to the orientation of the surface rugae. These blocks were taken from the tooth bearing portion of the hard palate for the reasons outlined in Section 3.3.5. Five cuts were made to produce four blocks running obliquely forward from the molar teeth to the midline. The anterior edge of each block was cut just behind the position of one of the main rugae.

The blocks produced were labelled 'U', 'V', 'W' and 'X'. The label given to each block corresponded to the position of the equivalent ruga in all cases. For example, the posterior boundary of each section 'U' was the ruga which ran from just behind the last molar tooth to the midline, and the anterior boundary was the ruga which ran from between the second and third molar teeth to the midline. The position and

the label for each section cut from the right half of the maxilla in each of the five animals are shown in Fig. 4: 1.

In the left half of the maxilla two tissue blocks were produced running in the anteroposterior plane. These blocks ran the full length of that part of the hard palate bearing the molar teeth. The first cut was made on the palate just on the palatal side of the molar teeth and the second was made midway between the first cut and the midline. These blocks were labelled 'Y' and 'Z' and the position of these is also shown in Fig. 4: 1.

All the tissue blocks were processed in a Histokine automatic tissue processor and embedded in paraffin wax. Sections 5 μm in thickness were cut from each block on a rotary microtome. In the obliquely cut blocks from the right half of the maxilla the sections were taken from the anterior surface of each block. In the blocks taken from the left half of the maxilla which ran in an anterposterior plane the sections were taken from the lateral surface of each block. The sections were stained with haematoxylin and eosin for analysis.

4. 3. 3 Tissue analysis

The quantitative analysis of tissue sections produced from animal investivations undertaken in this project was undertaken using a system of computerised

planimetry. This technique was convenient to use and less time consuming than the point counting technique of stereological analysis utilised in Chapter 2.

The system utilised a microscope with a drawing tube attachment. The arrangement enabled a light spot to be projected into the field under view within the microscope from a light source on a cursor which was situated upon a bit-pad directly below the drawing tube. Whilst viewing the field and the light spot through the microscope, lengths or areas of tissue could be traced upon the bit-pad by the hand held cursor. As the cursor was moved upon the bit-pad the light spot could be seen to move a corresponding distance and direction within the microscope field. The bit-pad was linked to a computer (Fig. 4: 2). The computer provided a printout of the values of lengths and areas measured by this technique, and a visual representation of each tracing.

4.3.4 Length and area measurement techniques

Measurement of lengths and areas was undertaken by examination of the epithelial section in question on a Leitz Dialux microscope at a standardised magnification. The width of the tissue examined was restricted to that lying between the two vertical lines of a square grid marked on the microscope eyepiece. This width was standardised for all sections examined by the use of uniform magnification and was measured using a stage micrometer.

The lengths for measurement in this study were those of the epithelial surface, the interface between the keratin and cellular layers of the epithelium and the basement membrane (Fig. 4:3). In the measurements of length the light spot was passed along the appropriate surface from left to right between the vertical sides of the square grid. The length of the surface was tabulated by the computer.

The areas measured in the study were those of the keratinised layer and the cellular layer of the epithelium (Fig. 4:4). In area measurements the light spot was directed along the peripheral surface of the tissue component lying between the vertical lines of the grid, starting and finishing at the same point. The area of the tissue was tabulated by the computer.

4.3.5 Site selection for analysis

- oblique sections

Consistency of sampling technique is a fundamental requirement in the quantitative assessment of tissue. In order that the selection of fields be standardised in this study it was considered desirable that they be selected with reference to a fixed anatomical feature. In the sections cut from tissue blocks 'U', 'V', 'W' and 'X', from the right half of the maxilla, the major palatal blood vessels running approximately halfway between the teeth and the midline

were a constant finding. The field of epithelium which lay immediately below the palatal blood vessels was chosen for analysis. Also analysed were the fields which lay adjacent to it on either side. This provided three columns of epithelium for analysis in total, in each section (Fig. 4:5). The objective magnification was set at x 25 for examination of tissue in the oblique sections.

4.3.6 Site selection for analysis

- longitudinal sections

Examination of the tissue blocks cut in a longitudinal plane from the left side of the maxilla showed those sections cut from the lateral surface of the 'Y' blocks to be more regular than those taken from the 'Z' blocks. The tissue taken from the lateral surface of the 'Z' blocks showed irregularity associated with the proximity of the molar teeth. Analysis of the longitudinal sections was confined to those taken from the lateral surface of the 'Y' blocks. The sections in question were from that part of the hard palate bearing the molar teeth and running approximately halfway between the midline of the palate and the molar teeth.

The appearance of the longitudinal sections was in marked contrast to both the coronal sections described in Chapter 3 and the oblique sections described in section 4.3.5. There was however a uniformity in the appearance of the material from each

of the five experimental animals. The sections showed a repeating pattern of prominent rugae with an intervening valley or col between each, along the length of the section. The rugae were large and widely spaced towards the anterior end of the tissue block, becoming smaller and less regular posteriorly. The characteristic appearance of the first major ruga in comparison with the tissue anterior to it made it easily identifiable and allowed for a consistency of sampling across the experimental group. Tissue for quantification was sampled from the epithelium overlying the rugae and from the intervening col areas. The epithelia from these two areas were analysed separately.

The tissue containing rugae was considered first. Each tissue section under consideration was aligned on the microscope stage such that the most anterior ruga lay in the centre of the field, appearing within the grid on the microscope eyepiece. The objective magnification was x 16 for examination of the longitudinal sections. The section of tissue in question was analysed. The process was repeated for the second and then the third palatal rugae. In total, three columns of tissue containing the three most anterior rugae were analysed in each tissue section (Fig. 4: 6).

A similar procedure was followed for tissue selection in the col areas. Again three fields of

tissue were analysed and these were from the areas lying posterior to each of the three most anterior rugae. The position of the fields of tissue analysed in this case was midway between the rugae (Fig. 4:7).

Thus, one longitudinal tissue block only was analysed for each of the five experimental animals. The epithelium overlying the rugae was assessed by examination of the three most anterior rugae, and the epithelium of the col areas was assessed separately, again by examination of three fields.

4.3.7 Measurements in obliquely cut tissue blocks

Lengths and areas in the obliquely cut blocks were measured in three adjacent fields as described in Section 4.3.4. Each of the five parameters in each field was measured twice, and provided the values found were similar, an average of the two was the recorded measurement of the parameter for that field. If there was a discrepancy in excess of 5 per cent between the two values, measurements were repeated until a consistent value was found. From the values found in each of the three fields the mean value for each parameter was calculated to represent the tissue section as a whole.

4.3.8 Measurements in longitudinally cut blocks

Analysis of the longitudinally cut sections was carried out in a similar manner to the oblique

sections, with two notable differences. Firstly, the epithelium was assessed in two different components. These were, the epithelium overlying the rugae and the epithelium midway between the rugae. Secondly, the three fields of tissue assessed for each of the components were not adjacent. This procedure has been described in Section 4.3.6. As with the obliquely cut sections, each of the lengths and the areas was measured twice in each field and the mean value for each of the parameters for the section as a whole was calculated by consideration of three fields. This was done for the tissue of the rugae separately from the tissue of the cols.

4.3.9 Area measurements within this study

It was found within some of the sections of palatal epithelium that a split occurred between the keratinised component and the cellular component (Fig. 4:8). Measurement of areas of these two tissue components was calculated individually. The values for the components were added together to give the total epithelial area, thus avoiding errors due to the presence of splits within the epithelium. As the dimensions of the measuring grid were known from the use of a stage micrometer (4.3.4), the width of each column of tissue examined was also known. Knowledge of total epithelial area, the area of the individual components,

and the width of the column of epithelium, allowed calculation of the average thickness of the epithelium and of its cellular and keratinised components.

In those tissue sections cut obliquely, and in those from the col areas, the area measurements were used to calculate average epithelial thickness, and the results have been expressed in this form. In these sections the flat epithelial surface made this calculation possible and meaningful. This was not the case in the epithelium overlying the rugae, in which the epithelial surface was not flat, and area measurements have not been converted to represent average thickness.

4.3.10 Tissue column widths

The width of each column of tissue delineated by the eyepiece graticule, was measured using a microscope stage micrometer. The width of the column of tissue seen was dependent upon the objective magnification used. The higher the magnification used, the narrower was the column of tissue seen through the microscope eyepiece. The oblique sections were viewed with an objective magnification of x 25 giving column width measured at 370 µm. The longitudinal sections were viewed with an objective magnification of x 16 giving a column width measured at 580µm.

4.4 RESULTS

4.4.1 Sections not suitable for analysis

- oblique sections

In each of the five experimental animals, four different tissue sections were examined, one from each of the four tissue blocks prepared. This gave a total of twenty sections examined from the obliquely cut tissue blocks in this study. Within these twenty sections, four were deemed unsuitable for quantitative analysis. As shown in subsequent tables of results, two sections from animal number five and one each from animals numbered one and two, were excluded. In two cases there was no epithelium on the tissue blocks produced, in one case the presence of very irregular rugae made quantitative analysis impossible, and in the fourth case the entire tissue block was lost during the processing procedure. It was considered that sufficient material remained to make a critical assessment of the techniques under investigation.

4.4.2 Epithelial Surface length

- oblique sections

For the sections cut obliquely from the right half of the maxilla the values found for the length of the epithelial surface within each column are shown in Table 4:1. In one case the epithelial surface length was the same as the column width of 370μm. In all

other cases the length of the epithelial surface was only marginally greater than the column width and the highest value found was 381µm. This is indicative of a regular epithelial surface with minimal undulation in the oblique tissue sections prepared from the right side of the maxilla.

4.4.3 Basement membrane length

- oblique sections

When there is a flat regular epithelial surface, as was the case in the oblique sections, the ratio of the epithelial basement membrane length to the epithelial surface length gives an accurate comparable measure of the morphology of the basement membrane. Watson and MacDonald (1982) have defined this ratio of basement membrane to epithelial surface length as the epithelial morphology.

The values for the ratio of the basement membrane length to the epithelial surface length for each tissue section examined are shown in Table 4:2. The higher the value recorded, the more irregular and undulating is the basement membrane. The mean value for the whole group was found to be 1.56 with a range from a minimum of 1.19 to a maximum of 1.91.

The figures show the mean value for the 'X' sections, from the most anterior blocks, to be highest. The mean values from the 'U' sections, from the most

posterior blocks was the lowest. When the individual animals were considered this pattern was not consistent. A wide variation of irregularity of the basement membrane was noted with no pattern to the variation, dependent upon tissue block location, being evident.

4.4.4 Morphology of keratinised layer

- oblique sections

Within each column of tissue analysed the length of the interface between the keratinised surface layer and the deeper cellular layer of the epithelium was measured. This measurement reflects the morphology of the keratinised layer when considered in relation to the length of the keratin surface. The values for the ratio of the length of this interface to the epithelial surface length for each tissue section examined are expressed in Table 4:3, as the morphology of the keratin layer.

The ratio of lengths indicated that in all cases the values for both parameters was very close. It was noted in section 4.4.3 that the surface of the epithelium was almost flat. The findings recorded in this section confirm the subjective impression that the keratin layer itself forms a uniform layer on the surface of the epithelium in all the sections analysed in this study.

4.4.5 Keratinised layer thickness

- oblique sections

As previously described (4.3.9) the thickness of the epithelial compartments within the oblique sections was calculated from area measurements. The values for the thickness of the keratinised layer are expressed in Table 4:4. The mean value from the whole group for the thickness of the keratin layer was 47.0μm. There was a wide spread of values from 32.6μm to 66.6μm, both of these extreme values being found in Animal 3. However, ten of the sixteen values were within 10 μm of the mean value, indicating a consistency of value for the parameter examined.

The mean value for the keratin layer thickness for each animal on assessment of all four sites was close to the average value of 47.0μm. The mean value for each of the sites, considering all the experimental animals, was also close to the average, there being no pattern of variation between sites.

4.4.6 Cellular layer thickness

- oblique sections

The values found for this parameter are expressed in Table 4:5 The mean value for the thickness of the cellular layer was found to be 113.8μm. Eleven of the sixteen values were found to be within 20μm of the mean indicating some degree of consistency for the value of the parameter examined.

The variations between the thickness of the cellular layer in different animals and sites were small, as had been the case with the keratinised layer thickness. The pattern of these small differences was similar when the thickness of the cellular and of the keratinised layers are considered together. For both parameters the largest values are found in Animal 2 and the smallest values in Animal 3. For both parameters the greatest mean values are found in the section taken from tissue block 'W'.

4.4.7 Total epithelial thickness

- oblique sections

The values for total epithelial thickness are expressed in Table 4:6.

4.4.8 Length estimations

- longitudinal sections

All longitudinal sections were considered suitable for analysis. Because of obvious differences in morphology between sample sites the results were considered separately for the tissues of the rugae and the tissues of the cols.

