ORAL MUCOSAL RESPONSE TO DENTAL PROSTHESES

STEWART CAMPBELL BARCLAY

B.D.S., F.D.S. R.C.P.S. (Glasg.), D.R.D. R.C.S. (Ed.)

THESIS

submitted for

the Degree of Master of Science (Medical Science)

Glasgow Dental Hospital and School

University of Glasgow, September 1993.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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ACKNOWLEDGEMENTS

My thanks are due to a number of people who have helped in the preparation of this work. I am especially grateful to Professor D. G. MacDonald and Dr I. B. Watson for their advice, encouragement and patience. My thanks are due also to Dr. A.B. Lamb for his comments on parts of the literature review, and to Mr K.J. Jennings for advice on the experimental techniques used.

I gratefully acknowledge the expert technical assistance of Mr. M. T. Broad in construction of the experimental appliances. I am grateful to Mr. W. Marshall and staff of the Oral Pathology Unit, especially Mrs. J. Hope and Mrs P. Mackintosh, for their considerable help in the preparation of the tissue specimens.

I owe particular thanks to the staff of the research facilities at the Western Infirmary and Yorkhill Hospital, Glasgow, especially Mr. F. Darling and Mr D. Duggan, for their assistance in management of the research animals.

My thanks are also due to the staff of the Department of Medical Illustration for their help in preparation of some of the illustrations.

Finally, I owe special thanks to my wife Barbara for the understanding and tolerance she has shown during the many hours I have spent on this project.

PREFACE

The work presented in this thesis was undertaken in the Oral Pathology Unit and Department of Prosthodontics of Glasgow Dental Hospital and School. It was carried out between January 1990 and January 1992.

The techniques used were modifications of previously published work, with some being techniques originally developed by the Oral Pathology Unit. The application of the techniques described was carried out personally by the author. Preparation of appliances and histological sections was carried out by technical staff of the Department of Prosthodontics and Oral Pathology Unit under the direct supervision of the author and his supervisors.

Animals for the first part of the project were maintained at the Western Infirmary and for the second part at Yorkhill Hospital, Glasgow.

Part of the work has been presented at the annual conference of the European Prosthodontic Association in September 1992 in a presentation entitled "Quantitative analysis of rat palatal mucosa covered by acrylic prostheses" and at the British Society for the Study of Prosthetic Dentistry in April 1993 in a presentation entitled "Quantitative analysis of palatal mucosa covered by acrylic prostheses in an animal model".

SUMMARY

Oral mucosa is not optimally adapted to provide a foundation for prostheses and the provision of dentures requires it to function in a modified environment.

Previous studies have used a rat model for examination of the histopathology of oral candidiasis or mucosal coverage with dental prostheses. Some studies have been complicated by the presence of accumulated food debris between mucosa and prostheses.

The present study was undertaken to develop further the Wistar rat as a suitable animal model on which to study the effects of dentures and dental materials on oral mucosa, and was divided into two parts. The first part examined the effects of prostheses on oral mucosa while modifying the animals' diet. The second part examined the effects of materials used to modify prostheses.

The first part of the study compared the effects of three dietary regimes upon debris accumulation and measurable epithelial parameters. Individual heat-cured acrylic appliances were constructed. Within each dietary group, animals were allocated to a control group, or one of two test groups, wearing an appliance for periods of either 7 or 14 days. Tissue was analysed using computerised planimetry.

Results showed that a powdered diet used in a paste form gave the least accumulation of debris in appliance wearers. This dietary group exhibited the least differences between appliance wearers and controls. Unlike the other two groups, they showed no difference in the thickness of adherent keratin between the test and control animals.

The second part of the study used the Wistar rat fed on the powdered diet in a paste form to analyse the effects of a resilient soft

lining material and a hard "chairside" relining material upon the epithelium. The effect of relining in the experimental groups examined was to increase the thickness of keratin, while not increasing the total epithelial thickness.

The use of the Wistar rat model utilising the paste diet is indicated for future investigation of the effect of prostheses on oral mucosa.

ABBREVIATIONS

LSU	Length of surface of epithelium
LBM	Length of basement membrane
LBM/LSU	Epithelial morphology - the ratio of basement membrane length to epithelial surface length
TKC	Thickness of keratin compartment
TCC	Thickness of cellular compartment
TET	Total epithelial thickness
TCC/TET	Compartment ratio - the ratio of cellular compartment to total epithelial thickness

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

The present study was undertaken to develop further the use of the Wistar rat as a suitable animal model on which to study the effects of dentures and dental materials on the oral mucosa, and was divided into two parts. The first part examined the effects of prostheses on oral mucosa while modifying the animals' diet, while the second part examined the effects of materials used to modify prostheses.

Previous studies have used the Wistar rat as a model for examination of the histopathology of oral candidiasis (Jennings and MacDonald, 1990a) or mucosal coverage with dental prostheses (Jennings and MacDonald, 1990b). Some studies have been complicated by the presence of accumulated food debris between mucosa and prostheses (Francis and Farthing, 1989). This accumulation is suggested to vary in extent with the prosthesis design (Jennings, 1988). It is possible that this debris might have affected the results obtained from these studies.

In order to understand the effect of dental prostheses on oral mucosa, a review of mucosal structure and function is essential. The following is a summary of current knowledge and existing research on oral mucosa and the effects of prostheses on the oral tissues.

1.2 ORAL MUCOSA - DEVELOPMENT, STRUCTURE AND FUNCTION

Mucous membrane is the moist surface membrane of all internal tubes which communicate with the exterior, such as intestinal and respiratory tracts (Borysenko & Beringer, 1989). The mucous membrane which lines the oral cavity is known as the oral mucosa. Sited between skin and intestinal mucosa, it has some of the properties of each.

1.2.1 Development

Oral mucosa, as a result of the development of the oral cavity from both the embryonic stomatodeum and the foregut, is derived from both ectoderm and endoderm. The structures which are derivatives of the branchial arches including the tongue, epiglottis and pharynx, are covered by epithelium of endodermal origin, while the epithelium covering gingivae, cheeks and palate derives from ectoderm (Squier & Hill, 1989).

1.2.2 Structure

Oral mucosa comprises two separate tissue components: a covering epithelium and an underlying supporting connective tissue.

Oral epithelium is a stratified squamous epithelium. The interface between it and the connective tissue of the lamina propria or corium is usually irregular with interdigitating connective tissue papillae and epithelial rete ridges or pegs. This interface may be seen at the light microscope level as a basement membrane which is approximately 1-4μm thick. At the level of the electron microscope, the

interface is known as the basal lamina, the structure of which is discussed in greater detail in Section 1.4.

The basement membrane allows easy recognition of the interface between oral epithelium and connective tissue, but the junction between the lamina propria and the deeper submucosal layers is less readily identified. Unlike intestinal mucosa, no intervening layer of muscularis mucosae exists. In the cheeks, lips and some areas of the palate, a layer of loose fatty or glandular connective tissue, which contains blood vessels and nerves, is seen to separate the mucosa from the underlying bone or muscle. This represents the submucosa as it exists in the oral cavity. In other areas, the mucosa is directly attached to the periosteum of the underlying bone and is therefore known as mucoperiosteum.

Minor salivary glands lie in or just beneath the lamina propria. Other tissues which may be present in some areas include sebaceous glands and lymphoid tissues. The distribution of these tissues varies with the structural type of oral mucosa.

Oral mucosa exhibits regional variation such that three main types of oral mucosa exist in different areas of the mouth. They are masticatory, lining and specialised mucosae. The different types of mucosa fulfil different functional requirements and vary in their clinical and histological appearance.

Masticatory mucosa in dentate subjects includes the gingival mucosa and hard palate and in edentulous cases it also covers the alveolar processes. It is usually a thick orthokeratinised, stratified, squamous epithelium, but parakeratinised areas can also be present. To withstand functional stresses, there are rete ridges and long papillae at the interface between the epithelium and the lamina propria. The

lamina propria is formed by thick dense collagenous tissue, especially under the rugae. The submucosa of this area is dense collagenous connective tissue with a mucoperiosteal connection to bone.

Lining mucosa overlies the cheeks, vestibule, soft palate and floor of mouth and is normally non-keratinised. It is usually thicker than keratinised epithelium with a different rete ridge pattern at the interface with the connective tissue, the epithelial ridges being broader. It has a thicker lamina propria which contains fewer collagen fibres and more elastic fibres. It is more distensible than masticatory mucosa.

Specialised mucosa constitutes the covering of the dorsum of the tongue. Although functionally a masticatory mucosa, it is more extensible and has specialised papillae, some of which have a mechanical function while others have a sensory function.

1.2.3 Function

Oral mucosa has a variety of functions, including protection and provision of sensory information.

The oral epithelium provides protection for the underlying connective tissue from trauma of both a mechanical and a chemical nature. Oral mucosa must be able to withstand the forces of mastication which include compression, stretching, shearing and surface abrasion. It must also protect deeper structures from the potentially toxic effects of ingested materials and from the oral flora and its by-products.

Oral mucosal sensory receptors include those for temperature, touch, pain and taste. These in turn may initiate a variety of reflexes of a protective or functional nature, such as gagging, swallowing and salivation.

The moist environment of the oral mucosa is provided by the salivary glands. These are the minor glands, lying within or just deep to the mucosa, or the major glands with ducts passing through it.

1.3 HISTOLOGY OF ORAL MUCOSA - ORAL EPITHELIUM

Oral epithelium is a stratified squamous epithelium which may or may not be keratinised. It develops by a process involving cell division, differentiation, maturation, migration and desquamation.

1.3.1 Keratinisation

The process of keratinisation involves the synthesis of the fibrous protein keratin as the cells migrate through different layers to the surface (Davis, 1986). Two patterns of keratinisation are seen at a histological level. The superficial epithelial layer, the stratum corneum, may be orthokeratinised, the cells being filled only with packed fibrillar material and having no nucleus. By contrast, in parakeratinisation, shrunken pyknotic nuclei persist in many or all of the flattened cells.

1.3.2 Cell layers in keratinised epithelium

As epithelial cells or keratinocytes mature and differentiate, they lie within layers or strata, known as the stratum basale, the stratum spinosum, the stratum granulosum and the stratum corneum.

The stratum basale or basal layer is a layer of cuboidal or columnar cells which lies adjacent to the basement membrane. The stratum spinosum consists of several rows of larger elliptical or spherical cells. It is named after the "prickle" effect created by the maintenance of cell contact by desmosomes which appear as

intercellular bridges following histological processing. The desmosomes and networks of tonofilaments within the cells appear to work as a tensile supporting system for the epithelium with a greater density of desmosomes in cells of masticatory or lingual mucosa than in lining mucosa (Hill, 1984).

The stratum granulosum is a layer of larger flattened cells containing many small haematoxylin-staining granules. The layer is pronounced in orthokeratosis and scant or absent in parakeratosis. The granules are keratohyaline granules and are found in association with tonofibrils. This layer is a transitional zone between viable keratinocytes below and the anuclear cells above. Accordingly, the nuclei show signs of degeneration and pyknosis.

The stratum corneum in orthokeratinised epithelium is a layer of flattened dehydrated cells where all organelles and nuclei have been lost, the cells becoming composed of tightly packed filaments coated with the protein filaggrin. In parakeratosis, the cells show less evidence of this loss of organelles. Layers of intercellular lipid material, extruded from the cells as they mature, contribute to the low water permeability of the epithelium (Squier, 1987).

1.3.3 Cell layers in non-keratinised epithelium

The changes which take place in the upper cell layers in non-keratinised epithelium are less striking than in keratinised epithelium. The gradual change in cellular appearance in non-keratinised epithelium has resulted in the outer half of the tissue being divided into two zones, the stratum intermedium and the stratum superficiale. There is no stratum granulosum. The cells of the stratum intermedium are slightly increased in size with the occasional appearance of

keratohyline granules. The stratum superficiale shows few other changes, although the cells may be slightly more flattened and have fewer organelles. The surface layer of non-keratinised epithelium thus consists of cells that have loosely arranged filaments and are not dehydrated. They form a surface which is flexible and able to tolerate compression and distension (Squier & Hill, 1989).

1.3.4 Epithelial maturation

The maturation of the oral epithelium can be considered by looking at it in terms of its component parts. The epithelium can be divided into layers, identified by certain characteristics of the cells which comprise each layer, as described in the preceding sections, or into functional compartments (Squier & Hill, 1989). In order to maintain the structural integrity of the epithelium, there is continuous cell renewal. This is achieved by three functional compartments, a deeply placed progenitor population of cells, a maturing compartment and a superficial compartment.

The progenitor population of cells lies in the basal layer of thin epithelium as in the floor of the mouth (Squier & Hill, 1989), or in the deepest two to three layers in thicker epithelium like the palate or cheek.

There are two distinct sub-populations of progenitor cells. The first is a slowly-cycling group, which forms the stem cells and produces the remaining basal cells, while retaining the potential for proliferation. Stem cells appear to remain at a basal level for at least 72 days compared with the remaining cells which leave the basal layer within three days (Wright & Alison, 1984).

The second group of proliferating cells is a larger amplifying group, which increases the number of cells available for later maturation, and forms the maturing group by undergoing a series of divisions and subsequently migrating to the surface.

Control of epithelial maturation requires chemical modulation. Chalones, glycoproteins produced by the cells themselves, have in the past been suggested as such mediators, acting by negative feedback. However, epidermal growth factor, a polypeptide molecule, has been suggested as a more important stimulator of epithelial growth (Wright & Alison, 1984). It has also been suggested that a mild sub-epithelial inflammatory infiltrate might stimulate mitosis, while severe inflammation may reduce cell proliferation (Squier & Hill, 1989).

1.3.5 Non-keratinocytes

Within the layers of the epithelium, a number of other cell types of a different origin are seen. Under the light microscope, they are found to have a clear area around the nucleus, hence being known as "clear cells". They include melanocytes, Langerhans cells, Merkel cells and lymphocytes. They can amount to 10 per cent of the total cell population. With the exception of Merkel cells, they lack desmosomal attachment to adjacent cells.

Melanocytes are derived from neural crest ectoderm and lie within the stratum basale. They are responsible for the endogenous pigmentation of epithelium. They have no desmosomal connection to adjacent cells, but have dendritic processes which can pass between individual cells and through several layers of cells. Pigment forms within these cells as melanosomes and can be inoculated into adjacent cells via the dendritic processes. Within keratinocytes, aggregations of

melanosomes are called melanin granules and are responsible for the variation in pigmentation of different tissues. In the oral mucosa melanocyte activity is usually minimal resulting in less pigmentation than skin despite a similar density of melanocytes.

Langerhans cells are derived from bone marrow and may be capable of limited division within the epithelium. They lie within the suprabasal layers of the epithelium and have no desmosomal or tonofilament elements (Roitt et al, 1989). They contain a characteristic small rod or flask-shaped granule known as the "Birbeck granule" and express a variety of surface markers. Although not readily identifiable in conventional sections, they can be demonstrated by ATP-ase and by OKT6 antibody probes.

Functionally, Langerhans cells are antigen-presenting cells involved in antigen processing in cell-mediated immune reactions. On being stimulated they can migrate via the afferent lymphatics into the paracortex of adjacent lymph nodes to present antigens to T-lymphocytes.

The origin of Merkel cells is uncertain, although they are thought to migrate from the neural crest (Hill, 1984). They are situated in the basal layer of epithelium and are non-dendritic clear cells. They have some desmosomal tonofilament complexes and contain small membrane-bound dense core granules within the cytoplasm which may be responsible for the release of a transmitter substance into an associated nerve axon. Merkel cells are thought to function as touch receptors.

Other lymphocytes of the oral mucosa are part of the mucosa associated lymphoid tissue (MALT) population (Roitt, 1989). They form aggregates of non-encapsulated lymphoid tissue, especially in the

lamina propria and submucosal areas, although smaller numbers of lymphocytes can be seen infiltrating the epithelium.

1.4 HISTOLOGY OF ORAL MUCOSA - BASEMENT MEMBRANE / BASAL LAMINA

The interface between epithelium and its underlying connective tissue is undulating with connective tissue ridges or papillae projecting into the epithelium. This allows an increased area of attachment to disperse the forces applied to the epithelium during function. Masticatory mucosa has a greater number of papillae per unit area than lining mucosa, the papillae also being longer.

The basement membrane, as seen under the light microscope, is found under the electron microscope to be a bilaminar structure known as the basal lamina. The deeper layer is the lamina densa composed of finely granular and filamentous material approximately 50nm thick. It is composed mainly of type IV collagen. The lamina lucida is a glycoprotein-rich layer approximately 45nm wide which lies between the lamina densa and the epithelium.

The attachment of the epithelium to the lamina propria is by hemidesmosomal attachment of basal cells to the basal lamina and the presence of anchoring fibrils inserted from the connective tissue to the lamina densa, interlocking with the underlying collagen. The basal lamina is a product of the epithelium.

1.5 HISTOLOGY OF ORAL MUCOSA - LAMINA PROPRIA

The lamina propria consists of two main subdivisions, a superficial "papillary layer" which is associated with the epithelial rete ridges and a deeper "reticular layer" which is formed by a network of collagen fibres. It contains a number of different cell types, fibres embedded in amorphous ground substance, vessels and nerves.

1.5.1 Fibroblasts

Fibroblasts are fusiform or stellate cells with processes which lie parallel to the collagen fibre bundles which they produce. The nucleus often contains one or more prominent nucleoli. As active synthetic cells, they contain a number of organelles including mitochondria, the Golgi complex and granular endoplasmic reticulum.

Fibroblasts both produce and maintain the fibres and ground substance in which they are enmeshed. The cells also exhibit motility and contractility, these functions being important in determining the structural organisation of the connective tissue, particularly during development and healing (Ten Cate, 1989).

1.5.2 Macrophages

Macrophages are also stellate or fusiform cells which, unless actively phagocytosing can be difficult to distinguish from fibroblasts although macrophages have smaller and less densely staining nuclei. The presence of obvious and numerous lysosomes can be noted using electron microscopy. In addition to being phagocytic defence cells, they are involved in antigen processing, perhaps increasing the antigenicity before presentation to lymphoid cells.

1.5.3 Mast cells

Mast cells are spherical mononuclear cells with relatively small nuclei. The cytoplasm contains large numbers of metachromatic granules, the contents of which include histamine and heparin. Leukotrienes, prostaglandins and chemotactic factors are also products of mast cells. Mast cells act as mediators of the inflammatory and immune responses, releasing factors which affect blood vessels and other cells.

1.5.4 Collagen

Collagen is found in many tissues and is a family of structural proteins, comprising a number of chemically distinct types. It exists within the oral mucosa as two types - type I is present in the lamina propria and type IV in the basal lamina. Type I collagen is synthesised by fibroblasts, forming procollagen triple-helix protein macromolecules, which aggregate to form collagen fibrils by cross-linking. The collagen fibrils then form small bundles or collagen fibres. The variation in the different types of collagen molecules results from the order of assembly of the basic polypeptide units. Type IV collagen is derived from the epithelium.

Collagen acts as the supporting meshwork for the connective tissue.

1.5.5 Elastic fibres

Elastic fibres contain elastin and a glycoprotein. They form aggregates of microfibrils which, as they mature, contain approximately 90 percent elastin. They are associated with blood vessels in the lamina

propria, most commonly in flexible lining mucosa. The fibres stretch easily, but are not as strong as collagen. They require specific staining, such as resorcin-fuschin, to be visualised (Borysenko & Beringer, 1989).

1.5.6 Ground substance

The ground substance comprises tissue fluid and a variety of proteoglycans and glycoproteins, which include hyaluronic acid, dermatan sulphate, heparin sulphate and chondroitin sulphate. It acts as a supporting element of the connective tissue and functions as a molecular sieve, facilitating the diffusion of metabolites between blood and tissues and acting as a barrier to larger particles.

1.6 BLOOD SUPPLY

Oral mucosa has a rich arterial blood supply, the vessels lying in the submucosa where that is present, or in the deepest reticular layer of mucoperiosteum. A dense capillary plexus lies within the papillary layer of the lamina propria, capillary loops extending up towards the basement membrane. The greater blood flow in oral mucosa as compared with skin may allow more rapid development of inflammation and more rapid healing in response to injury (Squier & Hill, 1989).

1.7 NERVE SUPPLY

Sensory innervation of oral mucosa is derived mainly from the fifth cranial nerve, the Trigeminal, although the Abducens, Glossopharyngeal and Vagus nerves are also involved. Sensation in the

oral cavity includes touch, pain, taste and temperature and the nerve endings may be free or organised.

Free nerve endings may be present in the lamina propria or extend into the epithelium, where they can be associated with Merkel cells.

Organised nerve endings lie superficially within the lamina propria and consist of a connective tissue capsule surrounding clusters of coiled nerve fibres.

1.8 AGE CHANGES

It is suggested that the structure of oral mucosa changes significantly with age. However, much of the literature currently available suffers from poor control data from younger age groups or from the fact that parallels are drawn with ageing skin (Scott & Baum, 1990). It is reported that, with age, oral epithelium becomes thinner and less resilient (Mack, 1989), cell and nuclear size and shape alter, and the epithelium/connective tissue interface shows evidence of flattening (Scott & Baum, 1990). Epithelial metabolism may decrease, but the effects on mitosis and epithelial turnover are uncertain.

Within the connective tissue, there are physical and chemical changes in the collagen, principally increased stability of collagen molecule cross linkages, which reduce its extensibility (Ten Cate, 1989). The ratio of ground substance to collagen decreases, leading to increased fibre density. This is accompanied by a change from thick irregular fibre bundles to regular compact bundles.

1.9 ORAL DISEASE AND DENTAL PROSTHESES

1.9.1 Incidence of edentulousness

The most recent Adult Dental Health Survey (Todd & Lader, 1991) has revealed that, although the incidence of edentulousness has dropped since the last Adult Dental Health Survey in 1978, it still occurs in 21 percent of those over 16 years of age in the United Kingdom. The same report also indicates a significant correlation with age, with over 80 percent of individuals over 75 years being edentulous.

1.9.2 Denture-induced mucosal trauma

It has been suggested by some authors that coverage of mucosa by dentures can induce either a proliferative (Turck, 1965; Razek & Shaaban, 1978) or a degenerative response in the oral mucosa (Nedelman et al, 1970).

In a survey of complete denture wearers, Dorey et al (1985) found that 60 percent of patients exhibited some form of oral lesion attributable to prosthesis wear. This study examined 200 edentulous cases referred to an oral mucosal disease clinic and therefore considered a highly selected group. The types of denture-related mucosal problems identified included the effects of chronic trauma such as stomatitis, with or without candidal infection, papillary hyperplasia, non-dysplastic hyperkeratosis (production of excess keratin as a response to mild chronic trauma from ill-fitting dentures), ulceration and denture-induced fibrous hyperplasia.

In another study, Sheppard et al (1971) showed that 46.1 percent of a sample group of 3569 cases had oral lesions. This group was selected from out-patients or short stay in-patients at a District

General Hospital and can be deemed to be more representative of the population as a whole. Within this group, over 40 percent showed inflammatory changes, 30 percent showed hypertrophic or papillated lesions and just over 11 percent showed evidence of ulceration. Over 54 percent of patients who routinely wore maxillary dentures wore them both day and night. The study suggested that denture wearers were more likely to experience problems if they wore their dentures both day and night, if excessive or adverse forces were placed upon them or if denture hygiene was poor.

Oral mucosa may be affected by dentures in a number of ways including mechanical or chemical trauma or microbial infection associated with denture coverage.

1.9.3 Mechanical trauma

Intermittent compression and shearing forces during masticatory function may result in tissue changes (Kydd & Daly, 1982). This may occur with a well-fitting denture, but is more likely to occur with a poorly-fitting prosthesis as suggested by Nyquist (1952). Poor fit appears to be a very significant factor in relation to the severity of inflammation. Other factors are also relevant, including poor denture hygiene, increased periods of tissue coverage during both day and night, and an increased inflammatory response to mechanical trauma in younger subjects (Love et al, 1967).

1.9.4 Chemical trauma

As discussed in Section 1.11, modern denture base materials, although essentially inert in their polymerised form, comprise a number of potentially irritant constituent materials. They may also

contain other chemicals as products of the processing cycle such as formaldehyde. There is some uncertainty as to whether tissue reaction to some of these materials is a result of simple chemical irritation or a contact allergy (a type IV hypersensitivity reaction as a result of re-exposure to an allergen).

Methyl methacrylate monomer is irritant and significant amounts may remain in a processed denture, particularly when it is improperly processed or stored (Austin & Basker, 1982). Similarly, benzoyl peroxide initiator often remains in significant quantities in dentures, unless they are cured for considerably longer periods of time than are normally used (Devlin & Watts, 1984). Its role as a true allergen or simple irritant is uncertain (Poole et al, 1970). In temporary or long-term soft lining materials, plasticisers like aromatic esters and alcohols are used but gradually leach out (Gonzalez, 1977) and may potentially be irritant to oral mucosa. Formaldehyde can be a decomposition product of polymethyl methacrylate processing (Ruyter, 1980) and is allergenic.

1.9.5 Microbial infection

The presence of dentures overlying the oral mucosa may result in inflammation of that mucosa, commonly known as denture-induced stomatitis. Poor denture hygiene increases the incidence of inflammation in the mucosa (Love et al, 1967). A major component of denture related debris is microbial plaque (Budtz-Jorgensen, 1981).

Candida albicans is a normal commensal fungal organism in about half the human population and is often implicated as a causative organism in cases of denture-induced stomatitis (Budtz-Jorgensen, 1981). The organism is capable of adhering to the fitting surface of a

prosthesis (Davenport, 1970). Thus, poor denture hygiene resulting in infrequent removal of the organism and extended periods of denture wear, keeping debris in close proximity to the mucosa, can contribute to its effect on the tissues (Jennings and MacDonald, 1990a). Similarly, ill-fitting prostheses will induce mechanical trauma which can further predispose to fungal infection. The altered environment beneath the denture created by these factors could allow the transformation of *Candida albicans* from the commensal yeast or blastospore form to a more pathogenic or mycelial form (Shakir et al, 1986a), although there is not universal agreement about the relative virulence of the different forms (Shepherd, 1990). Actual epithelial penetration by fungal hyphae is infrequently reported (Cawson, 1966). A possible effect of *Candida albicans* on the permeability of epithelium has been reported by Martin et al (1987), who suggested that candidiasis results in an increase in permeability.

Denture bases tend to harbour organisms (Budtz-Jorgensen, 1981), with temporary soft lining or tissue conditioning materials also doing so (Razek and Mohamed, 1980; Wright et al, 1985). However, disagreement exists as to the effect of tissue conditioners on fungal growth. Some, such as Coe-comfort (Coe Laboratories), support the in-vitro growth of *Candida albicans* as reported by Graham et al (1991c) while other authors (Douglas & Walker, 1973) have reported that the same material inhibits growth for limited periods because of fungicidal agents contained within it. Wright (1980) in a study of seventeen soft lining materials, which included a number of tissue conditioners, reported no inhibition of candidal growth in vitro by tissue conditioners. He attributed inhibition of fungal growth seen with four of the materials to the effect of certain constituents.

Thus, although candidal infection is known to be important in denture-induced stomatitis, a number of factors appear to be involved in the production of the mucosal inflammation.

1.9.6 Mucosal changes arising from prosthesis wear

Oral mucosa is not optimally adapted to provide a foundation for prostheses and the provision of dentures requires it to function in a modified environment. Mucosal changes may occur even if forces applied to the tissues are within their physiological limits of tolerance.

Previous studies in both humans and animals have analysed the effect of prostheses on oral mucosa with regard to both qualitative and quantitative tissue changes. These include mucosal thickness or the relative thickness of epithelium and connective tissue components (Jani & Bhargava, 1976; van Mens et al, 1975; Nedelman et al, 1970, Watson & MacDonald, 1982, 1983), the type and degree of keratinisation (Turck, 1965; Sharma & Mirza, 1986), changes in maturation patterns (Shakir et al, 1986b), ultrastructural changes in epithelial cells (Akbay & Akbay, 1988), histochemical differences (Razek & Shaaban, 1978; Sharma & Mirza, 1986) and the presence and status of immunologically competent cells within both connective tissue and epithelium (Francis & Farthing, 1989).

1.9.7 Quantitative morphological changes arising from prosthesis wear

A wide variation in results has been reported. Measurement of epithelial thickness, following a period of denture wear, has been suggested as a method of indicating an effect of the prosthesis on the mucosa. Jani & Bhargava (1976) examined thirty edentulous males

between the ages of 35 and 70 years with no previous denture wearing experience. They took biopsies of the hard palate in the first molar region. The biopsies were taken prior to and three months after insertion of complete dentures. Their results showed that 70 percent of the patients exhibited an increase in total epithelial thickness after three months of prosthesis wear with the mean increase being 13 percent. Two thirds of the patients showed an increase in the thickness of the keratinised compartment alone, while one quarter showed a decrease and the remainder showed no change. The mean difference in keratin thickness was an increase of 17.6 percent. Akbay & Akbay (1988), while concurring with the study of Jani and Bhargava in reporting an increase in epithelial thickness, did not provide evidence for this in their paper.

In contrast, van Mens et al (1975), looking at biopsies from a similar area of the palate, could find no difference in epithelial thickness between denture wearers and non-denture wearers. The accuracy of their findings must be questioned as their technique involved tracing photographs of the histological sections, cutting the tracings out and weighing the pieces of paper. The tracings also did not include the stratum corneum, which was deemed unsuitable for accurate measurements. The same authors noted that the basement membrane in denture wearers was less irregular, with shorter rete pegs, than non-denture wearers. Watson and MacDonald (1982) reported no significant difference in mean epithelial thickness between patients with and without denture wearing experience.

Ostlund (1958), in a mainly descriptive rather than quantitative study, reported an increase in epithelial thickness in denture wearers where inflammation was evident, although the overall

mucosal thickness did not appear to increase. The samples were obtained by removal of a cylindrical piece of tissue from the posterior part of the hard palate, and it was acknowledged by the author that the method of separation of the tissue from the periosteum may have resulted in an under-estimation of the total mucosal thickness. He also suggested that changes occur within the epithelium over a period of denture coverage. This was described as a gradual decrease in the stratum corneum with thinning of the stratum granulosum. A tendency towards parakeratosis and evidence of acanthosis were also noted. Krajicek et al (1984) in an examination of autopsy specimens from the edentulous mandible, felt unable to draw definite conclusions about the differences between denture wearers and non-denture wearers because of the difficulty in accounting for the variables between the two groups. They were unable to find any statistically significant differences in epithelial parameters between the two groups.

1.9.8 Qualitative morphological changes arising from prosthesis wear

The type and degree of keratinisation seen beneath dentures also appears to vary widely. Orthokeratinisation present beneath a denture has been found in a number of studies, including that of Kapur & Shklar (1963) who reported mainly hyperorthokeratinisation with only occasional areas of parakeratinisation. Abbas et al (1987) concurred with these results, while Razek & Shaaban (1978) recorded similar findings, but only in the first year after insertion of the denture. Others, including Pendleton (1951) and Ostlund (1958) report parakeratinisation resulting from denture wear. Van Scotter & Boucher (1965) reported that the type of keratinisation differed under different

denture base materials, with parakeratosis predominating in mucosa covered by vulcanite dentures and similar amounts of ortho- and parakeratinisation with an acrylic resin prosthesis. Nedelman et al (1970) suggested that a greater degree of keratinisation is seen in edentulous tissues without denture coverage than those with coverage.

1.9.9 Factors modifying morphological changes arising from prosthesis wear

Other variables may explain the apparently conflicting views described in the previous sections. These include the regional variations in mucosal morphology seen within one subject as shown by Pendleton (1940) and Watson & MacDonald (1982, 1983). Other factors are time in respect of the length of the denture-wearing period (Abbas et al, 1987; van Scotter & Boucher, 1965) and also the age of the subject (Watson & MacDonald, 1982).

Some authors suggest that the mucosa can alter with time under the same prosthesis. Ostlund (1958) suggested that the thickness of both the stratum corneum and the stratum granulosum decreased for the first six months of denture wear, while van Scotter & Boucher (1965) suggested that there was no correlation between the period of denture wear and thickness of the stratum corneum. Razek & Shaaban (1978) found that, over three years of denture wear, an initial period of hyperkeratinisation was followed by a gradual adaptation of the mucosa to the prosthesis with a reduction in keratinisation. As reported by Watson & MacDonald (1983), regional variations occur in the quantity of the mucosal elements in the edentulous subject, with for example, an increased thickness of epithelium at the ridge crest and midline.

The connective tissue also shows qualitative changes with denture wear, including an increased density of fibres (Pendleton, 1951). He suggested that the connective tissue provides a cushioning effect and the resultant fibre bundle form and orientation are related to mechanical effects.

Some authors have related epithelial changes in edentulous ridges to underlying inflammation, signified by the presence of an inflammatory cell infiltrate in the superficial connective tissue. Nedelman et al (1970) noted that the presence of a well-fitting prosthesis reduced evidence of inflammation in the underlying tissues and this was associated with decreased keratinisation. Epithelial thickness was reported by Ostlund (1958) to increase in the presence of inflammation. Love et al (1967) reported increased inflammation under dentures in younger subjects and also in association with other factors such as poor denture base adaptation, poor denture hygiene and decreased periods of tissue rest.

The presence of a prosthesis is reported to affect the development and maturation of epithelial tissues. Ostlund (1958) and van Mens et al (1975) reported an increase in mitotic activity in denture wearers of up to three times, possibly contributing to an increased rate of turnover of the epithelium. Other authors suggest that there is an increase (Turck, 1965) or decrease (Shakir et al, 1986b) in mitotic activity depending upon the level of trauma experienced by the mucosa.

1.9.10 Histochemical changes resulting from prosthesis wear

In addition to basic morphological investigations, other factors have been investigated and have included histochemical analysis. A variety of enzymes has been examined to elucidate the effects upon

epithelial metabolism of denture wear. Nedelman et al (1970) looked at enzymes such as acid phosphatase which appear to be associated with keratinisation. Their study showed this enzyme to decrease in the epithelium of denture wearers as compared to non-denture wearers. Alkaline phosphatase levels within the connective tissues, which can be related to chronic inflammation, have also been shown by the same authors to decrease under dentures. The reduction of this enzyme with time in denture wearers was confirmed by the work of Razek and Shaaban (1978).

1.10 EFFECTS ON MUCOSA OF MODIFICATION OF DENTURE BASES

Oral prostheses may from time to time require modification by addition of new material to the fitting surface.

The nature of the material and the reason for its addition varies depending upon the clinical situation. The method by which it is added may also vary in that it may be added directly to the denture at the chairside and allowed to set at least partially in the oral cavity, or indirectly in the laboratory via an intermediate impression stage.

Whereas the indirect technique equates in many respects to the provision of a new prosthesis, the use of directly placed denture lining materials differs in that placement of these materials in direct contact with oral mucosa during part of the curing cycle, and immediately after, may have an additional effect on this soft tissue.

1.11 DENTURE BASE RESINS

The most commonly used material for denture construction is the acrylic polymer polymethylmethacrylate. The material is formed from polymerisation of the methacrylate monomer.

The usual presentation of the material is as a powder and liquid. The powder comprises beads of the polymer, an organic peroxide initiator and other elements for control of the material's appearance, including titanium dioxide, inorganic pigments and often dyed synthetic fibres for colour and aesthetics. The liquid contains the methyl methacrylate monomer, a hydroquinone inhibitor to extend shelf life and occasionally cross-linking agents. The liquid for autopolymerising resins also contains an organic amine accelerator to allow room temperature curing.

There are a number of processing techniques for acrylic denture bases. These vary depending on whether the material is polymerised at room temperature or heat-cured.

The most commonly used procedure for heat-cured materials is the dough moulding method. The material is mixed in powder to liquid ratios as specified by the manufacturer, the ratio usually being approximately 3:1. Deviation from these recommendations can result in undesirable polymerisation shrinkage and a rise in temperature which could cause gaseous porosity. The material is then allowed to stand in a sealed container where it passes through a sandy to a sticky to a dough-like consistency, at which point it is ready for packing.

The denture mould is made by investing the waxed-up denture in a split metal denture flask with a gypsum product and boiling off the wax. After placement of a suitable separator to prevent interaction of

the plaster and the acrylic, an excess of dough is placed in the flask. The flask is closed for one or more "trial closures" with a thin plastic sheet to act as a spacer, and then reopened to remove any excess or flash. It is then closed again under pressure and placed in a polymerisation bath, which is temperature controlled. A number of polymerisation protocols exist but it is usually recommended that periods of eight hours or longer, at temperatures below the boiling point of water are used. This helps to ensure thorough polymerisation of the monomer and minimises its vaporisation which can lead to gaseous porosity.

Autopolymerising resins may be used in a similar way to the heat-cured types, and polymerisation may be enhanced by placement of the mould in hot water in a pressure pot during the curing phase. They have the benefit of curing much more rapidly than the heat-cured materials. They may however exhibit greater porosity or the presence of greater amounts of unpolymerised monomer, particularly if incorrectly handled.

1.12 TYPES OF DENTURE LINING MATERIALS AND REASONS FOR THEIR USE

As described in Section 1.10, dentures may be lined either directly at the chairside, or indirectly in the laboratory. One group of directly applied materials used for a specific purpose is known as tissue conditioners. Other relining materials can be classified as directly applied soft or hard materials or indirectly applied soft or hard materials.

1.12.1 Tissue Conditioners

Tissue conditioners are materials which are applied temporarily to the fitting surface of an existing denture and are so called because of their main use in the treatment or "conditioning" of abused denture bearing tissues. These materials are soft on placement and for variable periods thereafter, acting as a cushion to minimise trauma to the underlying mucosa.

Tissue conditioners are perhaps best described as tissue "reconditioners" (McCarthy & Moser, 1984) in that their resilient properties can be utilised to cushion previously abused tissues to allow recovery. The materials are effective because they flow readily over the entire denture-bearing area, reaching a degree of set or gelation over a period of hours. They allow close adaptation to the tissues, provide stability and more equally distribute functional loading (Kawano et al, 1991; Gonzalez, 1977). Ideally the material should remain sufficiently plastic to continue to adapt to changing soft tissue morphology as it returns to health. The use of ethyl alcohol and aromatic esters as plasticisers of the polymer resin (usually polyethyl methacrylate) results in a slowly setting material (Graham et al, 1991a). The use of such materials is advocated for hyperaemic or traumatised oral mucosa, this being associated with ill-fitting dentures or denture-induced mucosal hyperplasia (Gonzalez, 1977).

These materials should only be used over a short duration because in the oral environment the plasticising molecules leach out and the materials become hard and brittle, thus rendering them ineffective. They also absorb water, resulting in dimensional changes and distortion. The resilience of tissue conditioners appears to be clinically useful for up to fourteen days before an excessive amount of

plasticiser has been lost, although the compliance of each material varies with its chemical constituents (Graham et al, 1990).