4.4.9 Epithelial surface length

- longitudinal sections

For the sections cut longitudinally from the left half of the maxilla the values found for the length of the epithelial surface within each column are shown in Table 4:7. It is apparent from the individual and mean values that the length of the epithelial surface within each column of tissue is considerably greater overlying the rugae than it is in the region of the cols. With the general plane of the epithelial surface aligned horizontally across the field, the epithelial surface overlying each ruga assumes the form of a parabola, and in the area of the col midway between the rugae it is almost flat. Further examination of epithelial lengths within each column of tissue examined shows that the epithelium overlying the rugae exhibits a small range of values for length measurement across the group. The epithelial lengths of tissue from the col area also exhibit a small range of values across the group.

4.4.10 Morphology of keratinised layer

- longitudinal sections

Within each column of tissue analysed the length of the interface between the keratinised surface layer and the deeper cellular layer of the epithelium was measured. The value of this parameter is expressed

as the ratio of the keratin-cell interface to the epithelial surface length in Table 4:8. It is apparent that for both sites the form of the keratin-cell interface follows that of the epithelial surface closely across the whole group. This corresponds with the subjective impression that in the sections examined the keratin forms a uniformly thick surface layer.

4.4.11 Basement membrane length

- longitudinal sections

The basement membrane length was measured in all sections examined and the values found were expressed as a comparison with the epithelial surface length for the particular column of epithelium under consideration. This allows for some comparison between the two sample sites within the group. Comparison of direct length measurements would have been unsatisfactory as the length of epithelium present in each column of tissue sampled from the rugae has been shown to be greater than that taken from the area of the cols. Consequently any higher value in the basement membrane length over the rugae could have been attributed to the increased length of epithelium, rather than any increase in the undulation of the basement membrane. The values found for the ratio of the basement membrane length to the epithelial surface length are shown in Table 4:9. The results indicate the basement membrane to have shown more undulation in

the epithelium overlying the rugae in all specimens examined. The results confirmed the subjective impression that in the epithelium taken from the area of the col the basement membrane was flat and regular.

4.4.12 Area estimations

- longitudinal sections

With regard to area estimations the results from the tissue in the cols was considered separately from the tissue overlying the rugae. In addition there is a variation in the manner in which the data from these two areas is presented. Because the tissue in the col was regular and flat it was considered logical to calculate the average thickness of the epithelial components from the areas measured. In the less regular tissue overlying the rugae, estimations of the average thickness were considered to be less meaningful due to the greater variation found within the tissue. Measurements of area of the epithelial components overlying the rugae are presented as such without translation to measurements of average thickness. This allows comparison with tissue from equivalent sites between specimens, but precludes comparison with tissues taken from areas other than the rugae.

4.4.13 Epithelial thickness - col tissue

Measurement of the areas of the two elements

comprising the epithelium within a column of tissue and knowledge of the column width allowed calculation of the average thicknesses of the keratin and the cellular layers. The values found for these parameters and for the total epithelial thickness are expressed in Table 4:10. The column width was measured to be 580µm with the objective magnification of x 16. The values show a small variation around the mean for epithelial thickness within the group. The relative proportions of keratin and cellular elements making up the total epithelial thickness shows a similar pattern across the group.

4.4.14 Epithelial area - rugal tissue

The values found for the areas of both epithelial components in a column of tissue through the palatal rugae are expressed in Table 4:11. These results indicate a small variation around the mean for the epithelium overlying the rugae. Across this group there was a uniformity in the quantity of epithelium covering the rugae in the longitudinal sections.

4.5 DISCUSSION

4.5.1 Preparation of tissue blocks

Despite the small dimensions of the Wistar rat palate, trimming of tissues into blocks running longitudinally and obliquely across the palate was carried out in such a manner as to produce material

which, on analysis, produced consistent results. The methods of preparation produced tissue which was suitable for analysis on a quantitative basis, and allowed examination of tissue over a wide area of the palate.

4.5.2 Methods of analysis

As the use of quantitative data allows a more sensitive assessment of the structural features of tissue than subjective observation, the principles of planimetric morphometry (2.3.7) were used to evaluate the lengths and areas measured in this study. Stereological analysis of inflamed palatal epithelium was undertaken in the study of human oral mucosa (Chapter 2), and the technique involved superimposition of a grid of squares upon areas to be measured and a grid of parallel lines upon surfaces for length estimation. The technique used in Chapter 2 corresponded to that used by Watson and MacDonald (1982) in their study of healthy human oral mucosa. Thus some comparison of the results in the two patient groups could be made. However the technique of point counting is time consuming.

Provided the structures to be quantified can be clearly delineated, the use of a computerised system of quantitative analysis is more convenient than point counting **stereology**. The present study introduced the author to the technique of computerised planimetry.

4.5.3 Epithelial surface lengths

In the tissues examined from the oblique sections and the col areas of the longitudinal sections, the epithelial surface lengths were found to approximate closely with the column widths examined. This implies that there was a flat surface to the epithelial layer. This finding was consistent for all animals in the study. In the oblique sections which were cut at four different levels though the palate it was true for each of the four sites.

In the sections from the rugae the epithelial surfaces were found to be of greater length within each column of tissue. It is noteworthy that across the group the length of the epithelial surface present was found to be similar in all the specimens, indicating a uniform rugal surface morphology in all five animals.

4.5.4 Form of the keratinised layer

The subjective impression on visual examination of material was that, in all sections, the keratin formed a uniform layer on the surface of the epithelium. Analysis of the results gathered by objective methods of analysis provided confirmation that this was the case.

4.5.5 Form of the basement membrane

The form of the basement membrane showed a variation dependent upon the site sampled. Results, which were correlated as a comparison of basement membrane length per unit of epithelial surface length, showed minimal irregularity of the basement membrane in the region of the cols in the longitudinal sections. This finding was consistent in all five experimental animals. In the rugal tissue of the longitudinal sections there was a finding in all the animals of a more irregular basement membrane in comparison with the epithelium of the col. The degree of irregularity was measured as being at a consistent level throughout the group.

In the oblique sections the degree of basement membrane irregularity was measured to be of a similar average magnitude to that found in the rugal epithelium of the longitudinal sections. There was in the case of this parameter some indication of a small variation related to the site of the oblique sections. Average measurements indicated that sections from tissue blocks 'U' at the posterior part of the palate showed the most regular basement membrane. The tissue from the two centre blocks 'V' and 'W' were of intermediate value and the blocks with the most irregular basement membrane came from the most anteriorly placed tissue

block 'X'. The differences which established this trend were small. It is noteworthy that in the mean value for this parameter, counting all four blocks in each animal, four of the five animals had values for the irregularity of the basement membrane which were of similar magnitude.

4.5.6 Epithelial thickness

There was a uniformity of epithelial thickness across the group of animals when any one of the three sample sites was considered individually. Comparison of the obliquely cut sections with the longitudinal sections showed the epithelium of the col to be slightly thinner than that found in the oblique sections. Because of methods of analysis the epithelium overlying the rugae is not directly comparable with the other two sites. The relative proportions of the two elements comprising the epithelium were directly comparable in all three sites. The keratin formed approximately 30 per cent of the total epithelial thickness in all areas analysed.

4.6 CONCLUSIONS

It would appear from this study that, provided tissue sections are chosen and prepared with care, the palatal epithelium of the Wistar rat is suitable for quantitative analysis. It would also appear from this small group that in healthy Wistar rat

palatal epithelium there is a uniformity of appearance in each of the individual sample sites across the group. The use of all three sample sites, maximising the information from material available, would appear from this study to be a satisfactory procedure. In relation to some, but not all, parameters it is noted that some comparison between different sample sites may be valid.

Tissue blocks (oblique sections)

	u	v	w	x
Animal 1		379	378	370
Animal 2	380		372	373
Animal 3	373	378	376	372
Animal 4	381	377	378	380
Animal 5			379	372

TABLE 4:1 Average epithelial surface lengths per field (µm)

Tissue blocks (oblique sections)

	u	v	w	x	mean
Animal 1		1.80	1.54	1.68	1.67
Animal 2	1.38		1.51	1.91	1.60
Animal 3	1.19	1.24	1.60	1.22	1.31
Animal 4	1.77	1.55	1.60	1.76	1.67
Animal 5			1.37	1.91	1.64
Mean Value	1.45	1.53	1.52	1.70	1.56

TABLE 4:2 Epithelial morphology

Tissue blocks (oblique sections)

	u	v	w	x	mean
Animal 1		0.99	0.99	1.01	1.00
Animal 2	0.99		1.01	1.00	1.00
Animal 3	1.00	0.99	1.00	1.00	1.00
Animal 4	0.99	0.99	1.00	0.99	0.99
Animal 5			0.99	1.01	1.00
Mean value	0.99	0.99	1.00	1.00	1.00

TABLE 4:3 Morphology of keratin layer

Tissue blocks (oblique sections)

	u	v	w	x	mean
Animal 1		56.0	56.9	33.4	48.8
Animal 2	51.3		51.3	48.6	50.4
Animal 3	35.0	40.6	66.6	32.3	43.6
Animal 4	48.3	43.9	48.0	51.0	47.8
Animal 5			42.0	47.3	44.7
Mean Value	44.9	46.8	53.0	42.5	47.0

TABLE 4:4 Keratin layer thickness (μm)

Tissue blocks (oblique sections)

	u	v	w	x	mean
Animal 1		136.1	122.4	85.6	114.7
Animal 2	113.9		125.7	142.3	127.3
Animal 3	79.0	94.2	127.6	81.0	95.5
Animal 4	118.5	96.1	115.6	111.2	110.4
Animal 5			104.3	120.7	112.5
Mean Value	103.8	108.8	119.1	108.2	113.8

TABLE 4:5 Cellular layer thickness (μm)

Tissue blocks (oblique sections)

	u	v	w	x	mean
Animal 1		192.1	179.3	119.0	163.5
Animal 2	165.2		177.0	190.9	177.7
Animal 3	114.0	134.8	194.2	113.3	139.1
Animal 4	166.8	140.0	163.6	162.2	158.2
Animal 5			146.3	168.0	157.2
Mean Value	148.7	155.6	172.1	150.7	157.9

TABLE 4:6 Total epithelial thickness (μm)

	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	mean
Rugae	760	779	751	764	757	762
Cols	589	585	590	588	583	587

TABLE 4:7 Average epithelial surface lengths per field (µm) (longitudinal sections)

	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	mean
Rugae	0.93	1.00	0.97	0.93	1.01	0.97
Cols	0.99	0.99	0.99	0.99	1.00	0.99

TABLE 4:8 Morphology of keratin layer (longitudinal sections)

	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	mean
Rugae	1.44	1.31	1.36	1.57	1.39	1.41
Cols	1.11	1.05	1.08	1.06	1.0	1.07

TABLE 4:9 Epithelial morphology (longitudinal sections)

	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	mean
Keratin layer	43.1	54.5	38.7	36.1	35.6	41.6
Cellular layer	108.2	99.9	88.2	93.1	101.5	98.2
Total epithelium	151.3	154.4	126.9	129.2	137.1	139.8

TABLE 4:10 Average epithelial thickness (µm)
(longitudinal sections - col tissue)

	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	mean
Keratin Area	38,162	47,018	44,308	35,972	43,227	41,737
Cellular Area	95,001	117,161	98,090	94,612	107,209	102,415
Total Area	133,163	164,179	142,398	130,584	150,436	144,152

TABLE 4:11 Epithelial areas µm²
(longitudinal sections - rugal tissues)

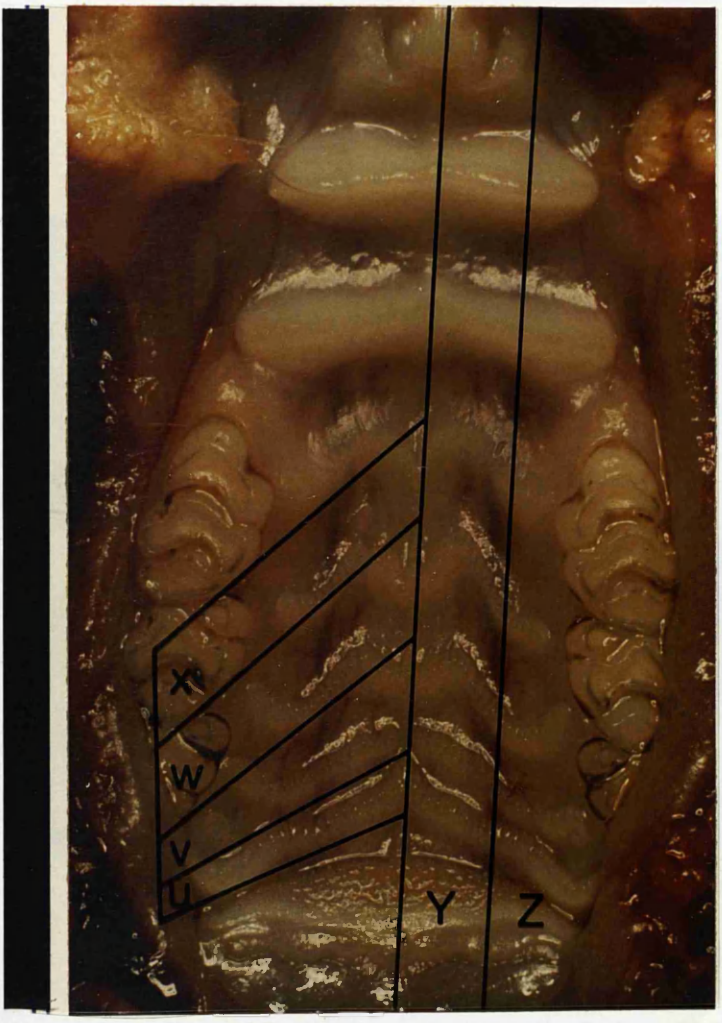


Fig 4:1 Location of tissue blocks prepared for analysis in the study of the anatomy of the Wistar rat palate.

Fig 4:2 Microscope with drawing tube attachment bit-pad and cursor, printer and computer used in computerised planimetry analysis

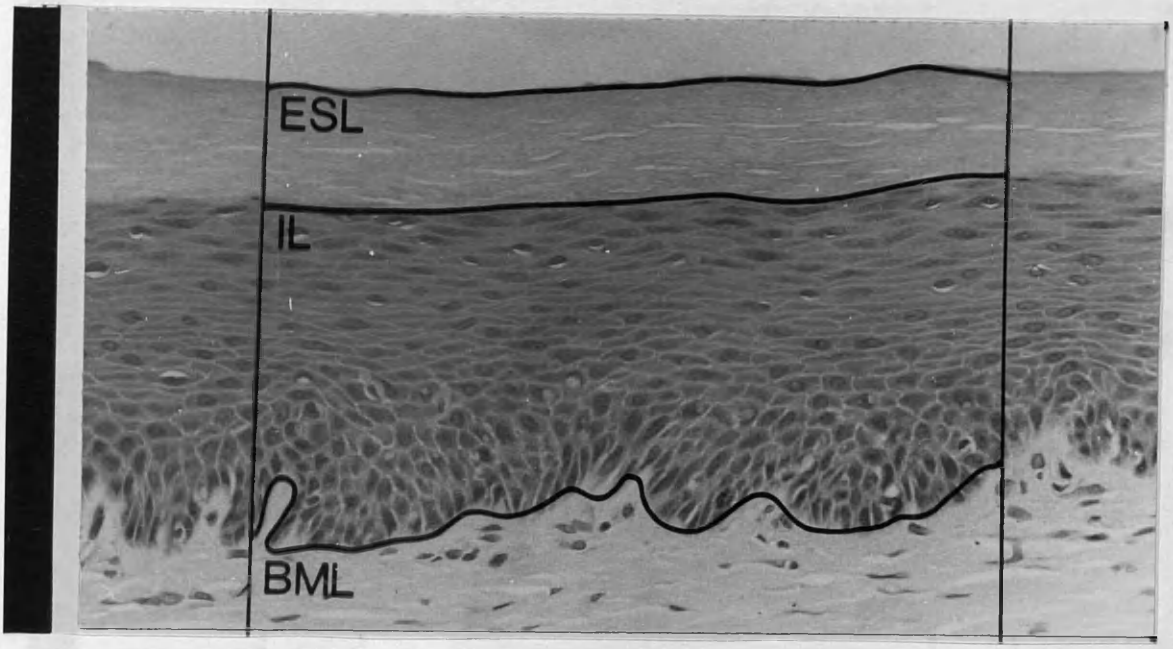


Fig 4:3 Epithelial surface lengths measured.

ESL Epithelai surface length
IL Length of interface between keratin and cellular component
BML Basement membrane length

H & E x 350

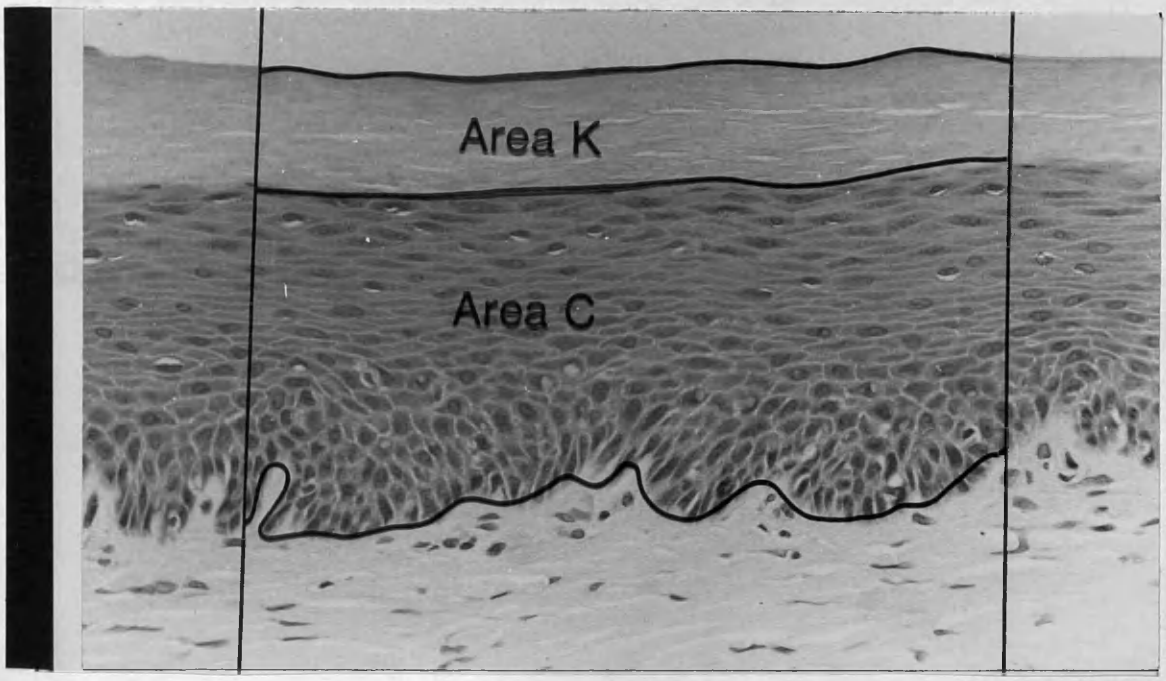


Fig 4:4 Epithelial areas measured.

AK Area of keratin
AC Area of cells

H & E x 350

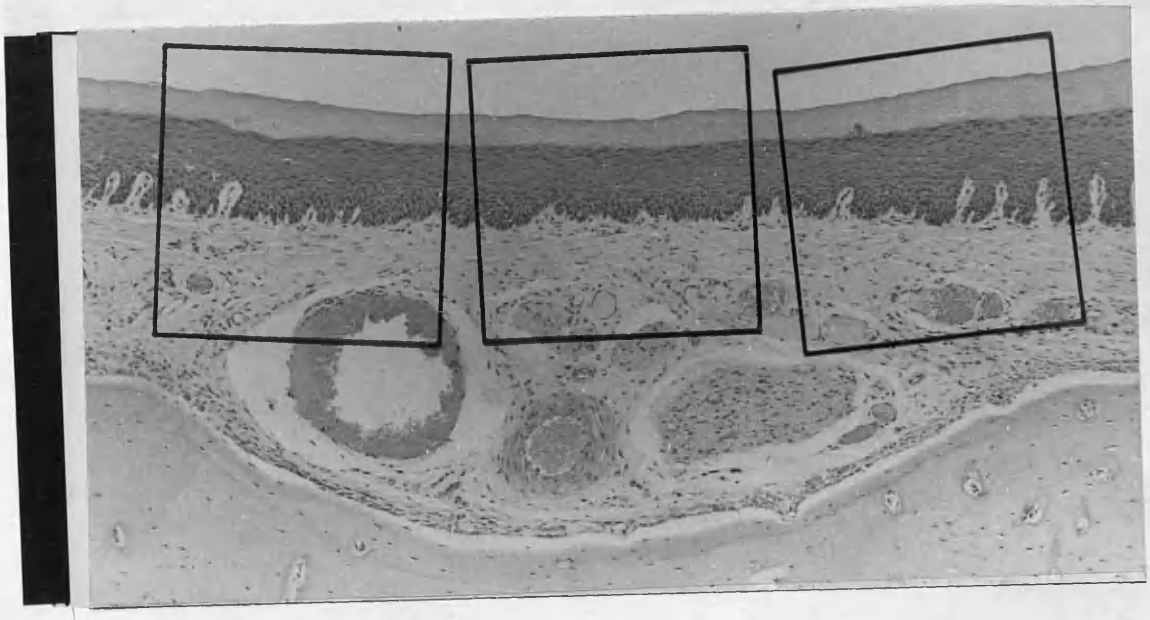


Fig 4:5 Location of tissue fields analysed in the oblique sections.

H & E x 90

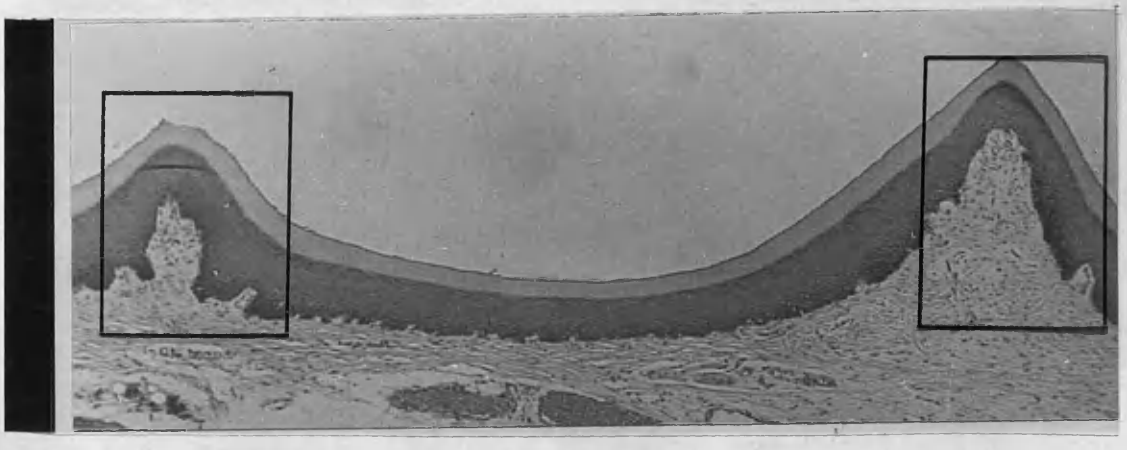


Fig 4:6 Location of tissue fields analysed overlying the rugae.

H & E x 60

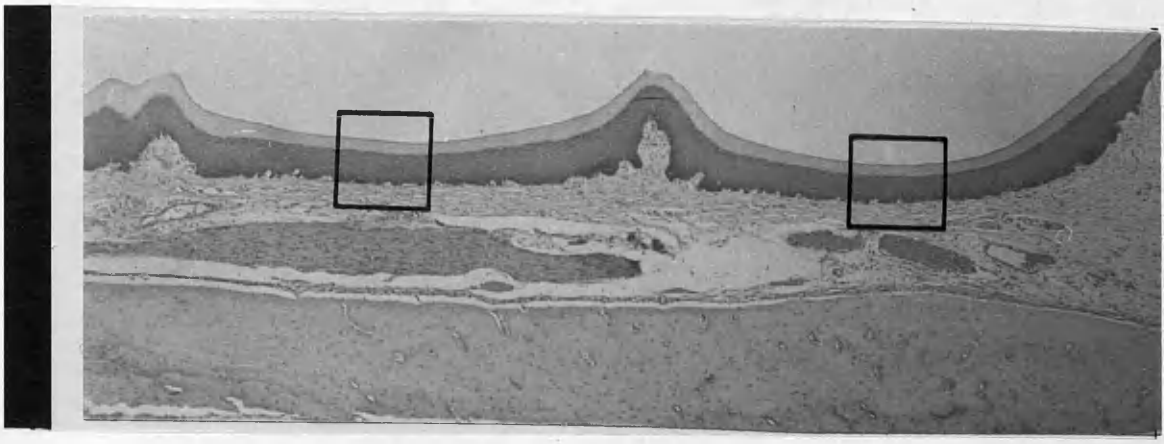


Fig 4:7 Location of tissue fields analysed from the cols.