Previous studies on the use of tissue conditioners have concentrated on the physical characteristics of the materials in relation to their clinical effectiveness. Such an approach has probably resulted from a desire to correct or control some of the inherent flaws in the materials. These flaws have existed since the earliest use of synthetic resins as tissue conditioners about fifty years ago (Qudah et al, 1990). Previous research has been directed towards examining and comparing a variety of commercially available products for the extent of leaching of plasticiser (Graham et al, 1991b), gelation and flow properties (Graham et al, 1991a), compliance (Graham et al, 1990) and pressure distribution (Kawano et al, 1991).

The ability of tissue conditioners to affect bacterial and fungal growth has been examined (Razek & Mohammed, 1980; Graham et al, 1991c; Wright et al, 1985). The latter two studies support the theory that tissue conditioners, probably as a result of difficulties in keeping the material clean, tend to accumulate greater quantities of organisms than normal denture base materials, particularly if used for any length of time.

Biocompatibility testing of some tissue conditioners has been carried out (Passeri & de Carvalho, 1985; Harsanyi et al, 1991), but has looked at the effect of the materials when implanted in bone and epithelial wounds and soft tissue pouches, rather than in circumstances which more closely mimic their clinical use. The work of Passeri and de Carvalho (1985) compared four tissue conditioners against a zinc-oxide eugenol impression paste in bone and soft tissue wounds and although the tissue conditioners were found to be less irritant than the paste,

they exhibited varying potential for inducing inflammation. The study by Harsanyi et al (1991) examined the effect of enclosing discs of dental soft polymers containing specific plasticisers in hamster cheek pouches for periods of between fourteen and thirty-five days. They suggested that these materials induced potentially pre-malignant changes in the epithelium.

1.12.2 Directly-applied soft relining materials

Directly-applied or "chairside" soft relining materials are mainly used as short or medium term measures to reline an ill-fitting denture and impart some resilience to reduce trauma to easily damaged denture-bearing mucosa.

An example of such a material, Coe-soft (Coe Laboratories), is described by its manufacturers as being indicated where soft tissue overlying bone is thin, unyielding, sensitive or easily irritated, or where the patient is unable to tolerate a denture with a rigid base.

The materials are intended for longer term use than tissue conditioners, but otherwise closely resemble them in constituents and handling. The ideal directly-placed soft lining should exhibit minimal solubility in saliva. The material should retain its resilience which depends mainly on the thickness and chemical composition of the material. The ideal thickness should be between 1 and 3mm (Gonzalez, 1977; Qudah et al, 1990). Any constituent plasticiser should not leach out. However most chairside soft lining materials leach plasticiser and therefore have a limited working life, requiring to be replaced at regular intervals. The material should be compatible with and preferably bond to denture base resin to prevent disruption during function. As with tissue conditioners, there should be little or no water

absorption to prevent distortion and dimensional change. Porosity as a result of polymerisation should be minimal to reduce accumulation of debris and plaque on the surface. However, in practical terms, because of the method of placement, porosity is almost unavoidable. The patient should be able to clean the material without damage. It should be non-toxic, tasteless and odourless.

1.12.3 Directly-applied hard relining materials

Chairside hard relining materials are marketed as a convenient permanent method of relining or modifying the periphery of an ill-fitting prosthesis in the surgery. An example of this group, Kooliner (Coe Laboratories), is described by its manufacturers as a hard, chairside denture reliner that does not exhibit the exothermic heat present in other chairside acrylic reliners. They suggest that it can be completely cured in the patient's mouth in 10 minutes and that "in-mouth" curing minimises potential distortion and ensures the patient a better-fitting denture. Other examples of hard relining materials for direct application are Flexacryl-hard (Lang Dental Manufacturing Co.), Total - hard (Stratford-Cookson Co.) and Coe-rect (Coe Laboratories).

Such materials are usually chemically-cured derivatives of the most commonly used acrylic polymer polymethyl methacrylate modified for direct use in the mouth. They are suggested by their manufacturers to produce less exothermic reaction and incorporate more biocompatible constituent materials. However, Bunch et al (1987) cited evidence for the likelihood of chemical burns on the underlying mucosa and significant porosity of the relined surface which may have an effect on tissue compatibility.

Despite these findings and other anecdotal reports, there is little evidence in the literature of studies looking at the effect on mucosa of such materials. Some studies (Bunch et al, 1987; Wyatt et al, 1986) have been directed towards investigating the physical characteristics of these materials, including temperature and consistency changes during the curing phase, colour stability, bond strength to denture base resins and surface texture of the cured material.

The studies by Bunch et al (1987) and Wyatt et al (1986) suggest a number of factors which could be important in the tissue response to these materials. Seven different materials were examined between the two studies with all showing a detectable rise in temperature during curing. This was measured in both intra- and extra-oral tests with peak temperatures ranging from 40^{o}C to as much as 70^{o}C depending on the material. The period of time during which the temperature remained at this peak also varied with the type and bulk of material present. Some materials showed only a gradual decline, suggesting a more prolonged period in contact with mucosa at a potentially irritant if not damaging temperature.

It was also suggested by Wyatt et al (1986) that a layer of surface porosity present on some materials results in a greater accumulation of microbial plaque than materials with no surface irregularities.

1.12.4 Indirectly-applied soft lining materials

Laboratory processed soft lining materials have been available for some time. They can be incorporated at the time of fabrication of a new prosthesis, where a resilient surface is required, or added by an

indirect impression technique. As they are required to function over a much longer period, the materials should remain soft for the lifetime of the prosthesis. Like temporary soft lining materials, they require to be elastic to provide a cushioning effect, should adhere to the denture base and be readily cleaned without significant deterioration. The materials can be broadly grouped into four categories, either autopolymerising or heat-curing modified acrylic material, or autopolymerising or heat-curing silicone rubber.

The autopolymerising modified acrylics are usually regarded as temporary soft lining materials. They become hard within a few weeks and cannot be considered as permanent reliners as they require frequent replacement (McCabe, 1990).

The heat-cured acrylics, such as Palasiv 62 (Panadent), are normally applied to a new denture at the time of production. They rely for their softness on the use of a higher methacrylate and a plasticiser.

The heat-cured silicones, such as Molloplast B (Austenal) or Polyliner 40 (Orthomax), are usually applied at the time of production of the new prosthesis and comprise a polydimethylsiloxane polymer paste, which cross-links when heated. Autopolymerising silicone materials such as Flexibase (Flexico Developments Ltd.) form by a condensation reaction and cure at room temperature.

Although they are capable of remaining soft for longer than the chairside-applied materials, permanent soft liners do deteriorate with time, and it is essential to regularly review patients for whom they are provided (McCabe, 1990).

1.12.5 Indirectly-applied hard relining materials

A permanent hard relining of a denture can also be provided by an indirect rebase technique. This is a widely used standard technique whereby, after modification of any undercuts or inaccuracies in base extension, a wash impression in a material of low viscosity, such as light bodied polyvinylsiloxane or zinc oxide eugenol impression paste, is taken in the existing denture base and sent to the laboratory. There the impression material is replaced by heat-cured polymethylmethacrylate.

1.13 ANIMAL MODELS IN PROSTHODONTIC RESEARCH

Animal models have been used for the investigation of the histopathology of disorders associated with the wearing of prostheses. Extensive work has been carried out on oral candidosis in relation to prostheses with both monkey and rat models having been used.

Monkeys have only been used to a limited extent (Budtz-Jorgensen, 1971; Grossman and Forbes, 1990) probably because of cost and difficulty of management whereas rats have been much more widely used. As previously discussed in Section 1.9, experiments have involved quantitative analysis of tissue changes with palatal coverage of mucosa (Jennings & MacDonald, 1990a), the effects of *Candida albicans* on rat palate mucosa, either by simple oral inoculation without a prosthesis (Jones & Adams, 1970), or by use of acrylic prostheses for variable periods with and without inoculation with the organism (Olsen & Bondevik, 1978; Shakir et al, 1981, 1986a,b; Martin, 1989; Jennings & MacDonald, 1992).

The studies examining candidal infection of palatal mucosa show that the presence of the *Candida albicans* organism in addition to palatal coverage with a prosthesis is required to cause a denture-induced stomatitis in a rat model. The studies using palatal appliances in the rat have included quantitative analyses of changes in epithelium, connective tissue and keratin thickness, suggesting that the presence of an appliance causes palatal epithelial hyperplasia (Jennings & MacDonald, 1992). Other studies have considered the effect of palatal prostheses on mitotic activity (Shakir et al, 1986b) and on Langerhans cells (Francis & Farthing, 1989). The latter authors examined changes in the number of Langerhans cells as a result of coverage of palatal mucosa by acrylic appliances. They suggested that the increased number of cells present after seven days of mucosal coverage in a rat model was in response to alteration in the nature or number of antigens arising from microbial growth and food retention penetrating the mucosa. The antigenic effect of the particular appliance material used in these studies, Peripheral Seal (Dentsply), and its constituents does not appear to have been considered.

Despite their obvious advantages, animal studies can be criticised for a number of reasons. Changes seen in the tissues of one species may not correlate with changes seen in the human subject, particularly as the gross morphology of the tissues being examined in the animal model differs from the human. The effect of diet and possible differences in mastication in terms of damage to, or stimulation of, the tissues under investigation may also vary. In most animal models, it is not practicable for appliances to be removed for cleaning on a daily basis, as most human denture wearers do. The effect on mucosa may therefore differ as a result of the increased periods of tissue coverage by

the prostheses. Similarly, the practicalities of appliance design mean that such prostheses will not necessarily equate in function with mucosa-borne dentures. In an animal model it is necessary to provide rigid fixation of the appliance to teeth, thus more closely resembling a tooth-borne partial prosthesis. The limited inherent flexibility of denture base resins may overcome this difficulty to some extent allowing masticatory loads to be applied to mucosa as well as teeth. However, designs which allow such movement of the appliance will also tend to allow excessive amounts of food debris to accumulate between the appliance and the mucosa. This difficulty was acknowledged by Jennings and MacDonald (1990a,b; 1992). The tendency for food debris to accumulate beneath appliances constructed for rats has been recognised by some authors, but not by others, despite essentially similar appliances having been used. Shakir et al (1981) noted the existence of diffuse erythematous areas of palatal mucosa with impaction of food. Martin et al (1987) and Martin (1989) made no mention of food debris, while Francis and Farthing (1989) regarded the food debris as a contributing factor to an alteration in the nature and number of antigens which led to their finding of increased numbers of Langerhans cells.

Despite these disadvantages, animal models provide a readily accessible source of material for analysis, with the further advantage that many of the variables which can affect human studies can be controlled or eliminated.

1.14 THE WISTAR RAT MODEL

The albino Wistar rat has been used widely as a model for examination of the histopathology of the palate. Although the jaws,

teeth and mucosa differ in a number of respects from humans, examination of the gross and microscopic anatomy of the rat palatal mucosa shows evidence for the suitability of the animal for such investigations (Kutuzov & Sicher, 1952; Gibbins, 1962).

1.14.1 Rat palate anatomy

An example of the palate of the Wistar rat is shown in Figure 1.1. The rat palate may be divided into four regions. The most anterior is the roof of the oral atrium, which is defined as the area between the incisors and the incisive papilla. Just distal to the roof of the oral atrium is the antemolar region. This is identified by the presence of three straight transversely-aligned rugae. A fairly thin layer of epithelium overlies the rugae, the core of which consists of a loosely arranged network of fine collagen fibres with a high proportion of elastic fibres.

The intermolar region lies, as its name suggests, between the molar teeth and is characterised by five low ridges. The anterior three are "M" shaped, while the other two show less curvature, forming a simple arc which is convex anteriorly.

Histologically, the mucosa nearer the molar teeth resembles that of the antemolar region, while the remainder is effectively a mucoperiosteum with an underlying layer of dense connective tissue firmly adherent to the bone of the hard palate. The connective tissue consists of thick bundles of collagen fibres.

The most distal area is the post rugal field which lies between the rugae and the terminal ridge which forms the boundary between the hard and soft palates.

The areas of tissue in the cols between rugal crests in the intermolar region exhibit a mucosal morphology similar to many parts of the human palate. Examination of these sites following a period of coverage by a suitably designed appliance provides an indication of the effects one might expect to see on human tissue.

1.15 OBJECTIVES OF PRESENT STUDY

The intention of the first part of the study was to evaluate different dietary regimes to attempt to minimise formation of debris beneath denture-like appliances. This is described in Chapter 2.

Subsequent to this, analysis of the histology of the palatal mucosa under the prostheses was carried out. This is also reported in Chapter 2.

The model utilising the dietary regime shown to be the most appropriate by the first part of the study was then used to compare the effects of different types of denture base materials on oral mucosa. The findings are described in Chapter 3.

A discussion of the findings of the thesis in relation to previous research is given in Chapter 4.

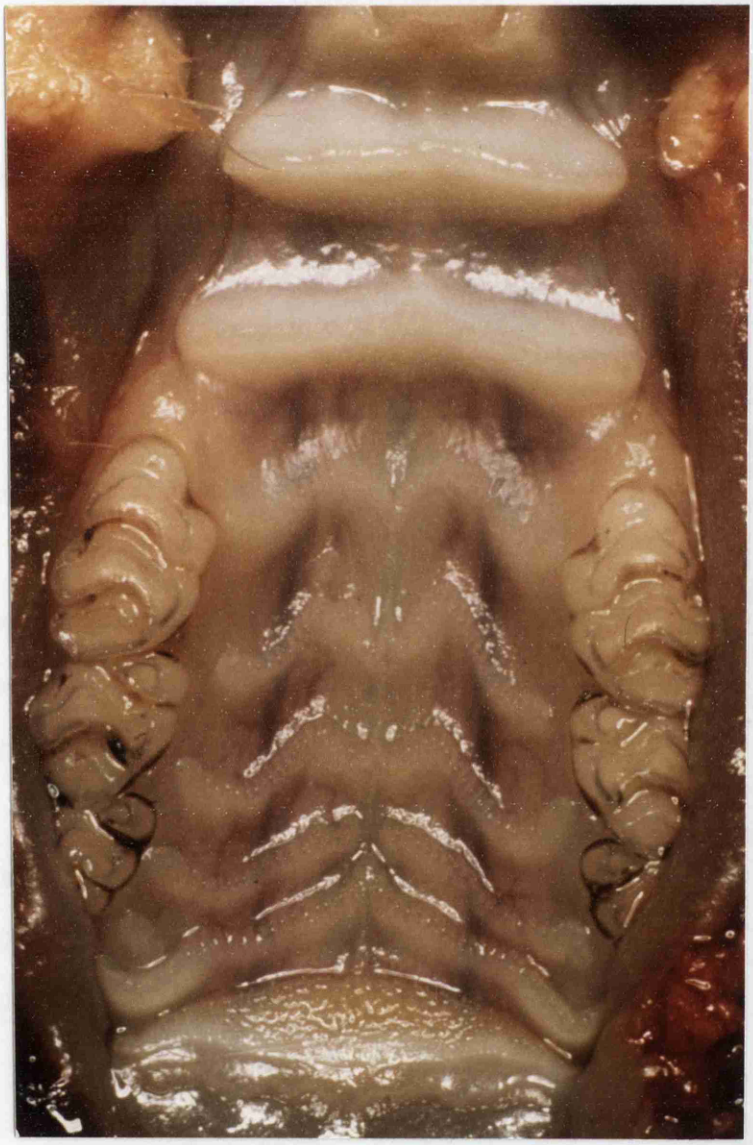

FIGURE 1.1 Rat palate

CHAPTER 2

DIETARY COMPARISON STUDY

2.1 INTRODUCTION

This study was carried out to examine the effect of different dietary regimes on the response of oral mucosa to palatal prostheses in a rat animal model. It also served to familiarise the author with the techniques involved in handling the experimental animals and processing the tissue samples obtained.

2.2 AIMS

Having recognised the potential problems with the rat animal model (Section 1.13), it was the aim of the study reported in this chapter to examine the effects of variation in diet, particularly with regard to the effect of appliance wear on the tissues under different dietary regimes. The amount of food debris accumulating between the appliances and the denture-bearing mucosa was also examined.

An additional aim was to determine which diet was most appropriate for this type of study. The animal model could then be used to examine the effect of materials employed to modify prostheses in tissue conditioning or relining procedures.

2.3 MATERIALS AND METHODS

2.3.1 Animals

Forty-eight adult male Wistar rats of an approximate start weight of 300g were used for this study. They were weighed regularly prior to the experiment to establish that the main growth phase had ceased and that they were fully mature. They were maintained in separate cages and randomly allocated to one of three experimental groups, each on a different diet.

Each dietary group in the experiment was further divided into three subgroups of five, with one subgroup wearing a test appliance for one week, the other test subgroup doing so for two weeks and a control non-appliance-wearing subgroup. One other animal was allocated to each of the three dietary groups to allow replacement of any animals which died before insertion of the appliances.

2.3.2 Diet

Animals were fed on one of three diets. The first group was given a standard rodent pellet diet (Special Diet Services; Witham, Essex). The other two groups received the same basic diet, this being a nutritionally complete powdered diet (Special Diet Services; Witham, Essex). The only difference in the diet fed to the latter two groups was the dilution of the base powder resulting in a different consistency. The second group was fed on 20g per day of powdered solid diet mixed with enough tepid water to form a thick paste. This was renewed daily. The third group was fed on a liquid diet, the powder being dissolved in water at a ratio of 20 grams to 80ml of water. This was renewed daily. All three groups were also given water ad libitum.

2.3.3 Anaesthesia

In order to take the master impressions and to fit the appliances, it was necessary to anaesthetise each test group animal twice. This was achieved by a 0.25ml intraperitoneal injection of Hypnorm (Jansen Pharmaceuticals Ltd, Oxford, UK) which gave a satisfactory working time of up to thirty minutes.

2.3.4 Appliance design and construction

Special trays (Drufoplast, Panadent) were constructed on a stone cast poured from a preliminary impression of a Wistar rat palate from a previous experiment (Jennings, 1988). Master impressions of each animal were taken in polysulphide rubber (Permlastic; Kerr) and the impressions obtained were highly detailed and individual (Fig. 2.1). The master casts upon which the custom-made palatal coverage prostheses were constructed were poured in improved dental stone (Kaffir D). The appliance design was as shown in Figure 2.2, having full palatal and molar tooth coverage. As suggested by the work of Jennings and MacDonald (1992), it was decided that in order to reduce food debris accumulation, coverage of molar teeth was preferable. A retentive element encompassing the upper incisor teeth was constructed from a band of 4mm wide stainless steel. A 15mm loop of 0.5mm diameter stainless steel wire was then soldered to this using low fusing solder. A wax pattern was laid down on the master cast to cover all of the palate and the molar teeth, with the lateral border being finished at the buccal margins of the occlusal surfaces of these teeth. The wire loop acted as retention for the acrylic. The appliances were processed in clear heat cure acrylic (Trevalon C, Dentsply), and stored

in tap water for at least 24 hours prior to insertion, to remove any residual free monomer.

2.3.5 Appliance insertion

Following anaesthesia with intraperitoneal Hypnorm, the appliances were secured in place by notching the distolingual aspect of each upper incisor with a carbon-steel fissure bur in a dental handpiece and cementing the appliance with autopolymerising acrylic (Palapress, Kulzer). Care was taken to seat the appliance accurately in place over the palate and molar teeth while setting of the acrylic lute took place.