H & E x 35

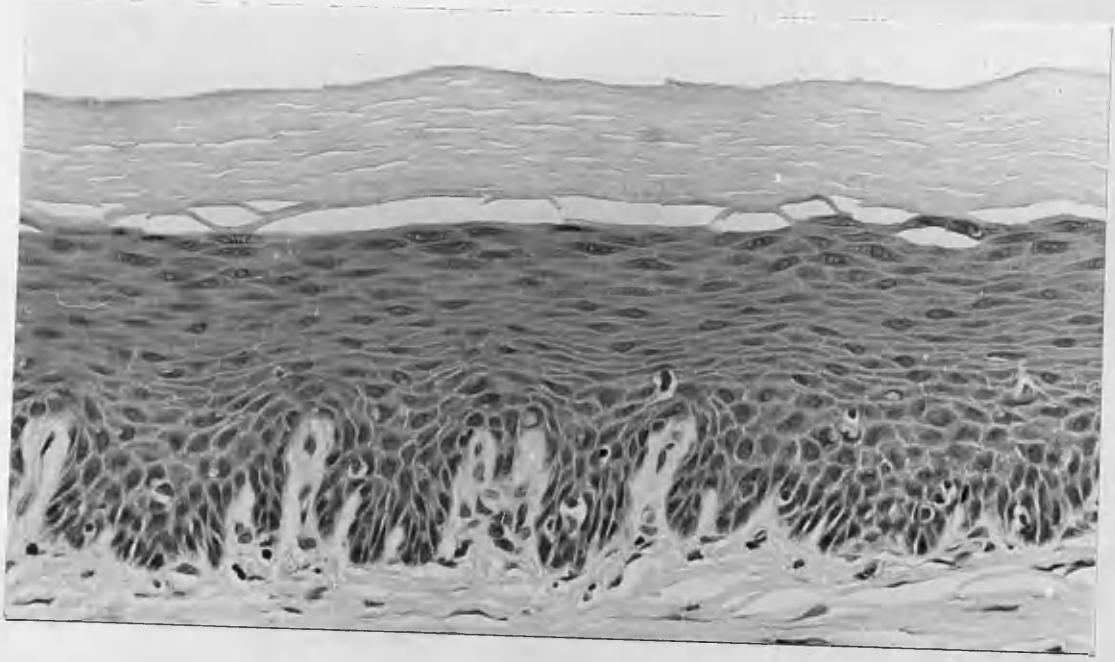


Fig 4:8 Separation between cellular and keratinised layers of the epithelium.

H & E x 350

CHAPTER 5

INVESTIGATION OF EPITHELIAL CHANGES INDUCED IN THE WISTAR RAT BY CANDIDA ALBICANS INOCULATION AND THE WEARING OF AN ACRYLIC APPLIANCE

5.1 INTRODUCTION

Subsequent to the initial investigation of human epithelium it has been the purpose of this research project to examine the use of an animal model in the study of prosthesis related palatal candidiasis. The initial animal investigation exposed difficulties related to sample site selection in the animal model used due to the presence of surface rugae over all of the palate of the Wistar rat. The initial animal study was followed by further investigation of normal epithelium to examine the suitability of different sites for sampling in the Wistar rat palate.

The final part of the study, now described, uses further the Wistar rat animal model in the study of palatal candidiasis.

5.2 AIMS OF STUDY

There were three aims of this part of the study, all of which were related to the further development of the Wistar rat animal model.

The first of these aims was to examine any

effects a period of continuous wearing of an intra-oral appliance constructed in heat cured acrylic had on the palatal epithelium of the Wistar rat. The second aim was to assess the variables introduced by the use of two different designs of appliance. The third aim was to examine tissue changes induced in a group of animals which had the organism Candida albicans inoculated under an acrylic appliance on insertion. In this group of animals both designs of acrylic appliance under investigation were used to assess any differentials introduced.

5.3 MATERIALS AND METHODS

5.3.1 Experimental animals

A total of 37 young male adult Wistar rats were used in this study. During experimental procedures there were 7 deaths within the group, leaving 30 animals from which results were tabulated. The use of male animals eliminated the potential complication of changes in epithelial structure related to sex hormone variations. The animals were weighed regularly during an initial period of caging prior to investigation to establish that obvious growth had ceased and the animals were fully mature.

There were five groups of animals. These comprised a control group, two groups to wear an acrylic appliance only and two groups to wear an acrylic appliance with Candida albicans inoculated underneath.

The animals were caged in groups of either two or three, such that in each cage there were animals from only one experimental grouping. The animals were fed commercial rat fodder and water ad libitum. As an indication of the wellbeing of the animals they were weighed twice weekly during the experimental period.

5.3.2 Design and construction of appliances

In the initial animal model (Chapter 3) it was found that debris considered to be largely food matter, became lodged between the fitting surface of the appliance and the palatal mucosa. This was an undesired occurrence and it is possible that the presence of debris underneath the appliance may have influenced the histological changes within the palatal tissue. It was proposed that in this study two different designs of acrylic appliances be used to examine if design modification induced a reduction in the accumulation of debris and to examine if design influenced the pattern of histological change seen within the epithelium.

In half of the experimental animals a similar design of appliance to that used in the initial animal model was constructed (3.3.4). It extended from the incisor teeth to just behind the posterior teeth, covering all of the palatal tissues. It was finished laterally against the palatal surfaces of the molar

teeth and contoured to avoid contact with the opposing teeth. With the remaining half of the experimental animals a modified design of appliance was constructed. In this design the appliance did not finish against the palatal surface of the molar teeth, but extended onto and covered the occlusal surfaces of the teeth (Fig. 5:1).

Impressions for appliance construction were taken whilst each animal was under general anaesthetic (3.3.2). As in the initial animal model, appliances were constructed on stone casts poured from individual master impressions in each animal. These impressions were taken using polysulphide rubber impression material in suitably shaped preformed trays. All trays for use in this study were constructed from a stone master cast of a fully grown Wistar rat palate made in the initial animal study (3.3.3). Following the pouring of a stone master cast and the forming of a closely fitting orthodontic band around the incisor teeth, an appliance was then constructed in heat cured polymethylmethacrylate (3.3.4).

5.3.3. Fungal inoculum

In the initial animal model (Chapter 3) no variation in response between the two experimental strains of Candida albicans used, was noted (3.5.5). Candida albicans strain RST A 20 from the Microbiology Unit, Glasgow Dental Hospital, was cultured for use in

this part of the study. The organism was incubated aerobically on Sabouraud's dextrose agar and 30 mg aliquots of the yeast culture were harvested.

5.3.4 Insertion of appliances

Both designs of appliance were secured to the notched incisor teeth by means of a closely fitting orthodontic band. Insertion of the appliances was accompanied by application of fungal inoculum in half of the animals. Half of the animals belonging to the group in which appliances covered the occlusal surfaces of the teeth had Candida albicans applied to the fitting surface of the appliance, as did half of the animals in the group without occlusal cover.

At the time of insertion of the appliances, 60 mg of pure culture of Candida albicans was applied, in the appropriate experimental groups, to the fitting surface of each appliance. Subsequent to notching of the incisor teeth, the orthodontic band attached at the anterior end of each apliance was filled with self curing polymethylmethacrylate and the appliance placed firmly in place covering the palatal mucosa (3.3.6).

5.3.5 Loss of appliances

During the experimental period appliances became loose and were dislodged in several animals. Failure of retention always occurred within a few days

of initial insertion of the appliances in question. In this event, appliances were cleaned and re-inserted with inoculation of Candida albicans where appropriate. The full experimental interval was then allowed from the time of appliance re-insertion.

5.3.6 Animal sacrifice and specimen preparation

After an experimental period of 28 days, the control and experimental animals were sacrificed by ether inhalation anaesthesia and fracture of the spinal cord. Each animal was decapitated, the mandible was dissected free and the tissues of the remainder of the head were fixed in 10 per cent buffered formalin for three days. Following fixation the upper part of the skull was removed with a rotary saw and the remainder of the palate decalcified in 20 per cent formic acid (3.3.7).

It was noted in the previous chapter (4.6) that provided tissue section sites were chosen and prepared with care, quantitative analysis of material from this animal model was meaningful. The sites for tissue sampling chosen for this part of the study corresponded with those of the previous chapter (4.3.2).

The maxilla was split in half along the midline. In the right half of the maxilla tissue blocks were cut obliquely in planes which corresponded with the orientation of the surface rugae. In each animal four blocks were cut in relation to the position

of the main rugae, corresponding in position to blocks 'u', 'v', 'w' and 'x' in the previous chapter (4.3.2).

In some instances accurate location of the rugae was more difficult than had been the case in the model described in Chapter 4. The wearing of an appliance encouraged the accumulation of material on the tissues of the palate and as it was considered undesirable to disturb the surface layer of the palatal mucosa, vigorous mechanical removal of all surface material prior to section preparation was not possible. It was the case in the few instances where there was adherent surface material, that the location of incisions for the preparation of tissue blocks was unavoidably less precise than where there was no surface covering.

The tissue for analysis from the left side of the palate was cut in a longitudinal plane. The palate was sectioned in an anteroposterior plane midway between the midline and the palatal surface of the molar teeth. This produced two blocks of palatal tissue similar in location to blocks 'y' and 'z' of the study of the anatomy of the Wistar rat palate (Chapter 4). The longitudinal section of the maxilla containing the molar teeth was used for analysis in this study.

This represents a modification of technique in relation to the method of site selection for analysis, in the longitudinal sections. In the study

of the anatomy of the Wistar rat palate (Chapter 4), sections for analysis were taken from the lateral surface of block 'y'. In this present study the sections were taken from the medial surface of block 'z'. The methods described both produced tissue for analysis from a similar location in the left half of the palate. This was approximately halfway between the midline of the palate and the molar teeth. However, the use of the tissue block containing the molar teeth simplified orientation of the block for section cutting.

The tissue blocks chosen for analysis were processed in a Histokine automatic tissue processor and embedded in paraffin wax. In the obliquely cut blocks from the right half of the palate, tissue sections were taken from the anterior surface of the block. In the longitudinal tissue blocks, tissue sections were cut from the medial surface. A section from each block was stained with haematoxylin and eosin for analysis of the epithelial structure. In tissue from those animals which had been inoculated with Candida albicans, a second section was cut and stained to identify if fungus was still present at the end of the experimental period. These sections were pre-treated with diastase to remove glycogen and stained with the periodic acid Schiff method (McManus 1946).

5.3.7 Methods of analysis

In this study morphometric analysis of the

tissue was undertaken using the computerised planimetry techniques described in the previous Chapter (4.3.3, 4.3.4, 4.3.5). The site selection for analysis, the techniques of analysis, the lengths measured and the areas measured in this present study corresponded with those of the study of the anatomy of the Wistar rat palate (Chapter 4), for both oblique and longitudinal sections (4.3.6, 4.3.7, 4.3.8, 4.3.9, 4.3.10, 4.3.11).

5.3.8 Column width analysed

For the study described in this chapter, the lens magnification used for tissue examination was standardised at X 16. Measurement, using a stage micrometer, showed that the resulting width of the column of tissue for analysis was 580μm.

5.3.9 Animal groups used in comparisons

Following the collection of results from the tissue sites analysed, comparison between the experimental groups was made and the findings were analysed statistically.

Each of the 4 experimental groups was compared individually with the control group. The control group was also compared in turn with 4 different combinations of 2 experimental groups.

Groups 1 and 2, in which appliances covered palatal mucosa but not teeth, were compared collectively

with the control group. Groups 3 and 4 in which appliances extended onto the occlusal surfaces of the teeth, were compared collectively with the control group. Groups 1 and 3 in which Candida albicans was inoculated underneath each appliance, were compared collectively with the control group. Groups 2 and 4 in which appliances were inserted but no Candida albicans inoculated, were compared collectively with the control group.

There was also comparison of Groups 1 and 2, in which appliances covered palatal mucosa only, with Groups 3 and 4 in which appliances extended onto the teeth. Groups 1 and 3 in which Candida albicans was inoculated under the appliances were considered collectively and compared with Groups 2 and 4 which collectively were those animals in which appliances were inserted, but no Candida albicans was inoculated.

In those instances where there was no significant difference between the control group and the experimental groups considered individually or in the combinations described, a comparison was then made between the control group and the experimental animals in total, from all four experimental groups.

5.3.10 Method of statistical analysis

In each of the comparisons made, two independent groups were studied. In order to examine if there was convincing evidence of a difference between

the groups in question, a nonparametric statistical test, the Mann-Whitney U Test, was used in the analysis of the data gathered. The probability level for the tests applied was $p<0.05$, unless otherwise indicated.

5.4 RESULTS

5.4.1 Premature mortality

At the onset of the experimental period there were 37 animals, divided into three groups of seven and two groups of eight. During the course of the experimental period seven animals died prematurely. Thirty of the animals survived until the end of the experimental period. Only the control group remained intact with all seven animals surviving. The numerical composition of the four experimental groups for which results were gathered at the end of the 28 day period was : two groups of five animals, one of six and one group of seven animals.

All of the premature deaths were related to general anaesthesia for experimental procedures. Three animals did not regain consciousness following anaesthesia, and four regained consciousness, but died within 24 hours of the procedure.

5.4.2 Animal weights

For up to the first ten days following

insertion of the oral appliances, the weight of each animal was reduced. Thereafter, in all cases, the animals showed a gradual increase in weight, approaching, and in some cases surpassing the original weight.

5.4.3 Experimental categories

The distribution of the 30 animals at the end of the 28 day experimental period, and the experimental conditions relating to each of the five groups are shown in Table 5:1.

5.4.4 Debris under appliances

There was persisting occurrence of a considerable amount of accumulated fibrous debris underneath both designs of acrylic appliance used in this study. The quantity of such debris was considerably less in association with those appliances in which there was full coverage of the occlusal surfaces of the teeth (Fig. 5:2).

5.4.5 Persistence of Candida albicans

Sections, stained using the periodic acid-Schiff method, from all twelve experimental animals inoculated with Candida albicans (Groups 1 and 3), were examined for the presence of fungal hyphae. Hyphae were present in tissue sections from three animals. In

one animal from Group 1, hyphae were evident in the section from the longitudinally cut tissue block. The hyphae were associated with the epithelium overlying one of the rugae (Fig. 5:3). Another two animals (one each from Groups 1 and 3) showed hyphae present within sections of eithelium from obliquely cut blocks. In both instances the hyphae were located adjacent to the molar teeth (Fig. 5:4).

Within each of the sections hyphae were restricted to a small area of tissue. The histological appearance of the tissue associated with hypae was in marked contrast to the well-ordered epithelial structure found elsewhere. There was hyperplasia and acanthosis, acute inflammatory infiltrate and micro-abscess formation within the epithelium, and a sub-epithelial chronic inflammatory response (Fig. 5:5).

5.4.6 Structure of surface keratin

Examination showed keratin on the surface of all sections prepared. The appearance was suggestive of a keratin surface comprised of two distinct components (Fig. 5:6). Keratin forming the deep layer was compact and in close contact with the underlying cellular epithelium. The superficial keratin, detached from the bound layer, was of less regular arrangement and often comprised several layers or strata of loosely arranged keratin squames.

Analysis of keratin in the tissue sections was confined to the deep compact tissue which was considered to be the equivalent of the normal stratum corneum of the palatal epithelium. The detached tissue was considered to consist of shed keratin which had been held in contact with the epithelium by the presence of acrylic appliances during the experimental period.

5.4.7 Analysis of epithelium of the rugae

Length measurements were with reference to the parameters of epithelial surface length (ESL), the length of the interface between keratin and cells (IL), and the basement membrane length (BLM). The results measured in microns, are shown in Table 5:2.

In all groups the epithelial surface length was found to be considerably greater than the column width of 580μm. This is consistent with the observed parabolic or pyramidal contour of the epithelial surface over the rugae. Similarity of values for length measurements of the epithelial surface and the keratin-cell interface indicated that in all groups keratin formed a layer of uniform thickness on the surface (Fig. 5:7). Comparison of groups showed that the epithelial surface form and the form of the surface keratin layer were not affected by experimental conditions.

Results indicated that the basement membrane was more regular in the experimental groups in which appliances covered mucosa only (Groups 1 and 2), than in

the other groups. Consideration of Groups 1 and 2 individually showed values for basement membrane length to be significantly less than the value found in the control group (Fig. 5:8). When Groups 1 and 2 were considered collectively the values found for basement membrane length were significantly less than the values found for Groups 3 and 4 considered collectively, and the control group. No other significant differences were found for this parameter.

Comparison between the experimental groups for parameters of area was with reference to the area of keratin (Area K), the area of cellular epithelium (Area C), and the total epithelial area in each column of tissue analysed (Total Area). Because of the irregular contour of the epithelium overlying the rugae, it was not considered meaningful to convert the areas measured to average thicknesses, in these tissues. The results, measured in microns, are shown in Table 5:2.

The area of the keratinised layer was found to be significantly less in all experimental groups than in the control group. There was no significant difference between any of the experimental groups.

The cellular component of the epithelium overlying the rugae was affected to a lesser degree than the keratinised layer by the experimental conditions. The cellular epithelium in those animals which had appliances covering the molar teeth (Groups 3 and 4) was

significantly thicker than in those animals in which appliances were borne by the mucosa (Groups 1 and 2). In Group 4 the cellular layer was significantly thicker than in the control group.

When total epithelial thickness was considered it was found that the epithelium which had been covered by the mucosa borne appliances (Groups 1 and 2) was thinner than the epithelium of the control animals. No other significant variations were found (Table 5:2).

5.4.8 Analysis of epithelium of cols

In tissue block preparation for animal 1 of Group 1 there was damage to the surface layer of the keratin. As a result, meaningful analysis of the epithelial surface length and the area of the keratin was not possible in this animal. The tissue block for one of the animals in Group 2 was lost in the processing procedure, and results for this group were gathered from the remaining four animals.

Comparison between groups for length measurement was with reference to the parameters of epithelial surface length (ESL), basement membrane length (BML) and the length of the interface between keratin and cells within the epithelium (IL). The values found for these parameters are shown in Table 5:3, and are measured in microns.

All groups showed a flat regular epithelial

surface with epithelial surface length values only marginally greater than the column width of 580 µm. There was little variation between groups.

It was a consistent finding in all groups that values calculated for epithelial surface length and the length of the interface between the keratin and cells were similar in all fields examined. This confirmed the subjective observation of a uniform surface layer of keratin (Fig. 5:9).

Statistical analysis showed no significant differences in basement membrane length between the groups sampled, indicating that there was no pattern of change induced in the tissues of the col by the variation in experimental conditions for this parameter

The values found for the parameters of keratin area and thickness, the area and thickness of cellular epithelium and the total epithelial area and thickness are shown in Table 5:3. Results are measured in square microns and microns.

Examination of the area and thickness of the keratin layer showed a small variation between the groups. The animals which had appliances constructed to cover the occlusal surfaces of the teeth (Groups 3 and 4) demonstrated the thinnest layer of keratin covering the epithelium. The reduced thickness of keratin in Group 4 in comparison with the control group was found to be statistically significant. When groups

3 and 4 were considered collectively and compared with the control group, the reduction in keratin thickness was also found to be statistically significant. No other statistically significant variations were found, although it is noteworthy that the control Group had the highest average value for keratin thickness.

In contrast, when the thickness of the cellular layer of epithelium was considered, it was found that in the control group of animals the value for this parameter was lowest. With one exception, the difference in thickness between the control group and the experimental groups was found to be statistically significant in all combinations tested. The exception was in the comparison of Group 3 with the control group. In this case, although the average thickness of the cellular layer was still found to be less in the control group, the difference was not significant statistically.

When total epithelial thickness was considered, the lowest value for this parameter was found in the control group. Statistical analysis showed that Groups 1 and 4, when considered individually, had an epithelial thickness which was significantly greater than the control group. On consideration of Groups 1 and 2 collectively it was found that the epithelium was significantly thicker in those animals wearing mucosa borne appliances than in the control animals ($P<0.005$). No other significant variables were noted.

5.4.9 Analysis of epithelium of oblique sections

The oblique sections were prepared from the right half of the palate. Four blocks of tissue were prepared and examined from this site in each animal. From the total of 120 sections analysed from the 30 experimental animals, 33 sections were considered unsuitable for quantitative analysis. The presence of irregular surface rugae was the primary reason for rejection of material for analysis (Fig. 5:10). This was caused by the previously described difficulty in tissue sectioning related to persisting surface debris. A few sections were rejected due to damage of tissue during processing or unsatisfactory block alignment prior to tissue sectioning.

Overall, 87 oblique sections, 73 per cent of the total prepared, were considered suitable for analysis. For each animal in the series, a minimum of two of the four blocks analysed produced sections suitable for use in the study. As the number and location of oblique sections suitable for quantitative analysis varied between animals, the mean value for each epithelial parameter was calculated from the suitable sections in each animal. The statistical assessment of differences between groups was based on the mean value for each parameter in each animal.

For the five animals in experimental Group 1,

the findings for histological parameters measured are shown in Table 5:4. For the five animals in Group 2, the findings for the histological parameters measured are shown in Table 5:5. The findings from the seven animals of Group 3 are shown in Table 5:6. The findings from Group 4 are shown in Table 5:7. There were six animals in this group. The control group, Group 5, consisted of seven animals and the results are shown in Table 5:8. The average values for each group for each of the parameters measured is shown in Table 5:9.

From the results it was seen that the surface of the epithelial layer was almost flat in all groups. The close correlation between the epithelial surface length and the length of the interface between keratin and cells, indicated a uniform surface keratin layer in all groups, not influenced significantly by experimental conditions. This corresponds with the subjective observation of a flat uniform surface layer of keratin in all groups examined (Fig. 5:11).

Basement membrane undulation was most prominent in the experimental groups wearing appliances covering mucosa only (Groups 1 and 2) and least marked in the control group (Fig. 5:12). This difference was found to be statistically significant when these experimental groups (Groups 1 and 2) were considered individually and collectively and compared with the control group ($P<0.005$). No other significant

differences were observed.

Average values for keratin area and thickness were found to be similar for all groups within this study. It was found on statistical analysis that the differences which did exist did not approach the level of being statistically significant.

There were, however, found to be differences in the values for thickness of the cellular component of the epithelium of sufficient magnitude to be statistically significant. On examination of the results collected for group averages in relation to this parameter (Table 5:9), it was observed that the values for the control group were markedly lower than the experimental groups. It was also observed that the average values obtained within the experimental groups fell within a small range. Analysis showed that with two exceptions, the cellular layer in the experimental groups showed an increase in thickness which, in comparison with the control groups, was statistically significant (Fig. 5:12). The increase in thickness was found not to be significant when the control group was compared with Group 1 individually and with Groups 1 and 2 in combination. There were no other significant differences found.

The findings noted for the keratinised and cellular components of the epithelium were reflected when the average thickness of the epithelium as a whole

was considered. Consideration of the average values showed the epithelial thickness of the experimental groups to be considerably greater than the control group (Fig. 5:12). Analysis of data revealed that in all combinations tested, the total epithelial thickness within the experimental groups showed an increase which in comparison with the control group was significant statistically. No other significant differences were found.

5.5. DISCUSSION

5.5.1 Changes induced in epithelial structure

Variations in epithelial structure induced by experimental conditions were dependent upon the experimental site analysed, the presence of an acrylic appliance or features of design of the appliance. The inoculation of Candida albicans did not appear to make a significant contribution to changes induced in the palatal epithelium, other than in three isolated instances.

Neither the form of the epithelial surface nor the morphology of the adherent surface layer of keratin were affected by variations in experimental conditions, regardless of the site sampled. Physical conditions appeared to exert an important influence on the changes which occurred in the other parameters of epithelial structure examined in the study.

It is noteworthy that in the tissues overlying the rugae, changes induced under experimental conditions were often in direct contrast to those occurring in tissues from the oblique sections. Changes induced in the tissues of the cols were often intermediate in relation to the changes induced in the other two sample sites.

It seems likely that the variation in response reflected the individual physical environment peculiar to each of the three sites. The contrast is highlighted by consideration of the changes induced under experimental conditions for each of the epithelial parameters of basement membrane length, keratin layer thickness, thickness of the cellular layer and total epithelial thickness.

Basement membrane length was unchanged by experimental conditions in the tissues of the cols. Changes induced in the two other sample sites were of a contrasting nature. The basement membrane length was found to be reduced overlying the rugae, but only underneath mucosa-borne appliances. The basement membrane length was found to be increased in the tissue from the oblique sections to a statistically significant degree in epithelium underlying mucosa-borne appliances. In relation to this parameter, site selection, presence and design of appliances all affected the results.

The keratin layer was found to show no change

in thickness related to experimental conditions in the tissue from the oblique sections. Reduced thickness of keratin was found in the other sites sampled. In the tissue from the cols change was confined to those animals wearing tooth-borne appliances. There was a marked reduction in keratin thickness in the tissues overlying the rugae in all experimental groups which may have resulted from a reduction in functional loading following coverage of the rugae.

In the tissues of the oblique sections and the cols the cellular epithelial layer showed an increase in thickness. This change was particularly marked within the oblique sections. In the tissues overlying the rugae, the type of change induced in the cellular layer of the epithelium was dependent upon appliance design. Tissues underlying tooth-borne appliances showed an increased thickness, the tissues underlying mucosa-borne appliances showed a reduced thickness of this layer. These differences were statistically significant and highlight the influence of appliance design on the findings within this animal model.

Total epithelial thickness reflected the values found within the cellular and keratinised compartments of the epithelium, showing variations dependent upon sample site and appliance design. In the oblique sections all experimental groups showed a marked increase in epithelial thickness. In the tissue

from the cols there was an increase in overall epithelial thickness which was most marked in those animals wearing mucosa-borne appliances. The epithelium was reduced in thickness over the rugae in those animals with mucosa-borne appliances.

Physical, rather than microbiological, factors appeared to be responsible for the experimental changes induced in the animal model. Due to the prominent location of the rugae, the physical effects of wearing an appliance are likely to have been maximised in this area. The exposed position, characteristic epithelial form and enhanced effects of physical change may have contributed to the distinctive epithelial changes seen in tissue from the rugae. Within the tissues from the oblique sections and the cols the increased thickness of the cellular layer contributed to the increase found in total epithelial thickness. It may be that reduced functional loading of the epithelium led to a slowing down of the process of cell maturation, and an increased thickness of this epithelial compartment.

5.5.2 Changes induced by Candida albicans

The presence of candidal hyphae in the epithelium of only three of the twelve animals initially inoculated suggests that in most cases the organism was eliminated from the tissues in the animal model.

Restriction of candida to a small area in each tissue section in which it was found implies that the presence of Candida albicans alone was not sufficient to promote generalised infection of the tissues underneath an appliance. It seems likely that an additional local factor encouraged the organism to persist and assume a pathogenic role.