2.3.6 Experimental protocol

Within each dietary group of sixteen animals, five were control animals, ten were experimental appliance-wearing animals and one animal was maintained as a potential substitute for any animals which died before fitting of the appliance. The experimental animals were in two groups of five, wearing appliances for either seven or fourteen days.

Animals were commenced on the appropriate diet on day 0 of the experiment. Impressions were taken on day 7 and the appliances fitted at day 14. The one week test animals were then sacrificed on day 21 while the control and two week test animals were sacrificed on day 28.

2.3.7 Weight record

The weights of the animals were recorded at intervals throughout the experimental period. Weights of both the test and the control groups were specifically recorded just prior to a number of procedures during the experiment. These included the impression

taking stage, the stage at which the acrylic palatal prostheses were inserted for the test groups, the day of sacrifice of animals wearing appliances for one week and the day of sacrifice of control animals and animals wearing appliances for two weeks. The weights should have been recorded immediately prior to the animals commencing on the experimental diets. However, due to an oversight the weights of the liquid group were not recorded at this stage. No values for this stage are therefore included, although it is acknowledged that a record of these weights would have been of benefit to confirm that all groups began the experiment at similar weights.

It was expected that control animals would continue to gain weight because although the animals were beyond their main growth phase, weight gain is expected throughout life if nutrition is adequate.

2.3.8 Animals withdrawn from experiment

The animal designated as the replacement in the pellet diet group failed to recover from the administration of the anaesthetic for impression taking. One animal from the one week experimental paste diet group failed to recover from the administration of the anaesthetic for fitting of the appliance. It was replaced by the extra animal in this dietary group.

Three animals were noted to have lost their appliances during the course of the experiment. They included one animal from the one week pellet diet group, one from the one week paste diet group and one from the two week liquid diet group. The animals in the pellet diet and paste diet groups were not substituted because the replacement animals in these dietary groups had already been allocated, while the animal in the liquid diet group was replaced with the extra animal.

One other animal was found to have died earlier on the day on which it was due to have been sacrificed. It was in the liquid diet test group to be sacrificed at one week and was noted to have suffered a substantial weight loss, being 235g at the time of death compared to 313.3g at the impression stage. It was not replaced.

All the animals which died prematurely or lost appliances were excluded from further analysis. It was therefore possible to obtain tissue for analysis from four animals in the one week appliance wear groups on each of the three diets and five animals from each of the two week test and control groups on each diet, giving a total of forty-two animals for histological analysis.

2.3.9 Animal sacrifice and specimen preparation

Animals were sacrificed by CO_2 asphyxiation and fracture of the cervical spinal cord. Each animal was decapitated and the heads were fixed in 10 per cent buffered formalin for a minimum of three days.

Following removal of the mandible, the maxilla was trimmed free from the cranium by sectioning through the nasal cavity, parallel to the palate, with a water-cooled saw. The appliance was then removed from the palate by sectioning the teeth with Mauns' cutters while stabilising the appliance against the tissues. It was then lifted vertically away from the tissues, taking care to minimise disturbance of any accumulated debris. In only a small proportion of the samples was there much debris adherent to the appliance.

The palatal tissue block was demineralised in 20 per cent formic acid and further blocked in a manner similar to that developed by Jennings (1988). This resulted (Fig. 2.3) in one longitudinal block (block A) taken to the left of the midline of the palate and three oblique blocks aligned with the rugae on the right of the palate (blocks B, C and D). The anterior cut for each of the latter three blocks was made on the upward slope of the more anterior ruga. The blocks were processed and paraffin embedded for sections to be cut from the anterior face of each block. Multiple sections were cut back at different levels through the block. This was carried out to identify the area at the deepest part of the trough between the rugae (Fig. 2.4) and

usually required three levels to be cut although in some cases up to six levels were required.

The longitudinal block was cut from the medial block face to give 5μm thick sections at right angles to the epithelial surface overlying the neurovascular bundle. The oblique blocks were cut to give 5μm sections from the anterior surface of each level. Blocks were sectioned with the palatal mucosa meeting the knife first because this orientation resulted in the least epithelial distortion. Sections were stained using haematoxylin and eosin.

2.3.10 Histological examination

In order to maintain consistency in measurement, all sections were examined to find the most appropriate one from each block. This was identified, as described in Section 2.3.9, as the tissue section which lay at the deepest point of the trough between the rugae and which exhibited the thinnest most uniform epithelium (Fig. 2.5). The fields for measurement were identified overlying the palatal neurovascular bundle, to allow reproducibility of specimens within and between animals. Three columns of tissue per section were identified and measured, the first directly overlying the neurovascular bundle and the other two immediately adjacent to this as shown in Figure 2.6.

In some of the sections, it was not possible to identify the most appropriate site for measurement because of poor sampling or blocking of the tissue. The main reason for this difficulty was that in the tissue block obtained, rugae overlay the blood vessel field. This, as noted by Jennings (1988), resulted in very variable thicknesses of epithelium, often with significant amounts of keratin and no well-defined split of

adherent and desquamated keratin (Section 2.3.12), preventing accurate measurement.

Where rugae lay in close proximity to only one of the three fields measured in each section, it was usually possible to take measurements from an adjacent field where the epithelial thickness remained fairly uniform. If this were not possible, the blocks were removed from the paraffin wax and remounted to have serial sections taken from the opposite face of the block. In most instances, this allowed identification of the appropriate field for analysis, while in others it was still impossible to identify suitable sections. One series of specimens required to be excluded as a result of such problems. These blocks were therefore deemed unmeasurable, and the animal was excluded from further analysis. This applied only to blocks B and D from animal 2, which had been in the one week pellet diet group.

Two sections on each slide from each block were analysed and the measurements averaged as a method of improving the consistency of the means. Data was expressed per animal, so that the values calculated were in most cases means of eighteen measurements - three fields in each of two adjacent sections in each of the three palatal blocks B, C and D for each animal.

2.3.11 Quantitative analysis of tissue - computerised planimetry

Tissue analysis was carried out using computerised planimetry. This allowed quantitative analysis of structural parameters of tissue by tracing using a bit pad with a light cursor and a microscope equipped with a drawing tube attachment (Fig. 2.7). Tracing of lengths or areas of tissue was carried out while viewing the microscope field on

to which was projected the movement of the light spot in the tracing probe. The bit pad was linked to a computer which printed out accurate numerical values for the lengths or areas traced, and also a visual representation of these measurements.

In the study being reported, measurement was carried out using the computerised planimetry technique of Al-Damouk & MacDonald (1987). The fields selected for counting were delineated by an eyepiece graticule as columns of tissue oriented perpendicular to the mean epithelial surface (Fig. 2.6).

Within these fields the lengths of the epithelial surface and the basement membrane were measured. In the experimental groups where the mucosa was covered by a prosthesis, the keratin surface showed a distinct split between adherent keratin and desquamated cells and debris (Fig. 2.8). This line was used as the epithelial surface for measurement.

The areas of the cellular and the keratinised compartments were measured and from these the mean thicknesses of the two compartments were calculated by division of the areas by the column width which was 370μm. Addition of these two thickness measurements gave the total epithelial thickness.

One other problem arose in histological analysis which made the measurement slightly more complicated. This was artefactual splitting of the tissue, most commonly within the keratin layer (Fig. 2.9). In most instances it was still possible to make the measurements by measuring the total area of keratin and subtracting the missing areas after tracing around the artefact spaces to calculate their area.

Grade 0 showed no evidence of debris over the whole of the tissue section, Grade 1 showed minimal evidence of debris over all or part of the section, Grade 2 showed a moderate amount of debris over all or part of the section and grade 3 was indicative of a substantial amount of debris over the section.

To minimise operator bias, the assessment of debris accumulation on the sections available was carried out without knowing from which dietary group they came. The sections were reassessed against the reference standards some time later and the scores found to be similar. This method of grading therefore appeared to be reliable and reproducible.

Figures 2.10 - 2.13 show representative examples of each grade of debris accumulation.

The ratio designated as "epithelial morphology" (Watson & MacDonald, 1982) was calculated by division of the basement membrane length by the surface length.

A further ratio was also calculated, the "compartment ratio", where the proportion of the cellular compartment to the total epithelium was derived. This ratio was calculated by division of the area of the cellular compartment by the total epithelial area.

2.3.12 Subjective analysis of debris

As discussed in Section 1.13, previous research of this type has been complicated by accumulation of debris between the appliances and palatal mucosa. This is usually a combination of particulate food debris and exfoliated keratin, which accumulates beneath the appliance.

By modifying the diet it was hoped to eliminate or reduce the dietary contribution to the accumulated debris. In order to make an assessment of debris among the three diets, the longitudinal A block sections were examined.

A four-point scale was devised to allow grading of each section to denote the exfoliated keratin and fibrous or powder debris accumulated.

All sections were examined and four chosen which were deemed representative of the four grades of debris accumulation. These were photographed and used as reference standards for all sections. (see opposite)

In order to maintain consistency in measurement, all sections were examined for the most appropriate section from each block. This was identified as the section directly overlying the main palatal neurovascular bundle, which runs anteroposteriorly along the palate.

This allowed reproduceability of site between animals. The sections were examined and graded for each animal.

2.3.13 Statistical analysis

Preliminary statistical analysis required comparison of measured parameters from three different groups of animals. These were either two experimental groups and one control group within each diet, or corresponding experimental or control groups between the three diets. Accordingly, a statistical method of examining three comparable groups was required. As only one factor needed to be defined between these groups, a one way analysis of variance was carried out. From this it was possible to select pairs of groups where a parameter was suggested by the one way analysis of variance to be significantly different.

A non-parametric method of analysing this data was required as it could not be assumed with the small sample size that the distribution was normal. Mann-Whitney U Tests were carried out and results were deemed to be significantly different where $p<0.05$.

Where no statistically significant results were seen when comparing the different parameters between the two experimental groups on each diet, the values for the experimental animals were pooled to allow direct comparison of the experimental and control animals.

2.4 RESULTS

As reported in Section 2.3.8, certain animals were excluded from analysis either as a result of early death or appliance loss. A total

of forty-two animal specimens were available for histological analysis. As stated in Section 2.3.10, the sections from one animal in the one week pellet diet group had to be excluded, resulting in analysis of tissue from forty-one animals.

2.4.1 Effect on weight of dietary variation between dietary groups

Table 2.1 shows the mean weights of all experimental and control animals at different stages throughout the experiment. From these results it is possible to investigate the effect of dietary variation and appliance wear on the weights of the animals during the experimental period.

Excluding the effect of appliance wear by considering only the control animal groups reveals the following results. After 7 days on the diet, at the impression stage for the test groups, the pellet diet control group had a mean weight of 345.8g (S.D.=10.6g). The paste diet group had an average weight of 371.6g (S.D.=22.4g) and the liquid diet group had a mean weight of 325.0g (S.D.= 22.9g). The only difference which reached significance was between the paste diet and liquid diet groups when compared by the Mann Whitney U Test (p=0.0367).

At 28 days, control animals on the pellet diet showed final weights which were significantly greater than those on paste (p=0.0122) or liquid (p=0.0122) diets, these latter two groups not being significantly different. The weights were 429.2g (S.D.=8.0g) for pellet diet animals, 347.1g (S.D.=12.9g) for paste diet animals and 341.5g (S.D.=24.2g) for liquid diet animals.

Comparison of weights of the control animals throughout the experimental period showed a continuous steady rise in weight for the

pellet diet animals. The mean weight for liquid diet animals tended to fluctuate during the experimental period. However, paste diet animals showed a gradual decline and levelling off thereafter.

The weight differences in the two test appliance-wearing groups on each diet were calculated. Examination of weights at Day 7 for the one week test groups showed the only difference to be that the liquid group was lighter than the paste diet group ($p=0.0304$). No differences were seen in the one week test groups at Days 14 or 21.

No differences were seen between the three diets in the two week test groups either at Day 7 or Day 28.

All six groups of animals wearing an appliance showed a mean weight loss. The pellet group did however show the greatest variation within a group with some animals actually gaining weight.

2.4.2 Effect of wearing an appliance on weight within dietary groups

The weights of the two test groups and the control group on each diet were compared at weekly intervals. Comparison of the groups at Day 7, the impression stage, showed no significant differences between the three groups in any of the diets. Similarly, there were no differences noted in any of the three diets at Day 14, the fit stage.

However, in the pellet diet group at day 21, seven days after appliance insertion, the control animals were significantly heavier than either the one week ($p=0.0369$) or two week test ($p=0.0122$) groups. A difference was also noted in the liquid diet group at day 21, where the control group animals were found to be heavier than the one week test animals only ($p=0.0373$).

By day 28, with the one week test groups having been sacrificed previously, the only difference to be seen was in the pellet diet group where again the control animals were significantly heavier than the test animals (p=0.0122).

In the paste diet group, no differences were seen between the weights of the test and control groups at any point during the experiment.

2.4.3 Subjective analysis of debris

As the amount of debris accumulated is likely to increase with time, comparison was only made between groups of animals maintained on the experiment for the same period of time, that is the two week test groups and the control groups. The Mann Whitney U test was used.

The grades given to sections from each animal are shown in Table 2.2. As might be expected, the control groups without mucosal coverage showed no significant differences in debris accumulation between the three dietary groups. Comparison of test and control groups within each diet showed both the pellet and liquid diet groups to have significantly more debris accumulation in the test, appliance-wearing, groups as compared to the control groups (pellet diet p=0.0160; liquid diet p=0.0193). The paste diet however showed no significant difference between the test and control groups.

Comparison of the two week test groups between the three diets showed no statistically significant differences in debris accumulation between the three diets, although the paste diet group had a lower mean score than either of the other two groups.

The reason for the difference in accumulation of debris between the liquid and paste diet appliance wearing groups is not very clear,

given that the two diets differed only in dilution. It may be however that the more fluid liquid diet infiltrated more readily underneath the appliances than the paste one.

Although there were limited differences between the three diets, the paste diet appeared to be slightly better in terms of reducing food debris.

2.4.4 Effect of diet or appliance wear on surface length

The lengths (in μm) of the epithelial surface (LSU) as measured by computerised planimetry are shown in Table 2.3. Values obtained for the epithelial surface varied from 375μm to 422μm with most values lying between 375μm and 385μm.

As might be expected from the method of measurement, the sites chosen for analysis and as previously reported by Jennings (1988), the epithelial surface was generally flat. No significant differences in surface length within the different test groups, both separately and pooled, and control groups in each diet were noted. Appliance coverage did not therefore appear to affect surface length.

Similarly, most groups showed no statistically significant difference when the length of the epithelial surface was compared between diets. The exception was the pellet diet control group as compared with the paste diet control group only ($p=0.0367$). This difference is most likely to be a result of sampling variables. The sections chosen for measurement in each of the two groups probably differed slightly in position relative to the previously described ideal at the base of the inter-rugal col.

2.4.5 Effect of diet or appliance wear on basement membrane length.

The lengths (in μm) of the basement membrane (LBM) as measured by computerised planimetry are shown in Table 2.3. The values for this parameter varied between 414μm and 980μm, with most lying between 430μm and 650μm.

When the length of the basement membrane was compared between test and control animals within the dietary groups the only result found to be statistically significant was in the paste diet. The length seen in the paste diet test animals wearing an appliance for only one week was less than either the two week test or control groups (test 1 week vs test 2 week - p=0.0200; test 1 week vs control - p=0.0234). Again this is most likely to be attributable to sampling variables between the groups. Appliance coverage had only a limited effect on basement membrane length.

No statistically significant difference was found between the three diets in any of the test groups, either separately or pooled, or the control groups. The different diets did not have an effect on basement membrane length.

2.4.6 Effect of diet or appliance wear upon epithelial morphology

Having considered the effects of diet or appliance wear upon the individual lengths, it is of value to consider the effect of these upon epithelial morphology, the ratio of basement membrane length divided by epithelial surface length (LBM/LSU). Table 2.3 shows the mean values for epithelial morphology. Values for this ratio varied from 1.0 to 2.4 with most values lying between 1.2 and 1.7.

A comparison of ratios between one week and two week test animals within each diet group was made. No significant difference was noted between one and two week test animals in either the pellet or liquid diets, but paste diet animals exhibited an increase in epithelial morphology from one to two weeks (p=0.0200)

Given this difference in one diet, it was not deemed possible to pool the results for one and two week test animals. Within each diet, comparisons were made between test and control groups, with the significant differences being in the pellet diet where the epithelial morphology ratio of the one week test group was significantly lower than the control group (p=0.0369), and in the paste diet where the one week test group had a significantly lower epithelial morphology than either the two week (p=0.0200) or control groups (p=0.0200). This latter difference is attributable to the differences seen between the groups in the basement membrane length. No significant differences were seen in the liquid diet groups.

Comparison of one week and two week test and control groups between diets all showed no significant differences. Variation in the diets did not therefore affect the epithelial morphology.

2.4.7 Effect of diet or appliance wear on thickness of keratinised compartment

Having measured by computerised planimetry the area of the different compartments of the epithelium, namely the cellular and keratinised compartments, it was possible, as described in Section 2.3.11, to calculate the thickness of each of these compartments.

Table 2.4 indicates the mean thicknesses (TKC) of the keratinised compartment. This compartment varied in thickness from 21.3μm to 53.5μm.

Comparison was made of the thickness of the keratinised compartment of each of the three groups, control, one and two week, between the three diets. The control animals in the pellet diet group exhibited a significantly greater thickness of the keratinised compartment than either the paste diet ($p=0.0122$) or liquid diet ($p=0.0216$) control groups, indicating an effect of diet alone upon the thickness of adherent keratin produced. No differences were seen between the diets in the test groups, either if taken independently or pooled. This suggests that the increased keratin thickness in animals not wearing appliances in the pellet diet group is a result of mechanical effects as appliance coverage appears to eliminate this difference.

Examination of measurements within each of the three diet groups showed no significant differences in keratinised compartment thickness between the one and two week test groups. The keratin did not therefore appear to alter in thickness with time.

Differences were seen in the thickness of keratin between test and control animals in the pellet diet group. The control animals exhibited a keratin layer of greater thickness than one week test animals ($p=0.0369$), two week test animals ($p=0.0122$), or the pooled group of test animals ($p=0.0043$).

Differences were also seen in the thickness of keratin in the liquid diet group where the keratinised compartment was seen to be of greater thickness in the control group as compared to the one-week test group ($p=0.0373$) and the two week test group ($p=0.0122$), these contributing to the difference seen when the two test groups were

pooled (p=0.0051). The results in these diet groups suggest that provision of a prosthesis results in reduced keratin formation.

The paste diet group however showed no differences in the thickness of keratin between test and control animals.

2.4.8 Effect of diet or appliance wear on thickness of cellular compartment.

Table 2.4 indicates the mean thicknesses (TCC) of the cellular compartment. This compartment was seen to vary in thickness between 76μm and 142μm.

Examination of the control animals only between the diets indicated the effect of diet alone upon the cellular compartment thickness. In the control animals, the pellet diet group showed an increased thickness as compared to the liquid diet group only (p=0.0216) and so only slight differences existed in this compartment as a result of dietary variation.

The thicknesses of the cellular compartment of the two test groups were examined between the three diets. Here again not many differences were seen. In the one week test animals no significant differences in cellular compartment thickness were seen between the diets. In the two week test animals, the pellet diet group showed an increased thickness as compared to the liquid diet group only (p=0.0367). If however the one and two week test animal group results were pooled, the thickness of the cellular compartment of the pellet diet group was noted to be significantly greater than either the paste diet (p=0.0304) or liquid diet groups (p=0.0033).

Although there was an apparent slight increase in mean cellular compartment thickness from one to two weeks in all three diet

groups, no statistically significant differences were seen in this parameter in any of the dietary groups between one and two weeks of appliance coverage.

The only difference in the thickness of the cellular compartment seen between test and control animals on any of the three diets was in the liquid diet group where the cellular compartment of the control animals was less than the two week test group only (p=0.0367), this contributing to a similar difference between the controls and the pooled test animals (p=0.0234). Appliance coverage in itself therefore appeared to have little effect on cellular compartment thickness.

2.4.9 Effect of diet or appliance wear on total epithelial thickness.

The total thickness of the epithelium was examined, with the effect on this parameter of either appliance wear or diet variation being considered. Table 2.4 indicates the mean thicknesses (column TET).

Comparison of the control groups between the three diets showed some difference between the diets, in that those animals fed on the pellet diet had a significantly greater total epithelial thickness than either the paste or liquid diet animals (pellet > paste - p=0.0122 ; pellet > liquid - p=0.0216).

Comparison of one week appliance wearers between diets to look for a possible dietary variation linked to appliance coverage showed no statistically significant difference between the three diets, although the trend of mean thicknesses suggested that the pellet diet gave a thicker epithelium than the paste diet, which in turn was greater than the liquid diet.

Comparison of two week appliance wearers between diets to look for a possible dietary variation also showed no statistically significant difference between the three diets, although again the mean differences suggested slight variation of a similar order to the one week test group.

When the two test groups in each diet were pooled, the only difference between the dietary groups was a greater thickness of the pellet diet group epithelium as compared to the liquid diet group ($p=0.0141$).

It would appear therefore from these results that appliance coverage eliminates the difference in epithelial thickness seen between the diets.

The effect in each dietary group of appliance wear over one or two weeks was examined to see whether the different period of coverage led to differences in thickness. The results showed that there was no significant difference in total epithelial thickness between animals wearing an appliance for one week or for two weeks in any of the diet groups. This meant that again it was possible to combine the results of both test weeks on each diet for comparison with the non-appliance wearing controls.

When all test animals were compared to controls in each of the three diet groups, again there was no statistically significant evidence of a difference in total epithelial thickness between those animals wearing an appliance and those not in all three diet groups.

2.4.10 Effect of diet or appliance wear on proportion of cellular and keratinised compartments.

Having examined the effect of appliance wear or dietary variation upon epithelial thickness, it was also of value to consider the effect of these upon the relative proportions of the different compartments within the epithelium. It is possible to consider the relative contributions to the total epithelial thickness by examining the relative proportion of one of the compartments to the total epithelial thickness.

The relative proportion of the cellular and keratinised compartments was calculated as the ratio "TCC/TET", being the cellular compartment thickness divided by the total epithelial thickness. Table 2.4 shows the values for this ratio in each group. The ratio of cellular thickness to total epithelial thickness increases if there is a higher proportion of cells, or a lower proportion of keratin present.

The differences between dietary groups was examined, looking at each of the control and experimental groups between the three diets. Only minimal differences were noted in the control animals, where the influence of appliance coverage was not a factor. The only statistically significant difference was seen between the paste and liquid diets where the ratio was higher in the paste group ($p=0.0122$). No difference was noted between the diets in the one week experimental groups. In the two week groups, the ratio was higher in the pellet group as compared with either the paste ($p=0.0122$) or the liquid group ($p=0.0216$).

In none of the diet groups was there a difference between the one and two week experimental groups. In both the pellet and liquid diets, there was an increased ratio in both the one and two week

experimental groups as compared to the control groups (pellet one week > control, p=0.0369; pellet two week > control, p=0.0122; liquid one week > control, p=0.0373; liquid two week > control, p=0.0122). In the paste diet the differences were less marked with the only difference being between the one week experimental and the control groups, with the experimental group having a higher ratio (p=0.0200).

2.5 DISCUSSION

As has been shown in other studies on oral mucosa (Jennings, 1988; Watson & MacDonald, 1983), individual variation is present in measurable parameters of the palatal epithelium. This was also seen in this study, where a large standard deviation existed in some of the parameters measured, most notably the basement membrane lengths. Some measurements appeared at first sight to be significant outliers, for example the basement membrane length of specimen 7 (Table 2.3), but findings such as this prompted a re-examination of the sections obtained and re-measurement. The measurements were found to be correct and the sections deemed to be the most appropriate available, although, as already stated, there was the possibility that some sections chosen were not in the ideal position. The accuracy of the measurements obtained was further confirmed by the fact that the values recorded for each site were in most cases means of eighteen separate measurements.

2.5.1 Weights

From the measurements obtained, it was apparent that the animals adapted to the different diets to varying extents and that the

most satisfactory in terms of weight gain was the standard pellet diet. Without the added difficulty of the appliance, the average animal in the other two groups also adapted to the altered diet.

The wearing of an appliance affected the animals' ability to eat with all groups losing weight. Animals fed on a pellet diet showed the greatest difference between test and controls in terms of weight, with a substantial weight gain on the pellet diet with no appliance in situ being replaced with a weight loss when an appliance was present. Animals on the liquid diet also showed a drop in weight as a result of appliance wear although this difference was considerably less than the pellet group. However, animals on the paste diet showed mean weight changes of a similar magnitude whether they were wearing an appliance or not.

There was considerable individual variation in weights as confirmed by the standard deviations within each group, with some appliance-wearing animals exhibiting a weight gain while others were losing weight.

2.5.2 Debris accumulation

Although no statistically significant differences in debris accumulation were seen in the appliance-wearing animals between the three diets, the paste diet group had lower debris scores as compared to the other two diets.

The trend seen within the pellet and liquid diet groups was for increased debris accumulation as a result of appliance wear compared to the control animals while no such trend was seen in the paste diet test animals as a result of appliance wear. The paste diet appeared to be better in terms of reducing food debris.

2.5.3 Histomorphometric parameters

As reported in Sections 2.4.4 and 2.4.5, only limited differences were seen in the length measurements of both the epithelial surface and basement membrane, both within and between the diets, suggesting that appliance coverage and dietary variation had little effect on these parameters.

As reported in Section 2.4.6, the epithelial morphology ratio will increase when the basement membrane length increases relative to the epithelial surface length or the epithelial surface length decreases relative to the basement membrane length. In this experiment the surface length did not vary significantly and therefore any change in epithelial morphology can be attributed to variation in basement membrane length.

There was no difference seen in epithelial morphology between the three diets, suggesting that they each had a similar effect on this ratio. Again only limited differences were seen within diets, mirroring the minor differences seen in the basement membrane length.

As reported in Sections 2.4.7 to 2.4.9, the thickness measurements appeared to be the parameters most affected by the variation of diet or presence of an appliance. If diet alone was assessed by consideration of the control groups, the solid pellet diet showed a significantly thicker keratin layer to be present than either of the other two diets. It is suggested that this results from greater epithelial stimulation from this solid diet as compared to the much softer alternative diets. This is supported by the fact that the differences between the three diets were less marked when appliances covered the mucosa.

It was also noted that provision of appliances seemed in the pellet and the liquid groups to reduce the thickness of adherent keratin formed when compared with animals not provided with an appliance.

In contrast, the cellular compartment thickness in all groups seemed to be less affected either as a result of dietary variation or appliance coverage, with only limited differences being noted.

These results for the separate compartments have an influence on the total epithelial thickness, with only the control animals without appliances showing significant differences between the dietary groups. No significant differences were seen between test and control animals within any of the three diets for the total epithelial thickness and therefore appliance coverage alone did not appear to affect total epithelial thickness.

As reported in Section 2.4.10, the compartment ratio showed some slight variations in the relative contribution of the two compartments to the total epithelial thickness. Little difference existed between the three diets, but there was evidence in all three diets for an increase in the proportion of epithelium contributed by the cellular compartment when the epithelium was covered by an appliance.

In summary therefore, it would appear that dietary modification had only limited effects upon the epithelium, its effects being more apparent in the absence of an appliance.

2.6 CONCLUSIONS

This study showed that it is possible to use the Wistar rat model for evaluation of the effect of dental prostheses on palatal mucosa.

It is possible to modify the animals' diet with no great effect on nutrition, as shown by the effect on animal weights. The use of alternative diets allowed a reduction of the layer of debris previously seen in such experiments. The powder diet used in a paste form gave the least accumulation of debris and the animals fed on this diet exhibited the least differences between the appliance wearers and controls. It is therefore appropriate to use this diet in any further experiments utilising this animal model.

The appliance wearers fed on the paste diet exhibited only limited differences in the appearance of the epithelium from non-appliance-wearing controls. This suggests that the use of an appliance of similar construction, both in terms of the design and the material from which it was made, would in animals fed on this diet be appropriate for studies comparing modifications of denture base materials.

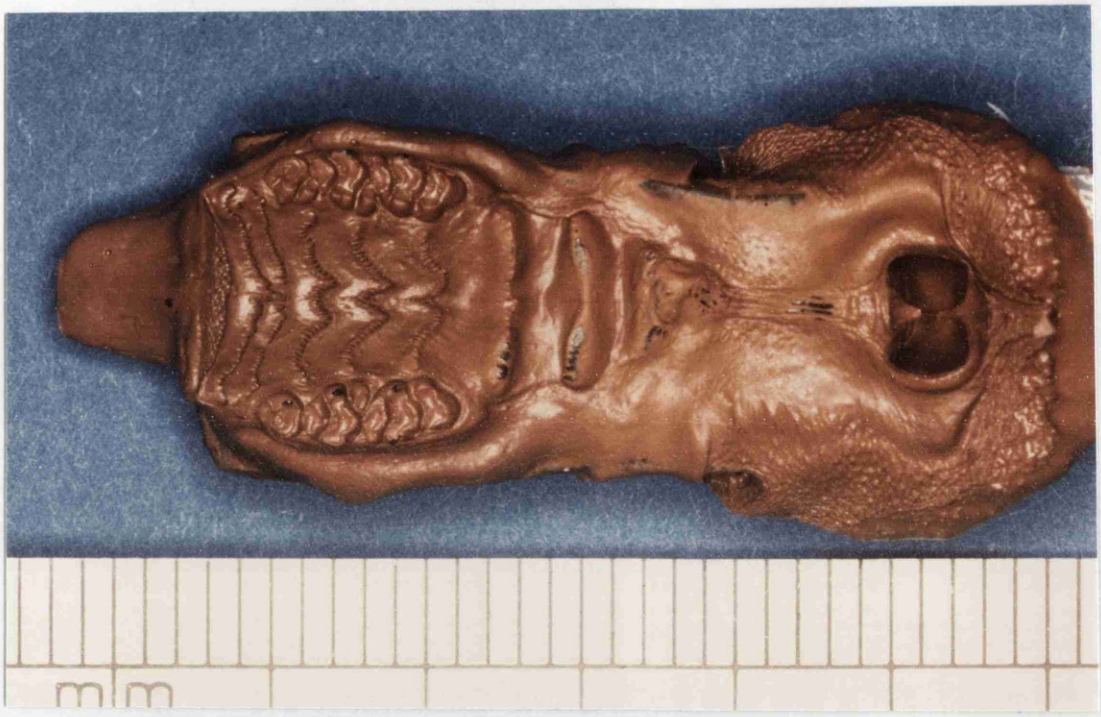

FIGURE 2.1 Impression of rat palate

in polysulphide rubber

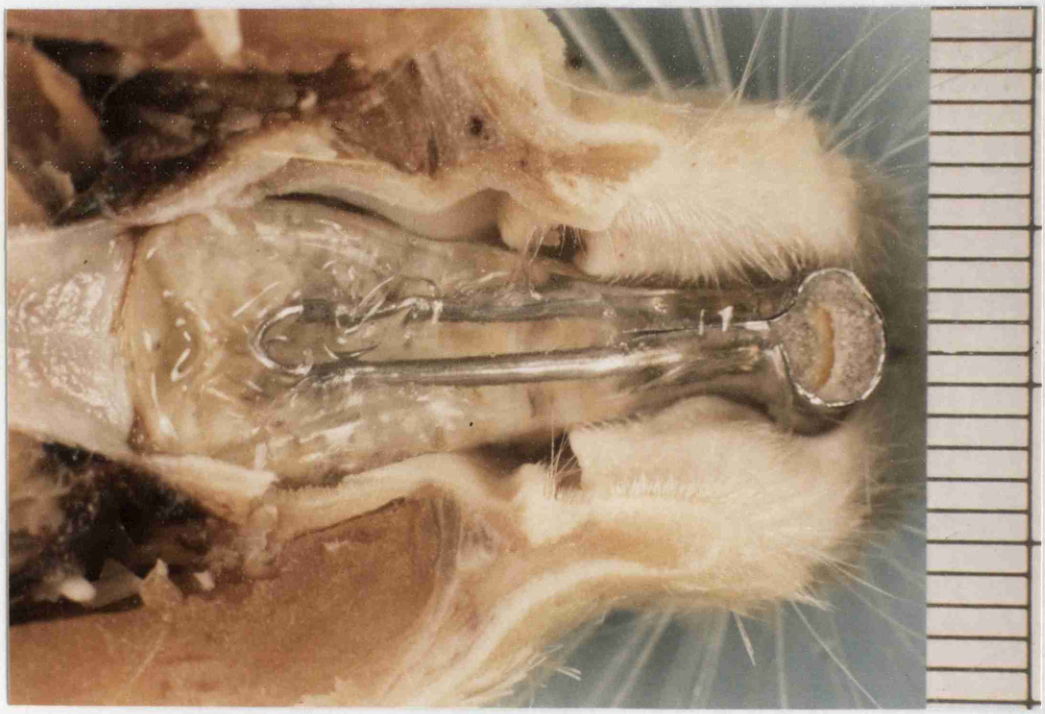

FIGURE 2.2 Appliance design

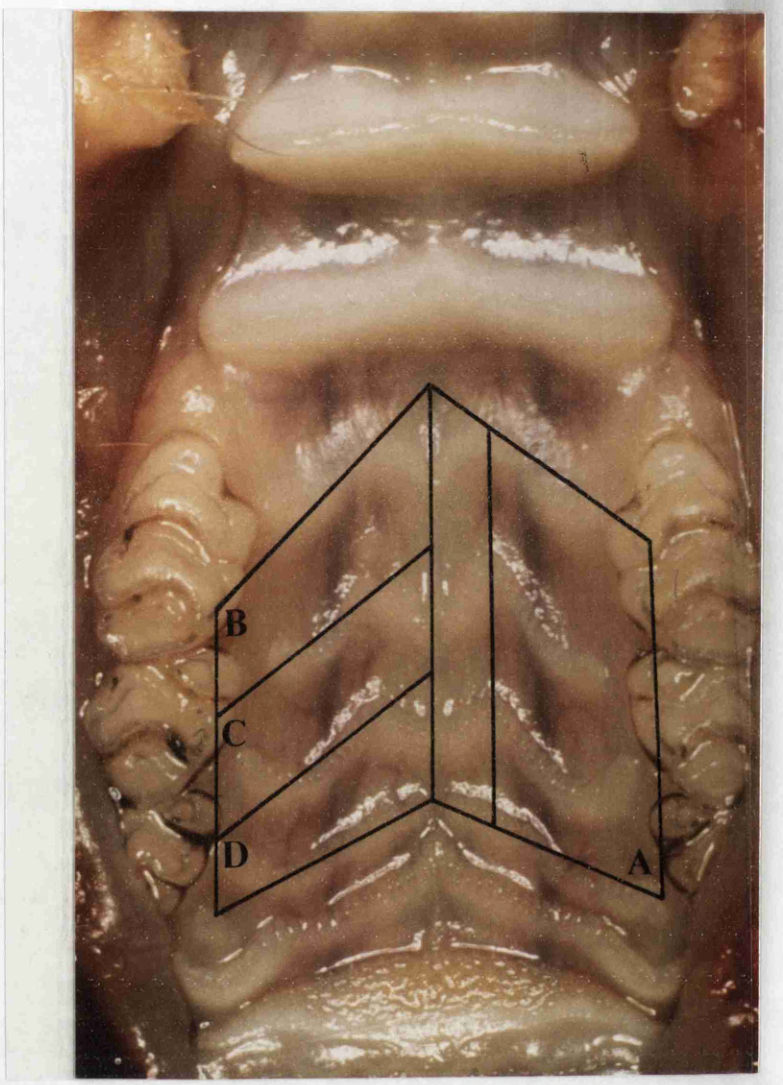

FIGURE 2.3 Method of blocking of rat palates

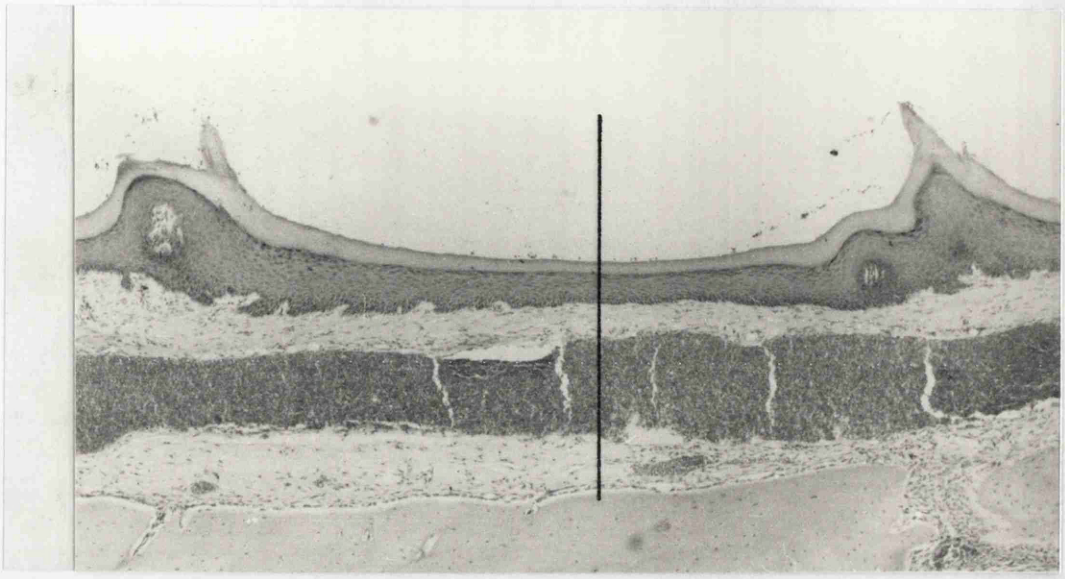

FIGURE 2.4 Longitudinal section identifying the base of the trough between rugae. H. & E. X 63.