Candidal hyphae were located either on a palatal ruga or adjacent to molar teeth. These are sites where trauma from an appliance is most likely to occur, and ulceration of the epithelium was evident in the tissue overlying the ruga in question. It may be that infection of the palatal tissues by Candia albicans was a secondary occurrence following localised ulceration of the epithelium by a rigid acrylic appliance.

5.6 CONCLUSIONS

This study, using the Wistar rat animal model, showed that changes in the epithelial structure within the experimental animals were evident using quantitative analytical methods. Changes in the morphology of the basement membrane along with changes in thickness of the epithelium and of its cellular and keratinised components were detected.

It was observed that changes occurring were related to the presence and design features of an acrylic apliance rather than inoculation of Candida

albicans. Accumulation of debris underneath appliances was a persisting occurrence which may have exerted an influence on the histological changes observed. Although changes in structure of epithelium were detected, the organisation of tissues largely remained ordered with no disruption of structured form evident other than in 3 small isolated areas. Although various generalised changes induced within the epithelium under experimental conditions were statistically significant, the quantitative methods of analysis used provided a sensitive form of assessment and it is unlikely that the main changes reported in the study would be fully apparent on subjective observation of the tissue.

The localised disruption of epithelial form which occurred in tissue from three animals was associated with the presence of candidal hyphae. It seems likely however that the role of the organism was in causing secondary infection within tissue initially traumatised by rigid appliances, rather than acting as a primary pathogen.

	Appliance Design	Candida Innoculation	Animal Numbers
Group 1	Mucosa cover only	Positive	Five
Group 2	Mucosa cover only	Negative	Five
Group 3	Tooth and mucosa cover	Positive	Seven
Group 4	Tooth and mucosa cover	Negative	Six
Group 5	No appliance	Negative	Seven

TABLE 5:1 Experimental Categories

		ESL	IL	BML	Area K	Area C	Total area
Group 1	1	785	698	846	35.742	119.146	154.888
	2	688	699	987	34.092	101.727	135.819
	3	761	774	1202	49.172	123.059	172.231
	4	663	648	978	36.926	93.391	130.317
	5	623	699	1002	39.220	79.689	118.909
	Mean	704	704	1003	39.030	103.402	142.432
Group 2	1	820	739	887	54.648	87.438	142.086
	2	698	714	1012	22.860	86.664	109.524
	3	851	788	1036	29.726	111.369	141.095
	4	709	715	1025	22.445	85.842	108.287
	5	630	632	964	26.503	99.813	126.316
	Mean	742	718	985	31.236	94.225	125.461
Group 3	1	752	782	1232	39.901	130.104	170.005
	2	659	713	1459	42.011	89.888	131.899
	3	657	679	955	28.254	92.857	121.111
	4	847	878	1133	37.436	123.409	160.845
	5	882	894	1279	68.744	146.431	215.175
	6	724	727	986	28.188	104.806	132.994
	7	638	642	1118	15.584	80.854	96.438
	Mean	737	759	1166	37.160	109.764	146.924
Group 4	1	667	645	773	34.449	104.995	139.444
	2	949	1040	1950	43.642	142.088	185.730
	3	942	942	1386	37.000	121.174	158.174
	4	734	710	1158	36.113	126.376	162.489
	5	686	683	1154	41.373	113.958	155.331
	6	699	697	1031	26.750	116.055	142.805
	Mean	780	786	1242	36.555	120.774	157.329
Group 5	1	753	695	1273	49.377	111.630	161.007
	2	806	790	1169	47.220	97.848	145.068
	3	708	682	1110	55.472	89.347	144.819
	4	735	693	1175	47.095	101.805	148.900
	5	706	733	1376	41.516	123.417	164.933
	6	840	755	1155	53.456	105.895	159.351
	7	705	704	1157	39.377	93.003	132.380
	Mean	750	722	1202	47.645	103.278	150.923

TABLE 5:2 Sections from Rugae
Epithelial Parameters (μm) (μm²)

		ESL	IL	BML	Area K	Area C	Total area	TK	TC	TT
Group 1	1		591	740		699.30				
	2	599	594	619	21.890	696.33	91.523			
	3	615	604	651	24.038	699.55	93.993			
	4	592	590	667	21.324	68.161	89.485			
	5	600	594	648	31.502	62.484	93.986			
	Mean	602	595	665	24.689	68.033	92.247	43	117	160
Group 2	1	603	597	657	36.136	65.433	101.569			
	2	588	584	611	15.155	59.029	74.184			
	3	583	583	614	24.622	64.413	89.035			
	4	596	593	644	20.987	66.865	87.852			
	Mean	594	589	632	24.225	63.935	88.160	42	110	152
Group 3	1	601	606	981	19.688	74.440	94.128			
	2	593	591	684	32.592	65.451	98.043			
	3	589	586	622	14.453	55.568	70.021			
	4	598	592	590	13.623	81.299	94.922			
	5	592	586	806	32.332	85.025	117.357			
	6	591	588	604	14.084	59.069	73.153			
	7	589	585	707	13.650	60.772	74.442			
	Mean	593	591	713	20.060	68.803	88.863	35	119	153
Group 4	1	591	589	695	15.085	75.571	90.656			
	2	607	595	685	21.610	81.949	103.559			
	3	595	589	644	18.784	57.279	76.063			
	4	596	591	758	23.150	85.444	108.594			
	5	602	595	610	24.170	72.109	96.279			
	6	600	588	611	23.120	69.395	92.515			
	Mean	599	591	667	20.987	73.625	94.612	36	127	163
Group 5	1	586	581	625	23.898	58.412	82.310			
	2	599	593	636	26.588	60.840	87.428			
	3	590	589	631	28.525	56.578	85.103			
	4	590	593	647	28.685	56.417	85.102			
	5	587	585	698	23.949	63.196	87.145			
	6	588	583	774	24.812	60.774	85.586			
	7	589	586	625	20.225	50.408	70.633			
	Mean	590	587	662	25.240	58.089	83.329	44	100	144

TABLE 5:3 Sections from Cols
Epithelial Parameters (μm) (μm²)

		ESL	IL	BML	Area K	Area C	Total area	TK	TC	TT
Animal	u	600	617	1116	29.014	83.189	112.203			
	v	596	602	969	22.945	91.649	114.594			
1	w	614	604	1075	18.946	99.474	118.420			
	x	596	594	830	14.560	63.547	78.107			
Mean		602	604	998	21.366	84.465	105.831	37	146	182
Animal	u	591	595	942	21.743	78.555	100.298			
	v	605	603	978	13.921	87.271	104.192			
2	w	592	590	929	20.444	87.762	108.206			
	x									
Mean		596	596	950	18.703	84.529	103.232	32	146	178
Animal	u	596	586	890	40.294	84.991	125.285			
	v									
3	w	596	590	1062	25.093	93.145	118.238			
	x	600	599	898	19.545	72.750	92.295			
Mean		597	592	950	28.311	83.629	111.940	49	144	193
Animal	u	645	636	1020	33.784	74.116	107.900			
	v	591	599	1289	21.957	81.692	103.649			
4	w	591	594	1295	19.978	78.093	98.071			
	x									
Mean		609	610	1201	25.240	77.967	103.207	44	134	178
Animal	u	587	593	829	21.472	48.050	69.522			
	v	580	582	849	26.912	54.669	81.581			
5	w									
	x									
Mean		584	588	839	24.192	51.360	75.552	42	89	130
Mean		598	598	988	23.562	76.390	99.952	41	132	172

TABLE 5:4 Oblique Sections Group 1
Epithelial Parameters (μ.m.) (μ.m.^{2})

		ESL	IL	BML	Area K	Area C	Total area	TK	TC	TT
Animal	u	590	592	826	20.454	69.842	90.296			
	v	616	608	1052	27.820	90.269	118.089			
1	w	588	587	741	28.257	64.802	93.059			
	x	589	587	702	19.629	70.797	90.426			
Mean		596	495	830	24.040	73.928	97.968	41	127	169
Animal	u	608	609	915	19.728	70.990	90.718			
	v	631	626	1138	14.879	71.568	86.447			
2	w	679	709	1934	22.565	96.566	119.131			
	x	592	589	1127	14.766	71.761	86.527			
Mean		628	633	1279	17.985	77.721	95.706	31	134	165
Animal	u									
	v									
3	w	595	597	829	27.702	73.277	100.979			
	x	605	613	872	28.124	89.214	117.338			
Mean		600	605	851	27.913	81.246	109.159	48	140	188
Animal	u									
	v									
4	w	596	634	1387	39.029	106.201	145.230			
	x	587	604	2532	35.972	128.557	164.529			
Mean		592	619	1960	37.501	117.379	154.880	65	202	267
Animal	u	581	582	651	21.133	55.815	76.948			
	v	590	594	856	19.643	64.191	83.834			
5	w	585	590	909	20.111	78.779	98.890			
	x	582	584	912	20.617	82.371	102.988			
Mean		585	588	832	20.376	29.376	90.663	35	121	156
Mean		600	608	1150	25.563	84.112	109.675	44	1459	189

TABLE 5:5 Oblique Sections Group 2
Epithelial Parameters (μm) (μm²)

		ESL	IL	BML	Area K	Area C	Total area	TK	TC	TT
Animal	u	587	590	681	22.102	70.611	92.713			
	v	598	597	758	14.839	69.238	84.077			
1	w									
	x	656	655	1246	33.596	98.900	132.496			
Mean		614	614	895	23.512	79.583	103.095	41	137	178
Animal	u									
	v									
2	w	585	587	751	19.333	64.579	83.912			
	x	645	631	1275	38.488	88.028	126.516			
Mean		615	609	1013	28.911	76.304	105.215	50	132	181
Animal	u	604	598	908	17.054	67.533	84.587			
	v	607	610	1125	28.598	111.287	129.885			
3	w									
	x									
Mean		606	604	1017	17.826	89.410	107.236	31	154	185
Animal	u									
	v	599	591	672	21.369	59.216	80.585			
4	w	580	580	755	11.985	75.843	98.828			
	x									
Mean		590	580	714	22.177	67.530	89.707	38	116	155
Animal	u	580	583	633	13.967	65.729	79.696			
	v	595	592	659	28.539	69.676	98.215			
5	w	598	588	639	24.226	72.924	97.150			
	x	603	595	670	22.981	67.880	90.861			
Mean		594	590	650	22.428	69.052	91.480	39	119	158
Animal	u									
	v	604	609	959	21.358	90.264	111.622			
6	w									
	x	586	586	952	13.709	77.497	91.206			
Mean		595	598	956	17.534	83.881	101.415	30	145	175
Animal	u	600	599	1161	16.646	74.620	91.266			
	v	608	602	1275	29.161	90.985	120.146			
7	w									
	x									
Mean		604	601	1218	22.904	82.803	105.707	39	143	182
Mean		603	600	923	22.185	78.366	100.551	38	135	173

TABLE 5:6 Oblique Sections Group 3
Epithelial Parameters (µm) (µm²)

		ESL	IL	BML	Area K	Area C	Total area	TK	TC	TT
Animal	u	590	589	694	21.867	84.692	106.559			
	v	602	599	919	17.695	73.970	91.665			
1	w									
	x	612	602	892	43.105	95.148	138.253			
Mean		601	597	835	27.556	84.603	112.159	48	146	193
Animal	u									
	v									
2	w	606	601	1017	37.407	100.603	138.010			
	x	593	595	1204	29.531	69.304	98.835			
Mean		600	598	1111	33.649	84.954	118.423	58	146	204
Animal	u									
	v	588	589	669	18.505	54.926	73.431			
3	w	584	583	785	14.554	64.513	79.067			
	x	588	588	705	12.839	63.865	76.704			
Mean		587	587	720	15.299	61.101	76.400	26	105	132
Animal	u	583	582	779	20.264	65.808	86.072			
	v									
4	w	586	600	1428	34.017	133.071	167.088			
	x	619	614	1003	17.421	95.562	112.983			
Mean		596	599	1070	17.421	95.562	112.983	41	169	210
Animal	u	586	588	710	20.291	61.161	81.452			
	v									
5	w									
	x	591	590	931	15.750	80.901	96.651			
Mean		589	589	931	15.750	80.901	96.651	31	122	154
Animal	u	594	592	618	25.487	62.827	88.314			
	v	588	586	670	25.504	81.227	106.731			
6	w									
	x	587	587	874	13.465	65.667	79.132			
Mean		590	588	721	21.485	69.907	91.392	37	121	158
Mean		594	593	880	23.289	78.290	101.579	40	135	175

TABLE 5:7 Oblique Sections Group 4
Epithelial Parameters (μm) (μm²)