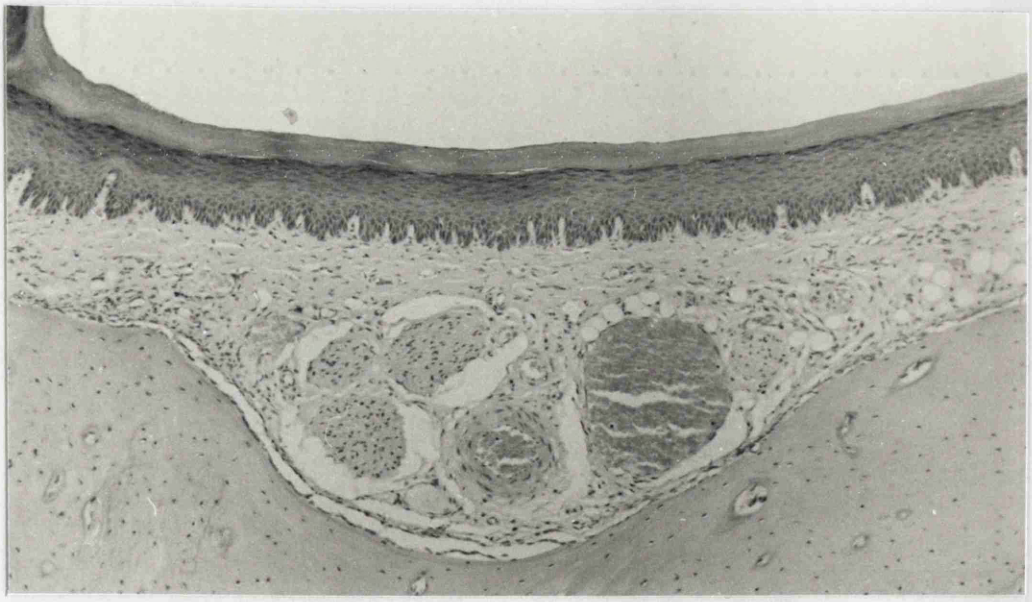

FIGURE 2.5 Typical section from the base of an inter-rugal trough overlying the neurovascular bundle.

H. & E. X 100.

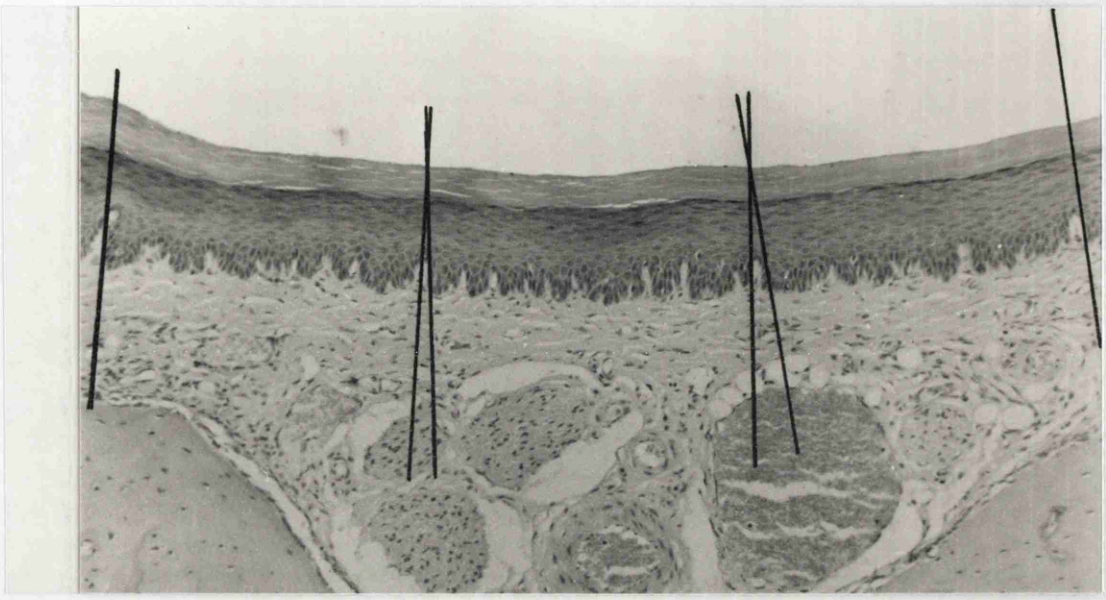

FIGURE 2.6 Fields for measurement with three measuring columns, each of which is 370μm in width.
H. & E. X 120.

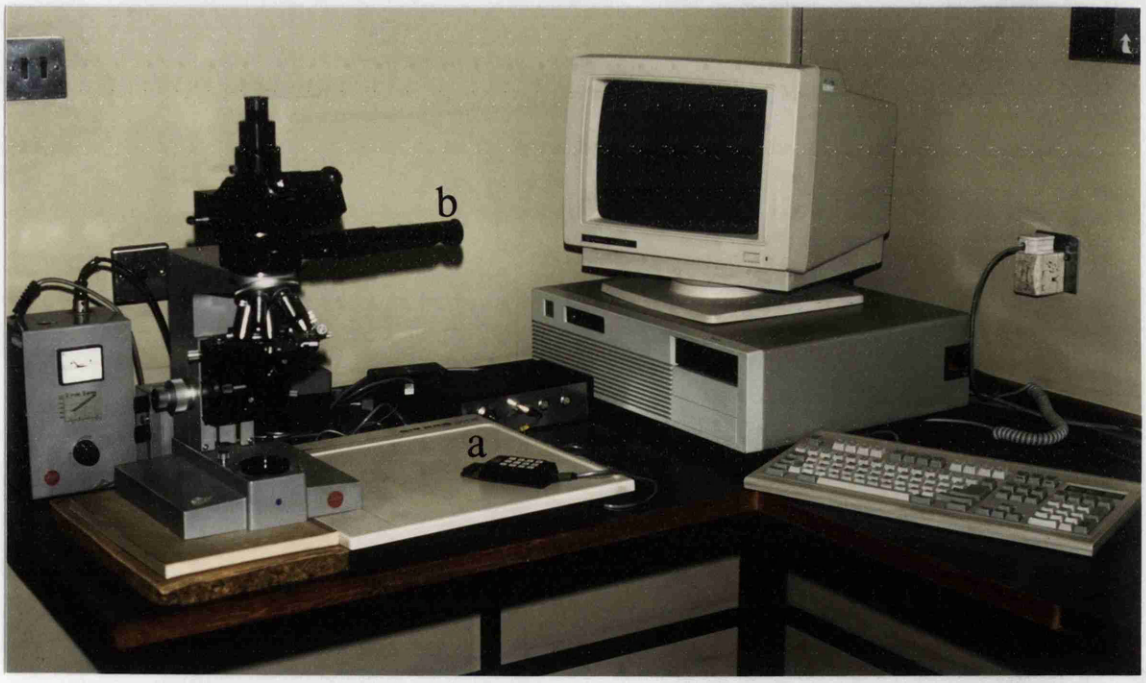

FIGURE 2.7 Equipment used for computerised planimetry. The light spot on the bit pad probe (a) is projected into the microscope field by way of the drawing tube (b).

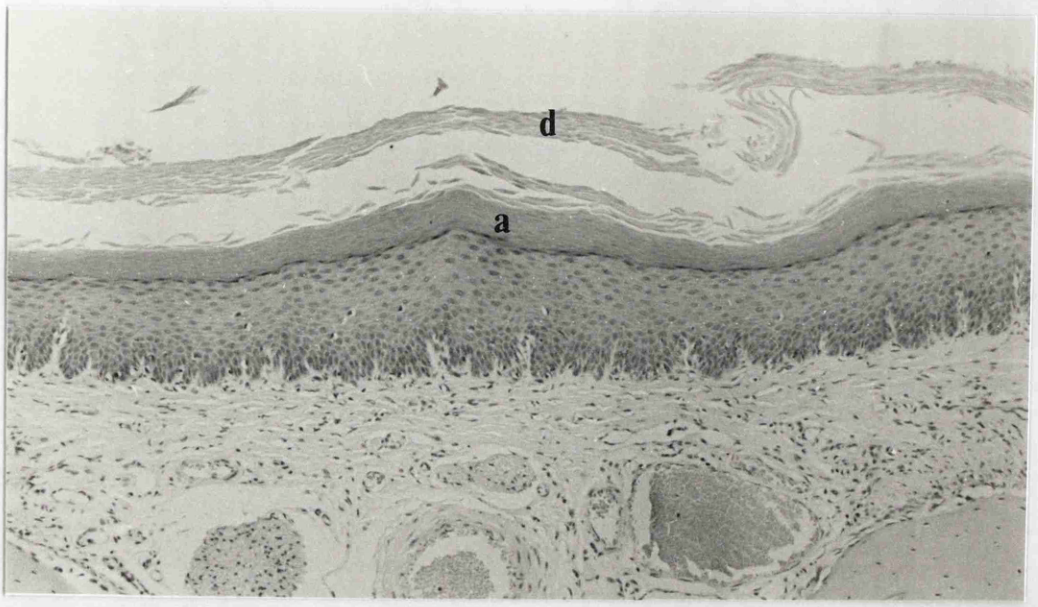

FIGURE 2.8 Keratin split used to demarcate adherent (a) and desquamated (d) keratin. H. & E. X 110.

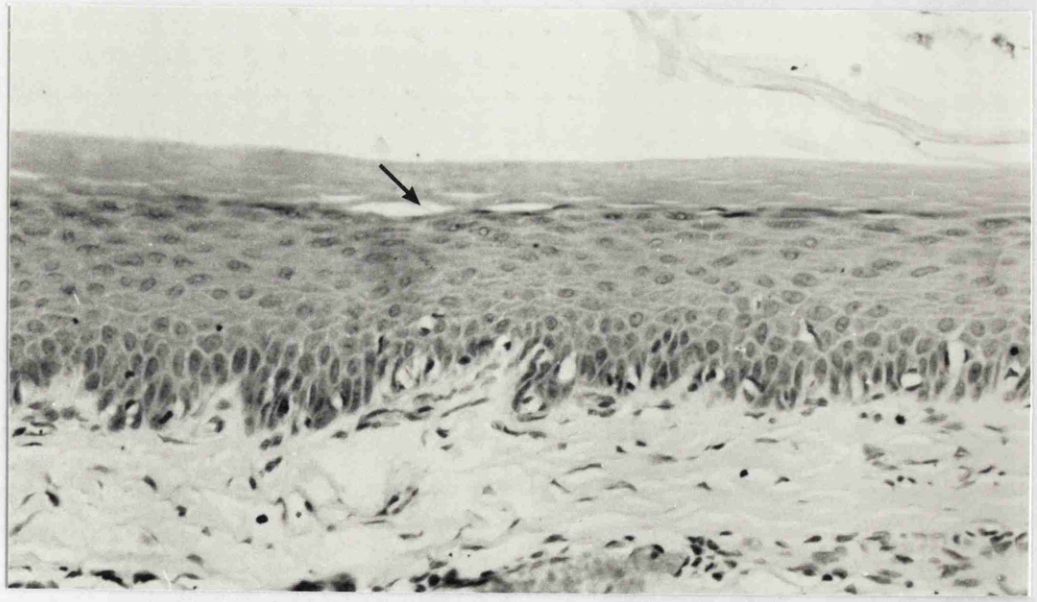

FIGURE 2.9 Artefactual splitting of keratin (arrow) due to section preparation. H. & E. X 170.

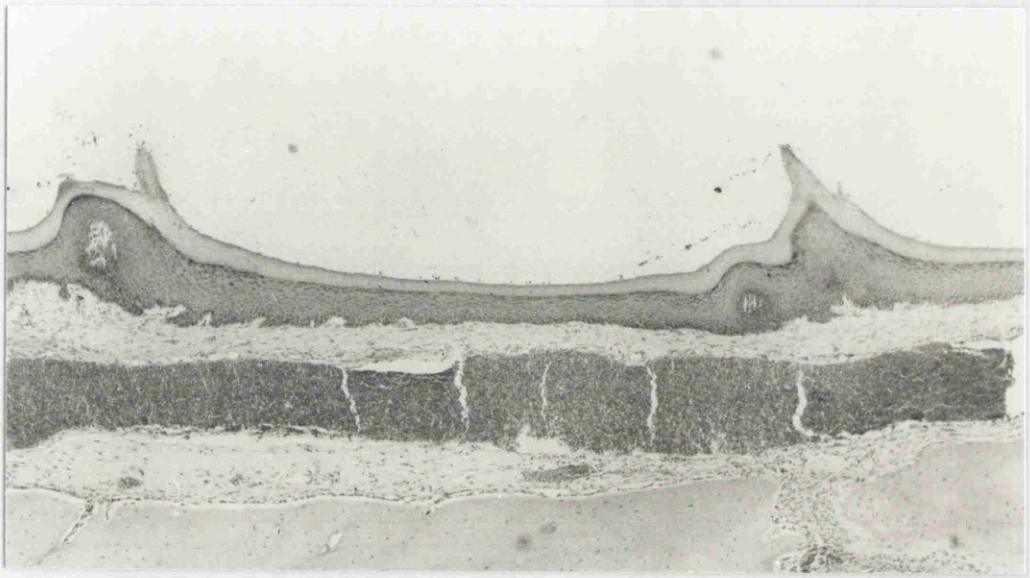

FIGURE 2.10 Debris accumulation - Grade 0. A control animal with no appliance. H. & E. X 57.

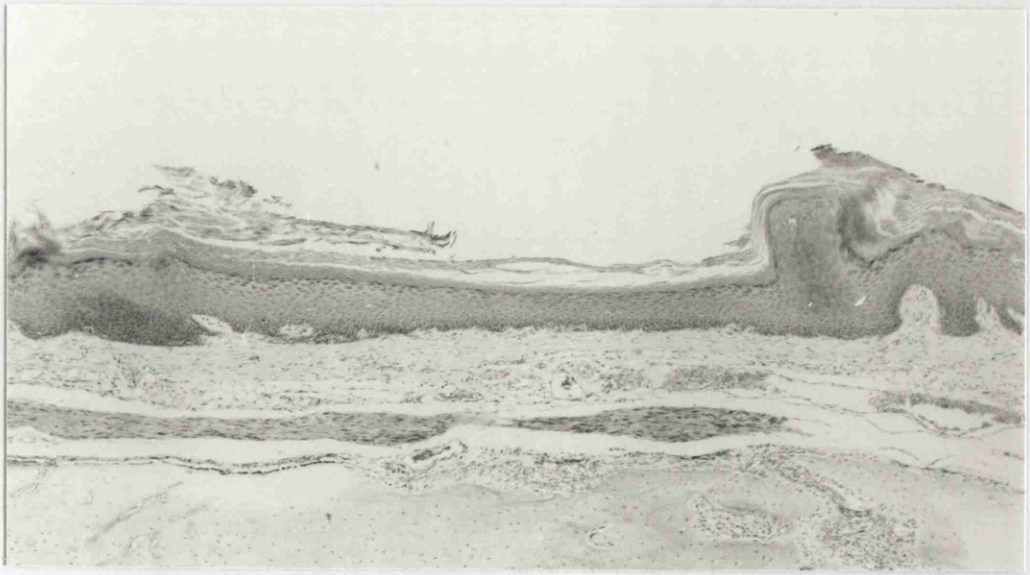

FIGURE 2.11 Debris accumulation - Grade 1. H.& E. X 59.

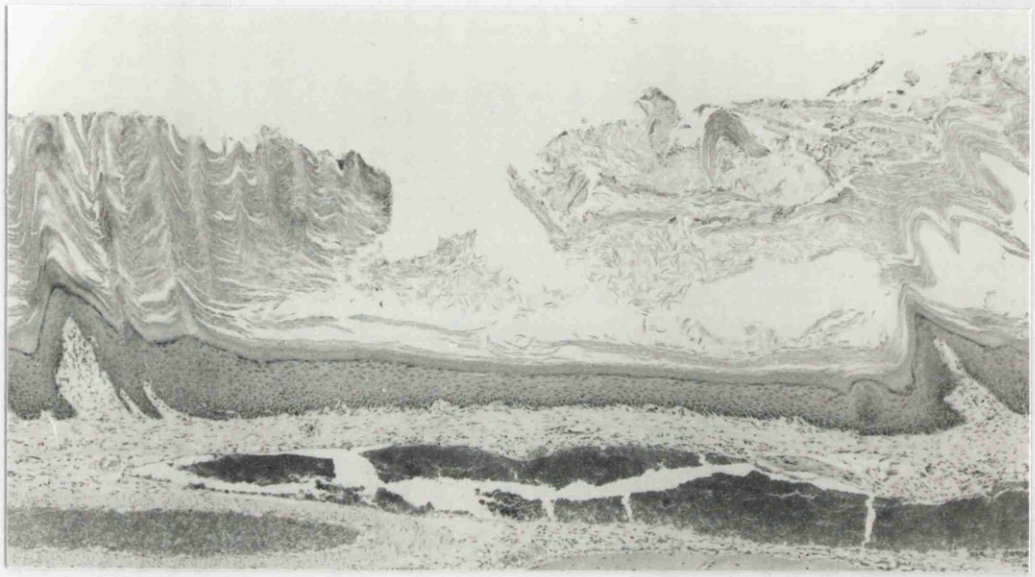

FIGURE 2.12 Debris accumulation - Grade 2. H.& E. X 53.

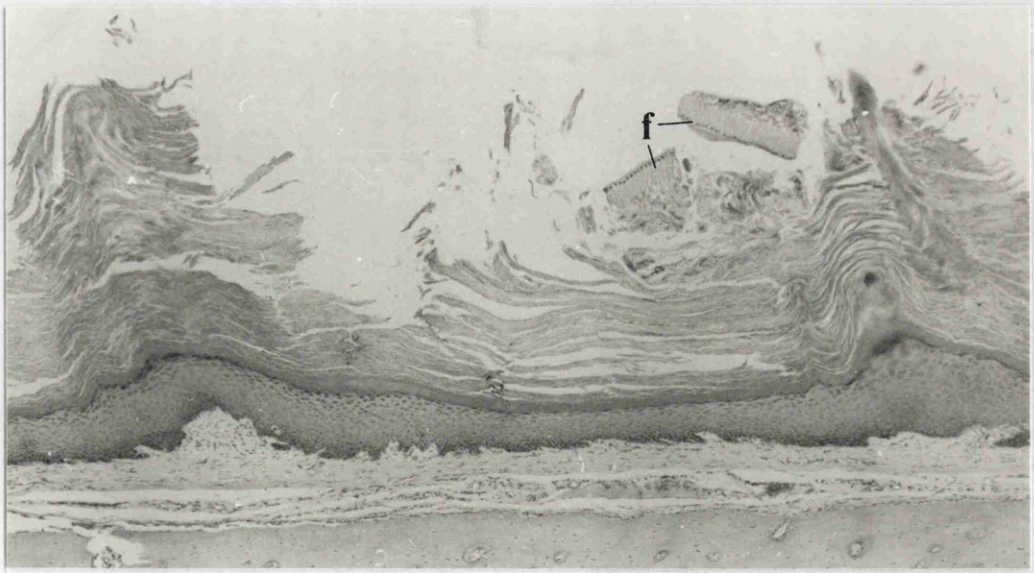

FIGURE 2.13 Debris accumulation - Grade 3. Keratin and obvious food debris (f) . H. & E. X 55.

SPECIMEN	DAY 7	DAY 14	DAY 21	SPECIMEN	DAY 7	DAY 14	DAY 21	DAY 28	SPECIMEN	DAY 7	DAY 14	DAY 21	DAY 28
PELLET TEST - 1 WEEK				**PELLET TEST - 2 WEEK**					**PELLET CONTROL**				
1	353.0	359.4	318.2	5	363.0	395.4	344.2	351.5	10	330.0	367.4	402.0	427.7
2				6	355.6	374.5	353.2	365.2	11	353.2	393.6	416.2	438.6
3	352.2	373.4	305.4	7	349.2	378.8	341.2	363.6	12	340.5	369.5	393.5	416.9
4	358.7	391.5	334.7	8	342.1	364.5	315.9	298.4	13	356.0	384.5	405.4	432.7
				9	334.8	358.5	297.7	258.7	14	349.3	383.5	402.0	430.1
MEAN	**354.6**	**374.8**	**319.4**	**MEAN**	**348.9**	**374.3**	**330.4**	**327.5**	**MEAN**	**345.8**	**379.7**	**403.8**	**429.2**
S.D.	**3.5**	**16.1**	**14.7**	**S.D.**	**11.0**	**14.2**	**22.9**	**47.1**	**S.D.**	**10.6**	**11.0**	**8.2**	**8.0**
PASTE TEST - 1 WEEK				**PASTE TEST - 2 WEEK**					**PASTE CONTROL**				
15	386.2	369.2	342.1	19	356.3	345.1	328.2	338.3	24	351.6	345.6	337.1	340.4
16	389.8	369.5	340.9	20	351.4	340.0	319.6	325.9	25	393.3	386.3	349.3	351.4
17	359.6	348.0	331.6	21	380.3	374.6	352.0	358.0	26	381.2	367.5	349.7	361.8
18	356.0	348.6	291.7	22	373.3	361.6	326.1	335.2	27	388.1	373.0	355.1	353.2
				23	365.9	355.7	340.3	348.4	28	343.9	345.8	324.6	328.5
MEAN	**372.9**	**358.8**	**326.6**	**MEAN**	**365.4**	**355.4**	**333.2**	**341.2**	**MEAN**	**371.6**	**363.6**	**343.2**	**347.1**
S.D.	**17.6**	**12.2**	**23.7**	**S.D.**	**11.9**	**13.7**	**12.9**	**12.4**	**S.D.**	**22.4**	**17.8**	**12.3**	**12.9**
LIQUID TEST - 1 WEEK				**LIQUID TEST - 2 WEEK**					**LIQUID CONTROL**				
29	325.0	340.3	295.6	33	370.1	385.8	335.7	342.4	38	326.1	363.0	335.3	355.9
30	291.6	318.5	277.6	34	299.2	319.4	284.7	287.1	39	359.0	388.6	343.8	354.7
31	319.5	311.8	283.3	35	294.3	317.0	261.2	258.7	40	332.0	363.6	345.0	366.9
32	343.2	362.4	303.8	36	332.8	347.3	309.8	304.9	41	304.9	326.5	310.0	317.8
				37	329.6	352.2	311.1	315.5	42	302.9	330.3	300.0	313.2
MEAN	**319.8**	**333.2**	**290.1**	**MEAN.**	**325.2**	**344.3**	**300.5**	**301.7**	**MEAN**	**325.0**	**354.4**	**326.8**	**341.5**
S.D.	**21.4**	**22.9**	**11.8**	**S.D.**	**30.5**	**28.1**	**28.4**	**31.3**	**S.D.**	**22.9**	**25.9**	**20.6**	**24.2**

TABLE 2.1 *EXPERIMENTAL WEIGHTS (in g)*

SPECIMEN	TEST	SPECIMEN	CONTROL
PELLET DIET			
5	1	**10**	0
6	2	**11**	1
7	1	**12**	0
8	3	**13**	0
9	3	**14**	0
PASTE DIET			
19	0	**24**	0
20	1	**25**	0
21	2	**26**	0
22	1	**27**	0
23	0	**28**	1
LIQUID DIET			
33	1	**38**	1
34	3	**39**	0
35	2	**40**	0
36	1	**41**	0
37	1	**42**	0

TABLE 2.2 *DEBRIS ACCUMULATION SCORES*

SPECIMEN	LSU	LBM	LBM/LSU	SPECIMEN	LSU	LBM	LBM/LSU	SPECIMEN	LSU	LBM	LBM/LSU
PELLET TEST - 1 WEEK				**PELLET TEST - 2 WEEKS**				**PELLET CONTROL**			
1	380.7	481.9	1.266	5	374.7	439.9	1.174	10	383.9	658.5	1.715
2				6	400.8	619.9	1.547	11	395.0	571.1	1.446
3	375.4	458.0	1.220	7	406.6	980.0	2.410	12	380.1	497.6	1.309
4	377.7	414.7	1.098	8	400.6	569.1	1.420	13	380.2	554.5	1.458
				9	395.1	587.3	1.486	14	382.5	672.7	1.758
MEAN	**377.9**	**451.6**	**1.100**	**MEAN**	**395.5**	**639.2**	**1.608**	**MEAN**	**384.3**	**590.9**	**1.537**
S.D.	**2.6**	**34.0**	**0.087**	**S.D.**	**12.3**	**202.4**	**0.471**	**S.D.**	**6.1**	**73.6**	**0.192**
PASTE TEST - 1 WEEK				**PASTE TEST - 2 WEEKS**				**PASTE CONTROL**			
15	422.7	448.4	1.061	19	381.3	517.9	1.358	24	379.4	629.0	1.658
16	376.0	470.2	1.251	20	382.0	514.7	1.347	25	378.2	551.9	1.459
17	380.2	438.2	1.152	21	397.9	523.7	1.316	26	378.1	545.9	1.444
18	379.1	482.3	1.272	22	380.6	528.8	1.389	27	380.5	581.3	1.527
				23	381.5	680.7	1.784	28	377.7	574.3	1.520
MEAN	**389.5**	**459.8**	**1.184**	**MEAN**	**384.7**	**553.2**	**1.439**	**MEAN**	**378.8**	**576.5**	**1.522**
S.D.	**22.2**	**20.1**	**0.097**	**S.D.**	**7.4**	**71.5**	**0.195**	**S.D.**	**1.1**	**32.9**	**0.085**
LIQUID TEST - 1 WEEK				**LIQUID TEST - 2 WEEKS**				**LIQUID CONTROL**			
29	392.8	430.3	1.096	33	376.7	473.9	1.258	38	378.1	487.2	1.288
30	377.2	491.3	1.302	34	378.9	564.7	1.490	39	377.5	529.4	1.402
31	379.4	596.0	1.571	35	379.4	440.1	1.160	40	377.4	506.7	1.342
32	379.6	591.7	1.559	36	380.2	573.0	1.507	41	377.2	630.0	1.670
				37	380.5	431.0	1.133	42	382.6	557.6	1.457
MEAN	**382.2**	**527.3**	**1.382**	**MEAN**	**379.2**	**496.6**	**1.309**	**MEAN**	**378.6**	**542.2**	**1.432**
S.D.	**7.0**	**80.8**	**0.227**	**S.D.**	**1.5**	**68.0**	**0.179**	**S.D.**	**2.2**	**55.7**	**0.147**

TABLE 2.3 *LENGTHS (in μm) AND EPITHELIAL MORPHOLOGY*

SPECIMEN	TKC	TCC	TET	TCC/TET	SPECIMEN	TKC	TCC	TET	TCC/TET	SPECIMEN	TKC	TCC	TET	TCC/TET
PELLET TEST - 1 WEEK					**PELLET TEST - 2 WEEKS**					**PELLET CONTROL**				
1	37.46	120.32	157.77	0.763	5	27.95	117.21	145.15	0.807	10	47.78	120.63	168.41	0.716
2					6	28.14	140.79	168.94	0.833	11	52.54	112.88	165.42	0.682
3	29.98	128.94	158.92	0.811	7	36.12	142.18	178.30	0.797	12	44.99	97.49	142.48	0.684
4	22.96	104.38	127.34	0.820	8	25.02	121.90	146.91	0.830	13	41.83	110.44	152.27	0.725
					9	25.36	122.27	147.63	0.828	14	53.52	124.22	177.74	0.699
MEAN	**30.13**	**117.80**	**148.00**	**0.798**	**MEAN**	**28.52**	**128.87**	**157.39**	**0.819**	**MEAN**	**48.13**	**113.13**	**161.26**	**0.701**
S.D.	**7.25**	**12.46**	**17.90**	**0.031**	**S.D.**	**4.48**	**11.70**	**15.21**	**0.016**	**S.D.**	**4.96**	**10.38**	**13.91**	**0.019**
PASTE TEST - 1 WEEK					**PASTE TEST - 2 WEEKS**					**PASTE CONTROL**				
15	32.19	106.18	138.37	0.767	19	26.69	88.65	115.34	0.769	24	39.79	100.85	140.64	0.717
16	22.30	76.96	99.26	0.775	20	41.99	120.10	162.08	0.741	25	36.33	86.11	122.44	0.703
17	35.86	119.06	154.92	0.768	21	47.93	130.38	178.31	0.731	26	38.16	97.79	135.95	0.719
18	29.32	97.57	126.89	0.769	22	32.39	110.38	142.77	0.773	27	37.65	100.24	137.89	0.727
					23	44.49	105.73	150.22	0.704	28	28.28	90.06	118.34	0.761
MEAN	**29.92**	**99.94**	**129.90**	**0.770**	**MEAN**	**38.70**	**111.05**	**145.94**	**0.744**	**MEAN**	**36.04**	**95.01**	**131.05**	**0.726**
S.D.	**5.40**	**17.68**	**23.4**	**0.004**	**S.D.**	**8.56**	**15.70**	**22.96**	**0.028**	**S.D.**	**4.51**	**6.57**	**9.98**	**0.022**
LIQUID - 1 WEEK					**LIQUID - 2 WEEKS**					**LIQUID CONTROL**				
29	30.90	93.6	124.50	0.752	33	25.80	90.30	116.10	0.778	38	40.22	89.62	129.84	0.690
30	21.26	100.74	122.00	0.826	34	36.40	104.92	141.31	0.742	39	39.35	89.44	128.79	0.694
31	38.27	97.58	135.85	0.718	35	37.47	122.09	159.56	0.765	40	40.06	88.50	128.56	0.688
32	38.84	90.19	129.03	0.699	36	22.95	93.96	116.91	0.804	41	38.60	89.96	128.56	0.700
					37	31.28	101.11	132.39	0.764	42	44.68	98.56	143.24	0.688
MEAN	**32.32**	**95.53**	**127.85**	**0.749**	**MEAN**	**30.78**	**102.47**	**133.25**	**0.770**	**MEAN**	**40.58**	**91.22**	**131.80**	**0.692**
S.D.	**8.21**	**4.60**	**6.08**	**0.056**	**S.D.**	**6.38**	**12.38**	**18.16**	**0.022**	**S.D.**	**2.38**	**4.14**	**6.42**	**0.005**

TABLE 2.4 *THICKNESS OF COMPARTMENTS (in μm) & COMPARTMENT RATIOS*

CHAPTER 3

MATERIALS COMPARISON STUDY

3.1 INTRODUCTION

Following the previous study where debris accumulation was reduced by use of a paste diet, a further study was undertaken to examine the effects of relining materials on oral mucosa in the Wistar rat animal model.

3.2 AIMS

It was the aim of this study to place selected examples of relining materials available for use at the chairside on the fitting surface of appliances covering the palatal mucosa of adult male Wistar rats, and to quantitatively analyse their effect on the mucosa by computerised planimetry.

The materials chosen were Coe-soft, a chairside resilient denture liner, and Kooliner, a hard chairside relining material, both marketed by Coe Laboratories. These are representative of the soft and hard immediate relining materials currently commercially available.

3.3 MATERIALS AND METHODS

3.3.1 Animals

Thirty adult male Wistar rats were used for this study, and their weights were checked regularly prior to the experiment to ensure that the main growth phase had ceased. They were maintained in

separate cages and randomly allocated to one of three groups, with control appliances of the design previously discussed (Section 2.3.4), or test appliances relined with one of the two materials under investigation.

The animals were caged separately as described in Section 2.3.1. Anaesthesia was required to allow master impressions to be taken and to fit the appliances and was carried out as discussed in Section 2.3.3.

3.3.2 Diet

The animals received a nutritionally complete solid powder diet (Special Diet Services; Witham, Essex), being given 20 grams per day, provided by mixing with enough water to form a thick paste. Water was also available ad libitum. This diet was chosen as a result of the work reported in Chapter 2, because it was the material giving the least accumulation of food debris and the least differences between the control and experimental animals.

3.3.3 Appliance design

Master impressions were taken for all animals and appliance construction for the control animals was as described in section 2.3.4.

In order to allow incorporation of the relining materials in the test prostheses, it was necessary to modify the appliance design. A well was created in each appliance in the intermolar region as shown in Figure 3.1, by using a 1mm thickness stainless steel spacer during curing of the acrylic appliance. The spacer was removed when the appliance had been processed. This well was filled with the test

materials at the insertion stage and allowed to cure according to the manufacturers' instructions (Section 3.3.4).

3.3.4 Materials handling

Coe-soft was mixed according to the manufacturers' instructions (1g of powder to 8ml of liquid) and stirred for thirty seconds. The material was then applied to the well in each of the appliances for the animals allocated to this experimental group. The prostheses were seated fully against the palate of the anaesthetised animals. After three minutes, the appliances were removed and the excess material removed by trimming with a scalpel to ensure that no material lay outwith the confines of the well. The appliances were then replaced for a further five minutes, prior to cementation to the previously notched upper incisors using autopolymerising acrylic resin as described in Section 2.3.5.

In the other experimental group, Kooliner was mixed according to the manufacturers' instructions (7g powder to 4ml liquid), allowed to rest for one minute and applied to the well in each appliance. The appliances were seated firmly against the palate, and the material allowed to cure for a further three minutes. Thereafter they were removed, excess relining material trimmed and the appliances cemented in place as the manufacturer's instructions state that complete polymerisation may take place in contact with the mucosa.

Both materials were found to be easy to trim at the times stated in the instructions and remained in close contact with the prepared well.

3.3.5 Experimental protocol

The thirty animals were randomly allocated in groups of ten to either of one control or two test groups. They were commenced on the paste diet one week prior to the impression stage. Fitting of the appliances took place seven days after the impressions were taken and the animals wore the prostheses for fourteen days before being sacrificed. Fourteen days was deemed to be an appropriate time scale as it is recommended as the maximum time a soft lining should be in situ (Graham et al, 1990).

The two experimental groups were provided with modified appliances as described in Section 3.3.3 and were designated the "soft-relined" (Coe-soft) and "hard-relined" (Kooliner) groups. The appliances were fitted as described in Sections 3.3.3 and 3.3.4.

3.3.6 Reasons for exclusion

During the experiment there were no anaesthetic-related deaths. One animal had to be sacrificed after the impression stage but before fitting of the appliance because it trapped and damaged its tail in its cage. This animal had been assigned to the hard relined group. Another animal, again from the hard relined group, was sacrificed three days early because of excessive weight loss. The tissue from this animal was subsequently examined and showed evidence of severe prothesis-related trauma. This is discussed further in Section 3.4.8.

The main problem found in this experiment was retention failure of the appliances. In the ten animals wearing appliances with a soft liner, only three appliances were retained without loss for fourteen days after cementation. The remaining seven appliances were lost within twenty-four hours of cementation. They were recemented using a

reinforced zinc oxide eugenol temporary restorative material I.R.M. (De Trey) and all except one were retained for the fourteen day experimental period. The one appliance lost again was lost one day before the sacrifice date. The animal was not included in the histological analysis.

A similar problem was noted in the group with hard liners, with only four appliances being satisfactorily retained for the fourteen day experimental period. Two appliances were lost within twenty-four hours and were recemented using I.R.M. One of these was lost in the next twenty-four hours. The remaining four appliances were lost at various periods after 24 hours.

It was not deemed reasonable to re-anaesthetise animals and replace appliances where loss had occurred more than twenty four hours after insertion and so it was necessary to replace these animals and appliances. A further six animals were entered into the experimental protocol with the intention of providing them with prostheses with hard linings. The animal which damaged its tail was in this group and therefore only five animals were provided with appliances. These were cemented from the outset with I.R.M. and remained in situ throughout the experimental period. The animal sacrificed as a result of excessive weight loss was also a member of this group. Four animals from the original hard relined group and four from the supplementary group were therefore included in the animals wearing hard relined appliances and subsequently underwent histological analysis.

The control appliance group of ten animals had appliances cemented as previously with self-cure acrylic and none were lost during the experimental period.

The total number of specimens analysed by computerised planimetry in each group was nine animals in the soft relined group, eight in the hard relined group and ten in the control group.

The extra animals used for replacement of the animals which were withdrawn from the hard relined group were stock animals of a slightly older age and consequently greater initial weight. The effects of this difference are considered in Sections 3.4.2 and 3.4.3.

3.3.7 Statistical analysis

Statistical analysis was carried out as described in Section 2.3.13, with comparison between the three appliance groups by a one-way analysis of variance and subsequent analysis by the Mann-Whitney U Test.

3.3.8 Animal sacrifice, specimen preparation and analysis

The animals were sacrificed by CO_2 asphyxiation and cervical dislocation. The tissue blocks and sections were prepared and examined using the techniques described in Sections 2.3.9 and 2.3.10. Quantitative analysis was carried out using the computerised planimetry technique described in Section 2.3.11.

3.4 RESULTS

As with the previous study, comparisons were made of the various epithelial parameters measured by computerised planimetry. Consideration was also given to the loss of the experimental appliances

and to the use of the stock animals to replace those animals which could not continue in the study.

3.4.1 Appliance failure

The appliances which were lost were examined to determine the point of failure of the lute. Nine appliances failed at the interface between the acrylic and the teeth, while two appliances failed between the acrylic and the stainless steel band. One appliance failed as a result of fracture within the acrylic lute. One appliance could not be assessed because the stainless steel band had been chewed and significantly distorted by the animal before being recovered from the cage.

3.4.2 Assessment of uniformity of hard relined group

As stated in Section 3.3.6, there were two sets of animals used for the hard relined group. The first set was allocated from the initial number of thirty animals for the experiment. The second group was taken from a group of stock animals when others had to be excluded from the experiment because of appliance loss. The second set of animals were older and consequently slightly larger.

There was therefore the possibility that the difference in size of these animals would affect the values of the parameters measured. Before the two sets were combined to form the hard relined group for comparison with the other experimental groups, it was necessary to compare the parameters measured between the two sets.

It was found when Mann-Whitney U tests were carried out on the length and thickness values for the two sets of animals that there were no statistically significant differences in any of the parameters.

Although the mean weights differed somewhat between the two groups, again the range of weights was such that there was no statistically significant difference between the weights of the two sets of animals.

It was therefore deemed possible to combine the two sets of animals to form the hard relined group of eight animals.

3.4.3 Weights

Weights were recorded throughout the experimental period and are shown in Table 3.1. The mean weights were found to fluctuate only slightly in each experimental group during the course of the experiment, with a slight rise in mean weights occurring for the soft relined and control groups and a slight decline being seen for the hard relined group.

Comparison of the weights at the impression stage showed the only statistically significant difference to be that the hard relined group was heavier than the soft relined group only ($p=0.0485$), this being attributable to the greater age and weight of the stock animals used in the hard relined group.

Comparison of the group weights at all other stages showed no statistically significant differences between the three groups.

3.4.4 Effect of different materials on length measurements

Measurements of surface and basement membrane lengths are recorded in Table 3.2.

No statistically significant differences were seen between the epithelial surface lengths of the three groups.

The only difference in basement membrane length which reached significance was that the hard relined group had a longer mean basement membrane length than the soft relined group (p=0.0304).

3.4.5 Effect of different materials on epithelial morphology

Measurements of epithelial morphology are recorded in Table 3.2.

The only difference seen in epithelial morphology was between the hard and soft relined groups where the mean value for the hard relined group was higher (p=0.0184). This is likely to be attributable to the difference seen in the basement membrane lengths.

3.4.6 Effect of different materials on thickness measurements

Measurements of the thickness of keratin and cellular compartments and total epithelial thickness are recorded in Table 3.3.

Examination of the thickness of keratin between the three groups showed both the relined groups to have a greater mean thickness of adherent keratin than the controls although only the hard relined group was found to be significantly thicker (p=0.0088) than the controls.