		ESL	IL	BML	Area K	Area C	Total area	TK	TC	TT
Animal	u	583	587	709	17.723	51.479	69.202			
	v	581	583	695	18.668	48.413	67.081			
1	w									
	x	582	583	991	31.424	68.380	99.804			
Mean		582	584	798	22.605	56.091	78.696	39	97	136
Animal	u	589	583	633	14.250	38.245	52.495			
	v	597	594	827	29.687	62.054	91.741			
2	w	583	584	890	22.106	56.112	78.218			
	x									
Mean		590	589	783	22.014	52.137	74.151	38	90	128
Animal	u	584	588	731	27.289	58.365	85.654			
	v									
3	w	584	584	710	23.851	51.216	75.067			
	x	583	585	777	22.468	52.111	74.579			
Mean		584	586	739	24.536	53.897	78.483	42	93	135
Animal	u	587	586	600	17.130	36.745	53.875			
	v	587	587	679	19.712	49.577	69.289			
4	w	586	586	844	31.935	67.423	99.358			
	x	582	586	985	29.440	57.623	87.063			
Mean		586	586	777	24.554	52.842	77.396	42	91	133
Animal	u									
	v	589	589	783	21.574	54.474	76.048			
5	w	582	583	847	23.233	58.745	81.978			
	w	589	589	698	18.807	46.214	65.021			
Mean		587	587	776	21.205	53.144	74.349	37	92	128
Animal	u	592	589	614	19.187	48.650	67.837			
	v	583	585	716	20.499	55.026	75.525			
6	w									
	x	583	582	832	27.261	59.027	86.288			
Mean		586	585	721	22.316	54.234	76.550	38	94	132
Animal	u	586	590	617	19.810	69.483	69.293			
	v	588	589	648	19.217	47.458	66.675			
7	w	591	592	739	21.998	52.845	74.843			
	x	594	590	1144	27.923	71.036	98.959			
Mean		590	590	787	22.237	55.206	77.443	38	95	134
Mean		586	587	769	22.781	53.936	76.717	39	93	132

TABLE 5:8 Oblique Sections Group 5
Epithelial Parameters (µm) (µm²)

	ESL	IL	BML	Area K	Area C	Total area	TK	TC	TT
Group 1	598	598	988	23,562	76,390	99,952	41	132	172
Group 2	600	608	1150	25,563	84,112	109,675	44	145	189
Group 3	603	600	923	22,185	78,366	109,551	38	135	173
Group 4	594	593	880	23,289	78,290	101,579	40	135	175
Group 5	586	587	769	22,781	53,936	76,717	39	93	132

TABLE 5:9 Oblique Sections
Average Values for Epithelial
Parameters (μm) (μm^2)

Fig 5:1 Acrylic appliance extending on to occlusal surfaces of the molar teeth.
(Compare with Fig 3:6).

Fig 5:2 View of palate following removal of appliance which had covered occlusal surfaces of teeth showing reduced quantity of accumulated debris underneath appliance.
(Compare with Fig 3:7).

Fig 5:3 Longitudinal section showing ulceration. disruption of epithelial architecture and hyphal invasion localised to one ruga. H & E x 35

Fig 5:4 Oblique section showing ulceration. disruption of epithelial architecture and hyphal invasion adjacent to molar tooth. H & E x 90

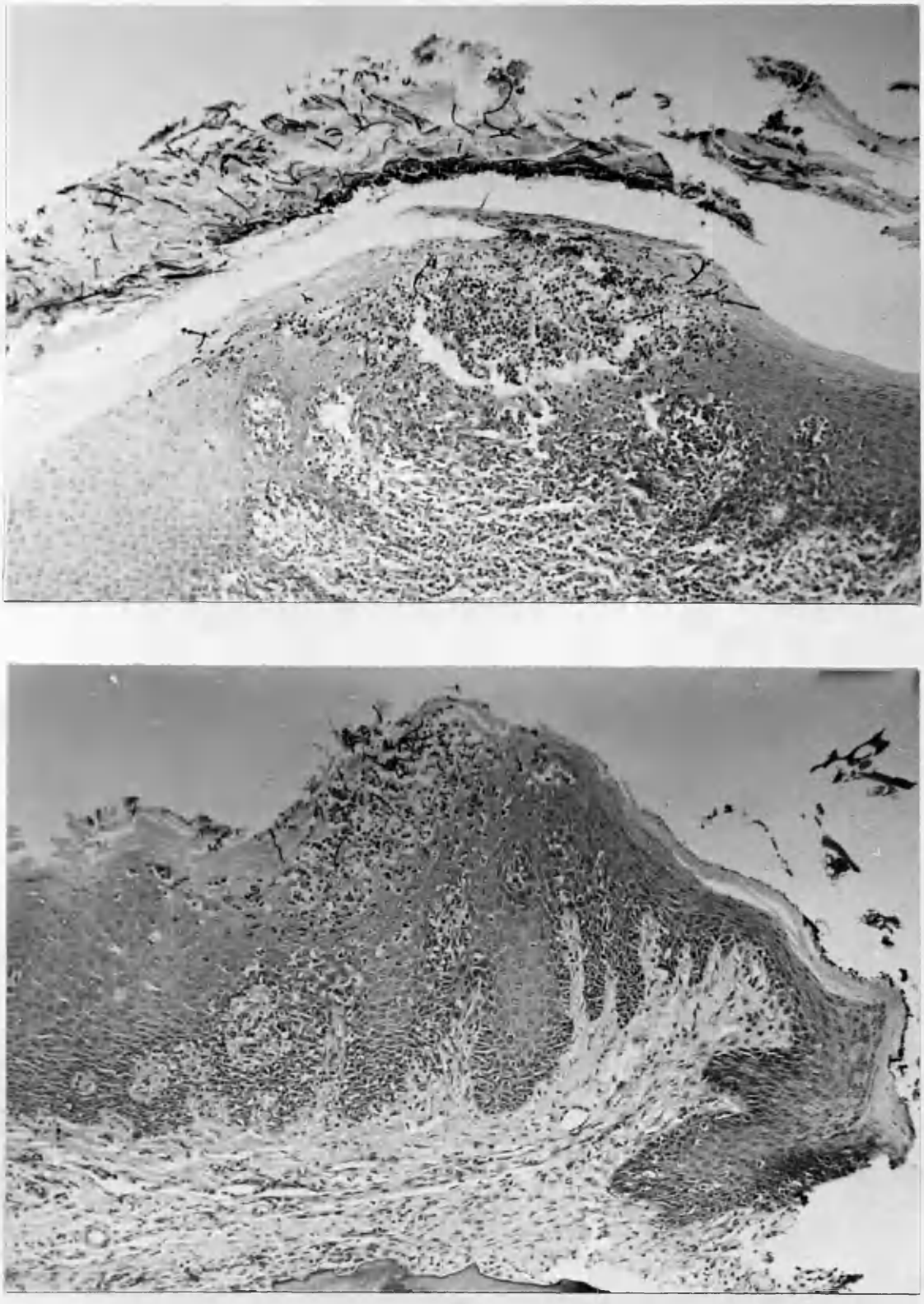


Fig 5:5 Intra-epithelial abscess formation. acanthosis. hyperplasia and chronic sub-epithelial inflammatory response associated with hyphal invasion of tissues.
H & E x 145

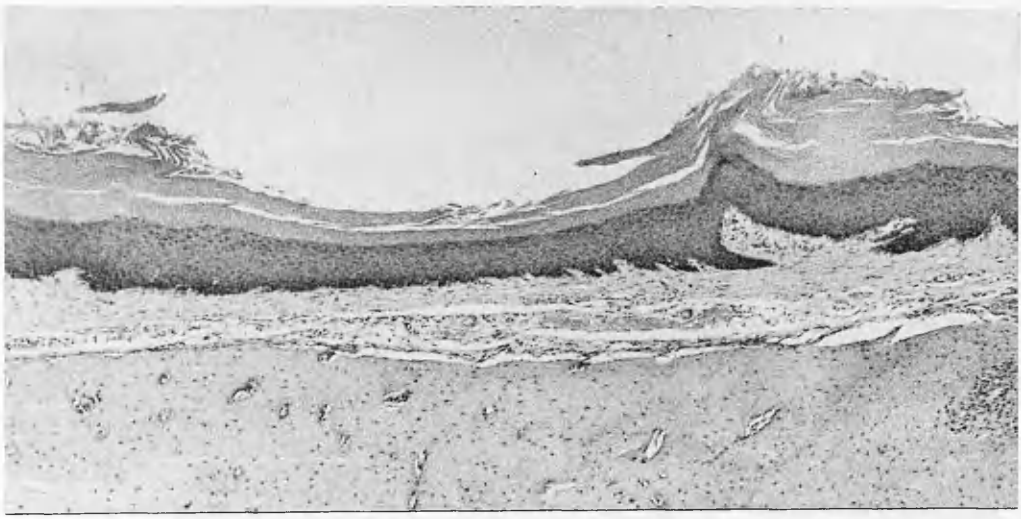
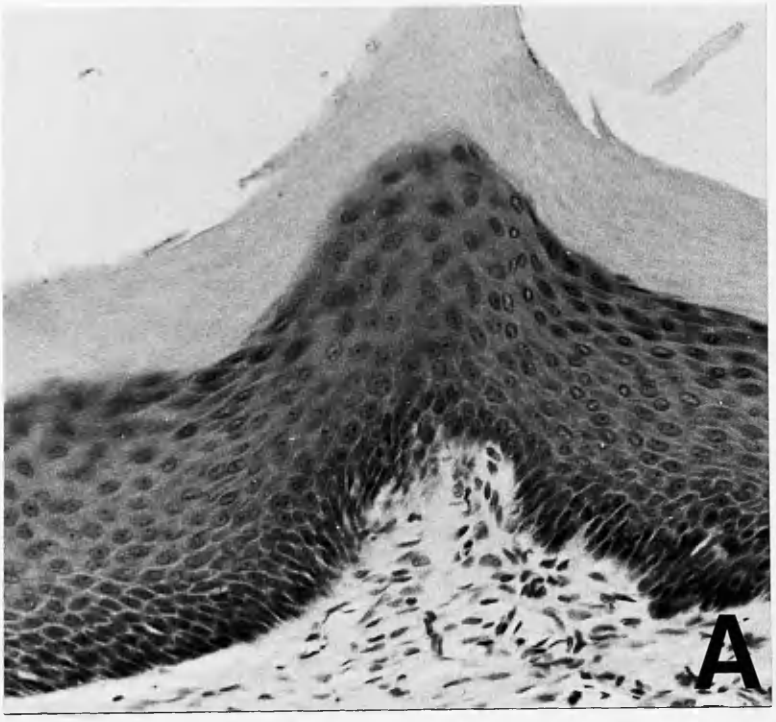


Fig 5:6 Longitudinal section showing compact and detached keratin.
H & E x 55

Fig 5:7 Rugae in longitudinal section showing conformity of epithelial surface form with underlying cellular tissue.
H & E x 60



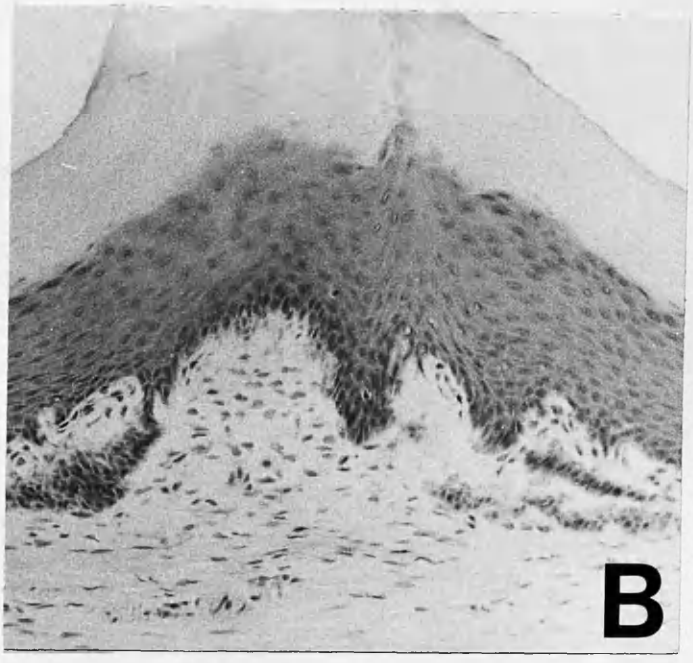


Fig 5:8 In the tissues of the rugae the basement membrane of Experimental Groups 1 and 2 (A) was found to be more regular than in the control group (B).
H & E x 240 (A) x 180 (B)

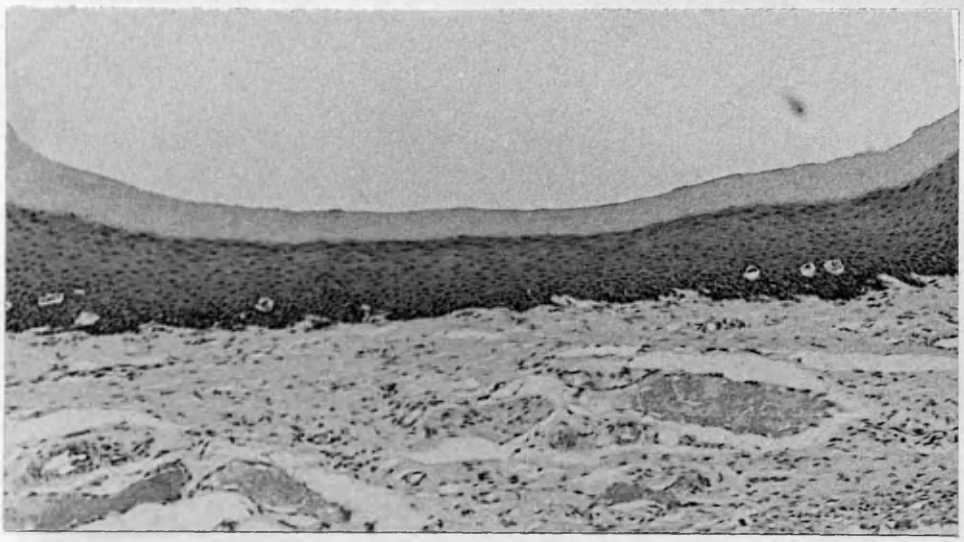


Fig 5:9 Uniform surface layer of keratin found in the area of the col.
H & E x 90