Comparison of the cellular layer thickness between the three groups showed the soft relined group to have a thinner layer than either the hard relined or control groups but this was not significant.

There were no significant differences in total epithelial thickness between any of the three groups.

3.4.7 Effect of different materials on compartment ratio

Measurements of the compartment ratio are recorded in Table 3.3.

The compartment ratio for both relined groups was essentially the same, but the control group had a higher ratio than both the experimental groups (control > soft relined, p=0.0200 ; control > hard relined, p=0.0368). There was therefore a difference in the relative proportions of the cellular compartment between the experimental and control groups, with the results of the thickness comparisons suggesting a higher proportion of keratin in the relined groups.

3.4.8 Histological examination of specimen exhibiting severe weight loss.

As discussed in Section 3.3.6, one animal in the hard relined group was sacrificed three days early because of significant weight loss. Examination of the blocks of tissue from this specimen showed evidence of trauma in the C and D blocks.

The C block showed ulceration of the epithelium with exfoliation of a small sequestrum of necrotic cortical bone (Figures 3.2 - 3.4). There was an acute inflammatory infiltrate immediately adjacent to the sequestrum. There was also evidence of a diffuse chronic inflammatory infiltrate within the adjacent connective tissue and of healing in the form of new woven bone on the periosteal surface. At a slightly greater distance there was remodelling with osteoclast activity on the periosteal surface.

There was also similar evidence of bone remodelling in the more distal D block, with evidence of osteoclastic activity and resorption.

The histological appearance was consistent with necrosis induced by marked overloading. It was also suggestive of recurrent trauma rather than a single episode of damage. This would therefore explain the animal's weight loss as a result of difficulty in eating related to trauma from the appliance. This trauma probably resulted from poor placement of the appliance, either during the addition of the relining material or at the time of cementation.

3.5 DISCUSSION

As discussed in Section 3.3.6, a major problem during this experiment was failure of the acrylic lute to the incisor teeth. The mechanism of retention between the teeth and appliances is mainly mechanical, relying on the notching of the incisor teeth to provide suitable retention of the set acrylic.

As reported in Section 2.3.8, only three appliances were lost in the preliminary dietary experiment, probably as a result of poor luting technique. In this experiment the loss rate was very high, with only seven of the twenty appliances originally placed in the two relined groups remaining in situ for the full experimental period without requiring to be recemented. The cause of this high failure rate is uncertain, but because it happened only in the relined groups it is likely that it resulted from an incompatibility of the relining materials with the luting acrylic. It has been reported in the literature (Davis & Carmichael, 1988) that leaching of the plasticiser from soft relining materials can affect the rigidity of other acrylic materials. This may have been the reason for failure in this case, particularly as failure occurred in most cases within twenty four hours of placement of the

appliances. In no case when the appliance was replaced using IRM, was the notching of the teeth altered and all except one of the replaced appliances remained in situ for the full experimental period.

As stated in Sections 3.4.1 to 3.4.7, only minimal differences were noted between the three experimental groups.

In comparison of the length and epithelial morphology results, there was only one factor which appeared significant. The hard relined group showed an increase in the length of the basement membrane as compared to the soft relined group . No difference was seen between the soft relined and control groups. As one might have expected the hard relined and control groups to be most similar in terms of their fitting surface, the results suggest that relining with a hard relining material may cause greater forces to be exerted by the new appliance on the epithelium than an appliance made on a stone cast or one which has been relined with a soft material.

Although not all results were significantly different in the measurement of thicknesses, it would appear that relining with either a soft or a hard material gave an increased thickness of keratin, while the total epithelial thickness remained similar. This suggests that relining may have an effect on the rate of transition of cells through the epithelium, with a more rapid formation of keratin. Alternatively, relining may result in a slower rate of loss of keratin.

As indicated in Section 1.11, there are no reports in the literature of the histological effects of relining materials on human oral epithelium under normal functional conditions.

3.6 CONCLUSIONS

Quantitative analysis of the tissue changes in the rat palate arising from coverage with directly applied relining materials suggested only limited effects on the epithelium of this procedure.

The materials appeared to cause a slight increase in the amount of keratin produced by the underlying epithelium.

The epithelium seems to progress more rapidly from the cellular to the keratinised compartment.

FIGURE 3.1 Design of reline appliances (showing well area into which the relining material was placed).

SPECIMEN	DAY 7	DAY 14	DAY 21	DAY 28	SPECIMEN	DAY 7	DAY 14	DAY 21	DAY 28	SPECIMEN	DAY 7	DAY 14	DAY 21	DAY 28
SOFT RELINED					HARD RELINED					CONTROL				
1	442	446	437	450	10	444	415	408	417	18	453	461	463	474
2	450	454	466	469	11	495	498	486	500	19	447	460	461	470
3	432	440	443	461	12	408	415	410	420	20	475	481	482	495
4	433	441	450	461	13	471	467	467	474	21	470	462	465	474
5	475	463	453	461	14	560	575	553	541	22	471	474	470	474
6	467	470	490	496	15	543	547	531	521	23	449	458	459	475
7	455	443	459	470	16	488	457	465	459	24	487	487	490	501
8	410	416	425	433	17	600	612	586	558	25	472	495	487	490
9	445	457	454	470						26	473	479	480	483
										27	476	474	471	477
MEAN	445.4	447.8	453.0	463.4	MEAN	502.9	499.0	489.6	488.3	MEAN	467.3	473.1	472.8	481.3
S.D.	19.5	15.8	18.4	17.0	S.D.	59.4	68.7	60.5	49.9	S.D.	13.1	12.6	11.2	10.5

TABLE 3.1 *EXPERIMENTAL WEIGHTS (in g)*

SPECIMEN	LSU	LBM	LBM/LSU
SOFT RELINING			
1	381.75	472.08	1.236
2	391.34	575.11	1.470
3	381.63	414.87	1.087
4	378.61	414.29	1.094
5	382.46	485.02	1.270
6	378.68	462.44	1.221
7	385.96	499.19	1.293
8	389.26	482.30	1.239
9	386.37	508.09	1.315
MEAN	**384.01**	**479.3**	**1.247**
S.D.	**4.49**	**48.9**	**0.115**
HARD RELINING			
10	382.54	644.27	1.684
11	385.58	502.49	1.303
12	388.14	619.59	1.596
13	382.32	507.78	1.328
14	379.49	486.44	1.281
15	383.26	536.01	1.398
16	385.65	638.95	1.657
17	381.10	481.03	1.262
MEAN	**383.51**	**552.10**	**1.439**
S.D.	**2.79**	**70.3**	**0.178**
CONTROL			
18	377.85	451.05	1.194
19	384.19	491.88	1.280
20	376.25	442.01	1.175
21	381.11	496.52	1.303
22	382.23	543.79	1.423
23	381.70	498.33	1.306
24	387.82	720.42	1.858
25	380.50	443.04	1.164
26	375.07	534.47	1.425
27	373.57	410.35	1.098
MEAN	**380.03**	**503.20**	**1.322**
S.D.	**4.38**	**87.4**	**0.217**

TABLE 3.2 *LENGTHS (in μm) & EPITHELIAL MORPHOLOGY*

SPECIMEN	TKC	TCC	TET	TCC/TET
SOFT RELINING				
1	37.52	102.10	139.62	0.731
2	30.34	101.11	131.44	0.769
3	30.08	94.66	124.75	0.759
4	35.84	69.08	104.91	0.658
5	58.57	124.14	182.71	0.679
6	32.68	76.11	108.79	0.700
7	39.90	82.04	151.94	0.672
8	47.85	91.24	139.09	0.656
9	52.38	108.82	161.20	0.675
MEAN	**40.57**	**94.37**	**134.94**	**0.700**
S.D.	**10.16**	**17.09**	**24.70**	**0.043**
HARD RELINING				
10	46.25	91.29	137.54	0.664
11	63.82	136.15	199.97	0.681
12	35.26	121.89	157.15	0.776
13	34.03	105.32	139.35	0.756
14	38.74	87.78	126.53	0.694
15	36.86	98.77	135.63	0.728
16	55.32	119.58	174.90	0.684
17	49.68	94.72	144.40	0.656
MEAN	**44.99**	**106.94**	**151.93**	**0.705**
S.D.	**10.72**	**17.18**	**24.48**	**0.044**
CONTROL				
18	27.10	101.47	128.58	0.789
19	33.32	107.46	140.78	0.763
20	26.25	94.93	121.18	0.783
21	30.96	105.16	136.11	0.772
22	54.07	96.62	150.69	0.641
23	30.34	114.43	144.77	0.790
24	36.22	133.90	170.12	0.787
25	33.63	85.55	119.18	0.718
26	33.05	106.74	139.79	0.764
27	35.32	100.76	136.08	0.740
MEAN	**34.03**	**104.70**	**138.73**	**0.755**
S.D.	**7.76**	**12.96**	**14.82**	**0.046**

TABLE 3.3 *THICKNESS OF COMPARTMENTS (in μm) & COMPARTMENT RATIO*

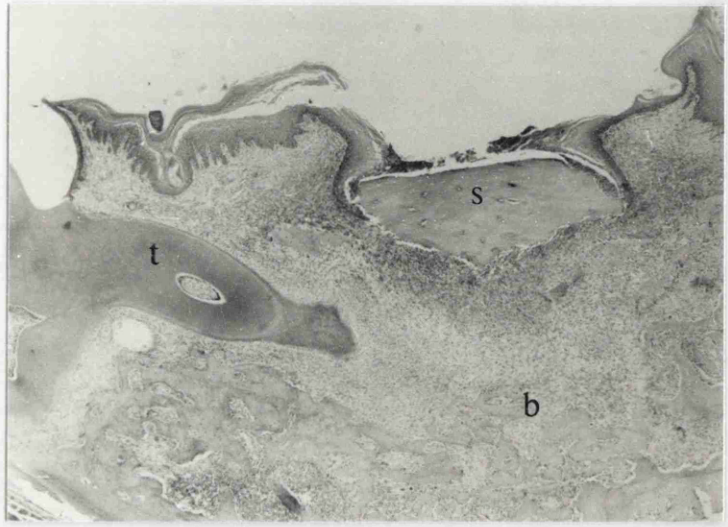

Figure 3.2
Low power view of sequestrum (s) being exfoliated. Note the tooth (t) and area of bone formation (b).
H. & E. X 30.

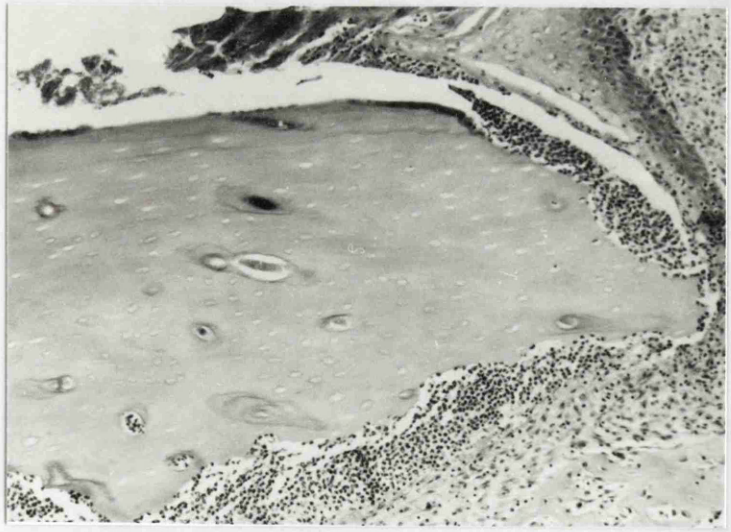

Figure 3.3
Exfoliating sequestrum of necrotic bone with related acute inflammatory cell infiltrate. H. & E. X 126.

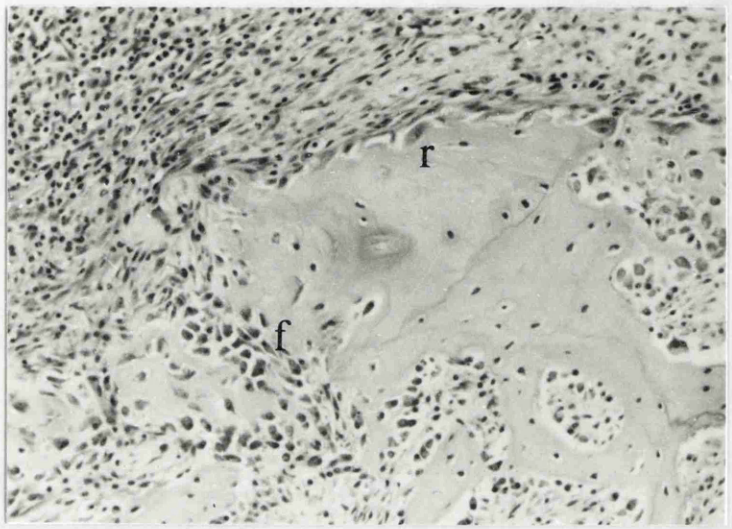

Figure 3.4
Active new bone formation (f)
and osteoclastic resorption of original bone (r).
H. & E. X 190.

CHAPTER 4

CONCLUDING DISCUSSION

The foregoing study has set out to establish a protocol whereby the Wistar rat model could be used as a viable model for examination of the effect of palatal coverage prostheses on oral mucosa.

Human studies have acknowledged the presence of significant variation, not only in terms of anatomical differences in the areas of tissue being examined within each individual subject, but also in terms of other factors, including age, systemic disease, habits like smoking and the length of time that the tissues are covered by the appliances. It is this degree of variability that has contributed to the conflicting results encountered in the literature (Sections 1.9.6 - 1.9.10).

It is acknowledged that animal models such as the one used in this study cannot be directly related to the human situation. They do however provide researchers with the ability to examine the effects of prostheses and their constituent materials on the oral tissues of subjects where most of the systemic and exogenous factors can be eliminated or modified. Regional variations within the epithelium of the rat palate are encountered, particularly in relation to the amount of keratin accumulating over palatal rugae. However the epithelium of the intermolar region of the hard palate shows almost no evidence of parakeratinisation (Kutuzov and Sicher, 1952) unlike the human palate and therefore the tissue is generally more uniform, making comparison between subjects easier. The model also allows more accurate control on the important variable of the period of time during which the oral tissues are exposed to the appliances.

Differences in a number of the parameters measured were noted between individual animals within each group. Individual variation in some of the length and thickness measurements of the palatal epithelium was seen. Animal weights during the experimental period also varied within the different groups. Most animals tended to lose weight if provided with an appliance, as this, at least initially, compromised their ability to eat. However other animals exhibited a weight gain.

The first part of the study was carried out principally to assess the benefits of dietary modification, given previous concern in the literature (Jennings and MacDonald; 1990b, 1992) that debris accumulation beneath appliances in this type of model is an undesirable variable.

Subjective analysis of debris showed only minimal accumulation in the non-appliance wearers on all three diets showing that an appliance was required for debris accumulation to occur. A significantly greater amount of debris accumulated as a result of appliance wear in both the pellet and liquid diet groups. The paste diet did not show any greater accumulation of debris in appliance wearers as compared to the control animals.

The reason for the difference in debris accumulation beneath appliances in the paste and liquid diet groups is rather uncertain as the basic material is the same. One possible explanation is that the liquid diet allowed more material to be deposited between the mucosa and the appliance due to its more fluid nature.

In the first part of the study the main differences seen in the parameters measured were in the thickness measurements and in calculations of the compartment ratio derived from them. The standard

pellet diet resulted in a greater thickness of adherent keratin in the non-appliance-wearing animals as compared to either of the corresponding groups on the other two diets or to appliance-wearing animals on the same diet.

This difference appears to be a consequence of a stimulatory effect of the pellet diet directly on the palatal tissues, rather than a protective effect of the appliance, as a difference in adherent keratin between controls and appliance wearers was less obviously present in the groups fed on the alternative diets, particularly the paste diet.

Minor differences were seen with regard to the cellular compartment and total epithelial thickness between the three diets in both the appliance wearing and control groups, with again the pellet diet groups being thicker.

This therefore suggests that previous animal studies which have compared epithelial thickness between appliance-wearing test and non-appliance-wearing control groups may have produced results which were affected by the particular diet used, although the exact formulation of the diets has rarely been stated. The results of the present study also conflict with those of the human studies of Kapur and Shklar (1963) and Jani and Bhargava (1976). These studies suggested a stimulatory effect of appliances on the underlying epithelium. Other workers (Watson and MacDonald, 1982) failed to find a difference in epithelial thickness between denture and non-denture wearers.

The effect of an appliance seemed to be minimised when the paste diet was used, with virtually no differences in any of the parameters measured being seen in this group. It was for this reason that this diet was thought to be the most appropriate for further study.

The materials comparison study reported in Chapter 3 was prompted by a review of the literature which suggested that denture polymers may be potentially irritant to tissues, either as a result of their constituents or as a result of their ability to harbour organisms. Previous biocompatibility testing in animal models has looked at the effect of the materials in situations which do not equate to normal clinical use. The tests have also been carried out on different tissues (Passeri & de Carvalho, 1985; Harsanyi et al, 1991). The keratinised epithelial coverage of palatal denture bearing mucosa exhibits limited permeability and this may provide greater protection against the ingress of potentially irritant or toxic materials than other non-keratinised tissues.

The materials used in the current study were chosen because they are examples of regularly-used soft and hard chairside relining materials. Soft relining materials are acknowledged to leach plasticiser and to have a potentially porous surface, while hard relining materials may also exhibit increased porosity in addition to possibly inducing epithelial changes as a result of their exothermic setting reaction, although the effects of this may not be obvious fourteen days after insertion.

However, the results described in Chapter 3 show only limited effects of "chairside" relining with such materials. Differences were seen between the basement membrane lengths in the hard and soft relined groups, with the hard having a significantly longer basement membrane length than the soft relined group. This suggests a difference in their mechanical effect on the underlying tissues.

Given that the hard relined appliance more closely resembles a conventionally-processed acrylic prosthesis, these findings differ from

some previous human studies (Kapur and Shklar, 1963; Watson and MacDonald, 1982) which suggest that dentures produce a more regular basement membrane by reducing mechanical stimulation.

The only other difference found was in the compartment ratio in the two test groups as compared to controls. This suggested that the keratinised compartment in the relined groups formed an increased proportion of the total epithelial thickness, again perhaps suggestive of a mechanical effect.

It is acknowledged that the analysis carried out is of a quantitative type and it might be expected that further differences could be seen by examining the tissues for evidence of an inflammatory infiltrate. The current study concentrated on effects within the epithelium. Evidence of inflammatory cells in this tissue is seen to only a very limited extent. The specialised techniques required to identify cells of the inflammatory series like Langerhans cells in the epithelium were not used in the present study.

This does however suggest that further work is indicated in this field. Further investigation in this animal model of the effect of prosthesis coverage on the deeper tissues of the connective tissue and bone should be considered. This could be looked at over different time intervals from those used in the present study.

The presence and number of inflammatory cells in both the epithelium and connective tissue when relining materials are applied to overlying appliances, particularly in the first few days after appliance placement, could also be investigated using this model.

Similarly, the effect of alternative materials or alternative methods of processing could be examined in this animal model.

Finally inoculation of *Candida albicans* beneath relined prostheses could also be investigated as previous in vitro studies (Douglas and Walker, 1973; Wright, 1980; Graham et al, 1991c) differ in their findings on the effects on candidal growth in the presence of soft lining materials and an in vivo model of the type used in this study might prove useful.

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