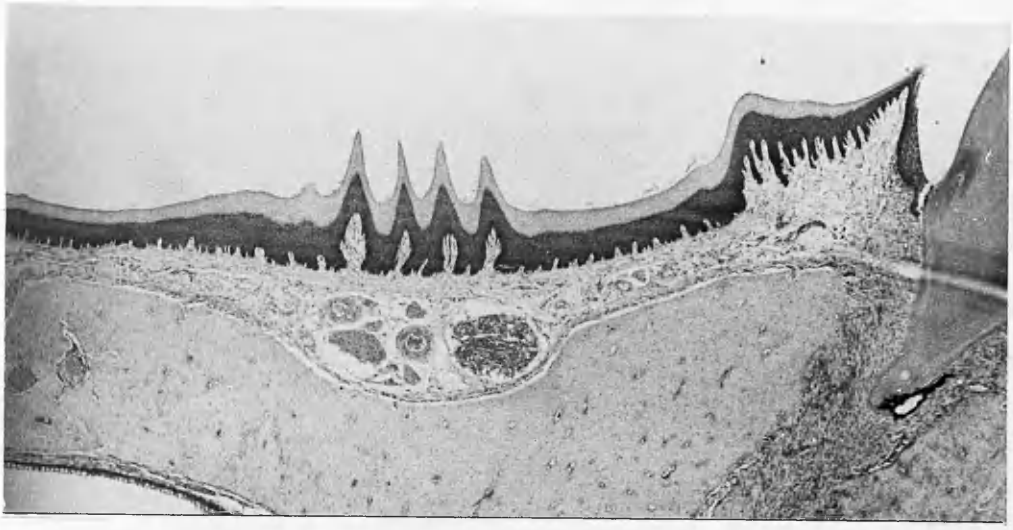


Fig 5:10 Tissue from oblique section unsuitable for analysis because of rugae overlying the palatal blood vessels. H & E x 40

Fig 5:11 Uniform surface keratin layer typical of tissue overlying the palatal blood vessels in oblique sections used in analysis. H & E x 40

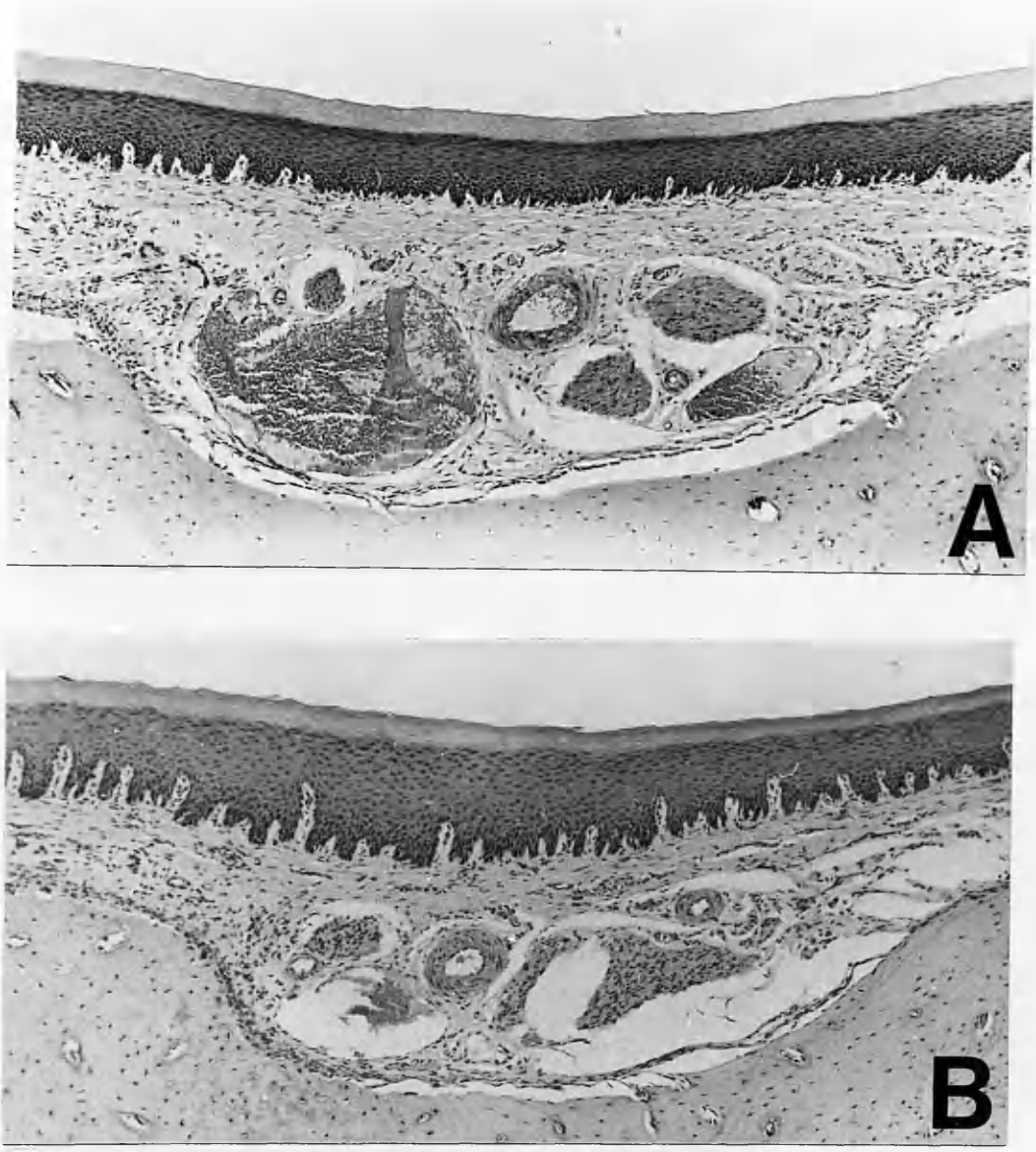


Fig 5:12 Tissue from obliquely cut sections illustrating increased irregularity of the basement membrane and increased thickness of the cellular layer and total epithelium seen in the experimental groups (B) in comparison with the control group (A). H & E x 85

CHAPTER 6

CONCLUDING DISCUSSION

In this research project the preliminary study of inflamed palatal epithelium in patients (Chapter 2) produced results which were, in part, subject to further consideration in animal studies (Chapters 3, 4&5). The animal investigations constituted the major part of the work.

There were several points of interest noted in the study of human subjects. Within the sample of ten patients examined, who presented with palatal inflammation, four were found to have values for haematological parameters outwith the range of normal. Within this group of four patients the number of microbial colonies enumerated from oral imprint cultures was found to be less than in those patients with normal values for the haematological factors examined. It was also found that conspicuous growth of Staphylococcus aureus occurred only in those patients exhibiting a low value for corrected whole blood folate. Although these factors merit further investigation it was the histological features evident within the preliminary study of human subjects which were of primary interest in the subsequent animal investigations of this thesis.

The average epithelial thickness found from investigation of ten patients exhibiting the signs of

denture-induced stomatitis was 373µm. This compared with a value for epithelial thickness of 240µm found by Watson and MacDonald (1982) in a study in which healthy oral mucosa, from the same site, in patients wearing dentures was analysed using similar techniques. However a wide range of values within the group was noted for epithelial thickness within the present study. It was also noted that the degree of irregularity of the basement membrane, measured as the epithelial morphology, showed considerable variation within the group. In view of these findings further investigations were undertaken to examine if the clinical appearance of the palatal epithelium in denture-induced stomatitis was associated with a consistent histological presentation. Work was undertaken to establish an experimental animal model for this purpose.

In the animal investigations the structure of palatal epithelium and changes induced by wearing a prosthesis and inoculation of Candida albicans were described in the Wistar rat. The preliminary animal study (Chapter 3) revealed problems in the selection of tissue for analysis due to irregular surface rugae covering all of the Wistar rat palate. Variations in epithelial structure dependent upon anatomical location had not been highlighted by Olsen and Bondevik (1978) or by Shakir et al. (1981) in their use of this particular

animal model. It was apparent from subjective analysis of material from the preliminary investigation that changes in epithelial structure were induced in experimental animals. Increased epithelial thickness and basement membrane undulation were noted. However the structure of the tissues produced by preparing the tissue blocks in a coronal plane (3.3.7) was such that only subjective assessment of these changes was possible.

It was considered important in this project that changes in epithelial structure induced under experimental conditions be analysed in definitive, quantitative terms. To that end, in Chapter 4, selection of sample sites and preparation of tissue to produce sections suitable for **quantitative** analysis were considered. In all three sample sites analysed, tissue suitable for quantitative analysis was obtained. When each of the sample sites was considered individually there was a consistency of the appearance of the epithelium in all of the animals in this study. It was noted that comparisons between the sample sites did not produce consistent results.

Subsequent to initial investigation of the Wistar rat animal model and examination of suitable methods of tissue preparation for analysis, the aims of the experimental study described in Chapter 5 were to examine if a period of wearing an acrylic appliance produced changes in palatal epithelium, to examine

variables introduced by modification of appliance design and to examine the effects of inoculation of Candida albicans underneath appliances, in the Wistar rat.

Debris was found to accumulate underneath appliances in all instances. The importance of the presence of debris in inducing epithelial change is unknown and this factor has not been highlighted by other workers. The absence of rudimentary hygiene measures may make the application of findings within the animal model to the clinical situation, questionable. The use of an appliance extending onto the occlusal surfaces of the teeth helped to reduce the amount of debris which collected under the appliances. However, modification of design changed the nature of the mechanical support of appliances from the mucosa to the teeth, and complicated the assessment of the role of mechanical trauma in the pathogenesis of the epithelial inflammation. Appliance design was found to be of significance in determining the pattern of tissue changes induced. However, the presence of any acrylic appliance and the location of the sample site appeared to be of greater significance in this respect.

The histological appearance of the palatal epithelium was dependent in particular upon the location of the sample site and changes induced within the epithelium considered overall, were difficult to identify. It is noteworthy, however, that there was a

marked increase in the total thickness of the epithelium in the experimental animals in the sections of col tissue and the oblique sections. This increased thickness was due to an increase in the cellular epithelial layer. Jani and Bhargava (1976) observed that in human subjects the wearing of dentures caused an increase in the thickness of the palatal epithelium.

Changes in basement membrane length induced in the sections from rugae and in the oblique sections were of a conflicting nature. It is likely that the epithelium of the rugae was subject to the greatest mechanical stimulation of the three sample sites, prior to the insertion of appliances. In the animal model, the basement membrane length of the rugal epithelium was seen to show a reduction following the wearing of a mucosa-borne appliance. Van Mens et al. (1975) and Watson and MacDonald (1982) found a more regular basement memrane contour in denture wearing subjects than in non-denture wearing subjects. Further information is required as to which of the three sites sampled provides information most relevant to the features of denture wearing and oral candidiasis in human subjects.

Candida albicans inoculation did not appear to play a role in inducing experimental tissue changes, other than where ulceration of the surface epithelium had occurred. This finding is contrary to the findings of other workers using the Wistar rat animal model

(Olsen and Bondevik 1978, Shakir et al. 1981, Shakir et al. 1983, Lamb and Martin 1983). Of interest is the role of trauma in relation to the persistence of Candida albicans. Further investigation using similar techniques to those used in the present study, modified by constructing all appliances from one master cast, may provide information on the role of trauma in inducing the type of tissue changes reported in other investigations. The virulence of specific strains of Candida albicans in the animal model also requires further clarification. The strain of Candida albicans 3091 (Serotype A) from the National Mycological Reference Laboratory, London, is no longer available. Other reference strains of Candida albicans have not been shown to be virulent in the Wistar rat. It may be the case that experimental candidiasis in the Wistar rat is induced only by a specific strain or strains of Candida albicans.

The continued development of the Wistar rat animal model offers scope for further investigation of the pathogenesis of palatal candidiasis and the effects on the palatal epithelium of wearing a prosthesis. Quantification of the histological appearance of normal palatal tissue and examination of variations dependent upon location carried out in the Wistar rat in this project, give a baseline from which measurement of induced changes can be made.

The use of therapeutic regimes, the role of iron deficiency and factors affecting the virulence of different strains of Candida albicans in candidiasis are areas of great interest in which the use of the animal model could be of value in future studies.

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