ORAL MUCOSA

With Particular Reference to the Edentulous Mouth

Volume 1 of Two Volumes

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THESIS

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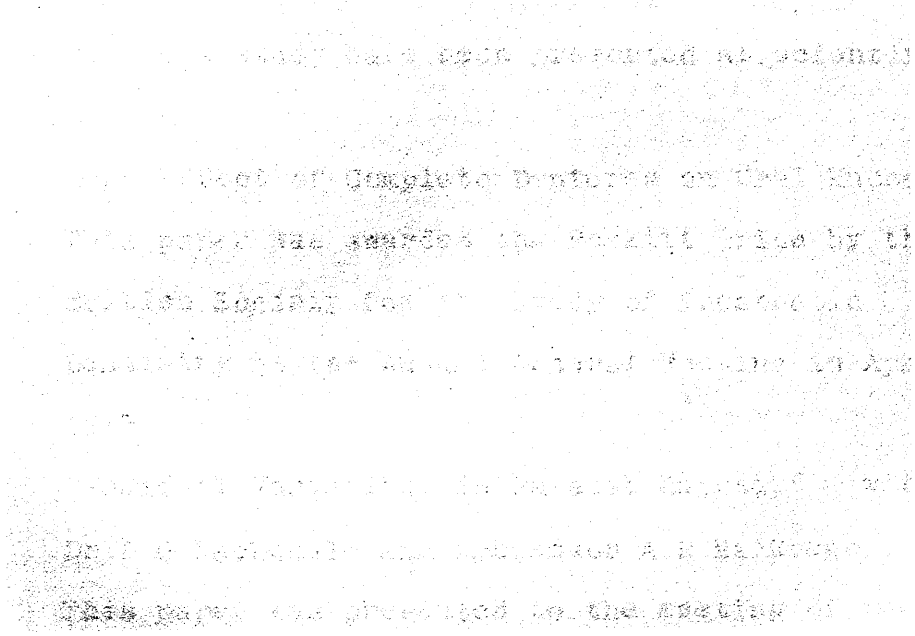

PREFACE

This work was undertaken in the Department of Prosthodontics and the Department of Oral Medicine and Pathology in the University of Glasgow Dental School during the period from October 1974 to September 1977.

Some of the techniques used in this thesis are modifications of previously published work and some are techniques developed by the author in conjunction with the supervisors. The application of these techniques in this work were undertaken by the author personally, except for the preparation of the histological sections which was carried out by technical staff under the supervision of the author.

Parts of this study have been presented at scientific meetings:

1. "The Effect of Complete Dentures on Oral Mucosa." This paper was awarded the Reckitt Prize by the British Society for the Study of Prosthetic Dentistry at the Annual General Meeting in April 1977.
2. "Regional Variations in Palatal Mucosa." with Dr D G MacDonald and Professor A R MacGregor. This paper was presented to the meeting of the European Prosthodontic Association in Amsterdam in March 1978.

SUMMARY

Surveys of adult dental health in recent years have shown that more than one third of the United Kingdom population over the age of sixteen years is edentulous. In spite of this, surprisingly little research work has been carried out into the nature of oral mucosa in the edentulous mouth.

The initial part of this work was to devise a method for the quantitative evaluation of oral mucosa. This was achieved by modifying the techniques of stereology described by Warnakulasuriya (1976). In addition, descriptive analysis was carried out on the stratum corneum to assess the type and degree of keratinisation.

These techniques were used to evaluate the differences between mucosa on the crest of the ridge in the maxillary first molar and mandibular anterior regions in post-mortem material. Intact edentulous palates were obtained post-mortem and cross-sections were prepared in the first molar region. These enabled an assessment to be made of the regional variations in the tissues across the palate from the surface layer to the periosteum.

In order to determine the effects of complete dentures on oral mucosa, it is necessary to have detailed information on the structure of normal oral mucosa.

Quantitative and descriptive analyses were therefore undertaken on a number of patients to provide baseline data. Having established this information, the study was extended to include patients who had been wearing dentures for a number of years and whose oral mucosa was clinically normal. These results enabled conclusions to be drawn on the effects of complete dentures on oral mucosa.

Throughout this work the effects of the age and the sex of the individuals on the mucosa were evaluated. In addition, in the clinical studies, the influence of the number of hours of denture-wearing per day, the length of denture experience and the smoking habits of the individuals were assessed.

CHAPTER ONE

ORAL MUCOSA AND DENTURES

1.1 INTRODUCTION

Oral mucosa is a tissue of fundamental importance to the practice of prosthodontics. The precise nature of its structure and regional variations are, however, not fully known, particularly in the edentulous mouth. Indeed it is not certain whether oral mucosa under a denture should be regarded as being in a physiological or pathological state.

Before discussing oral mucosa in relation to dentures, it is necessary to describe the histological features of normal oral mucosa.

1.2 NORMAL ORAL MUCOSA

1.2.1 General Features

Oral mucosa consists of a superficial oral epithelium and an underlying connective tissue layer, the lamina propria. The junction between the epithelium and the lamina propria, the epithelial-connective tissue interface, is the site of the basement membrane. This is an undulating boundary produced by the

interdigitation of the epithelial rete ridges with the papillae of the lamina propria.

1.2.2 Oral Epithelium

The oral epithelium is a stratified squamous epithelium. The cells in contact with the basement membrane are called the stratum basale or the basal cell layer. Above this layer is the stratum spinosum which consists of larger cells with a spiny appearance, giving the layer its name. The next layer, which is not present in all oral epithelia, is the stratum granulosum. This is a layer of flattened cells which contain basophilic keratohyalin granules in their cytoplasm. In keratinising epithelium the superficial layer is the stratum corneum, which consists of markedly flattened hexagonal discs or squames.

Three types of surface specialisation have traditionally been described (Squier et al, 1975). In orthokeratinisation the stratum corneum lacks nuclei or other organelles and consists of squames entirely filled with keratin. In parakeratinisation the stratum corneum stains like keratin with eosin but contains pyknotic nuclei. Another feature of parakeratinisation is that keratohyalin granules are either sparse or absent in the stratum granulosum. In the third type, non-keratinising oral epithelium, keratohyalin granules are rarely found and the superficial cells retain apparently normal nuclei which are less flattened and

show less eosinophilia than in keratinised epithelium.

1.2.3 Lamina Propria

Two layers are usually recognised within the lamina propria: a superficial zone of loose connective tissue adjacent to the basement membrane and called the papillary layer, and a deeper zone of denser connective tissue which, because of the net-like appearance of the fibre bundles, is called the reticular layer. Both of these layers are penetrated by nerves and blood vessels. Sensory branches of the nerves enter the epithelium but the blood vessels, although they run close to the epithelium, never enter it.

1.2.4 Structural Differences in Oral Mucosa

Oral mucosa is traditionally divided into lining, masticatory and specialised types. Lining mucosa is non-keratinised and is present on the cheeks, lips, the floor of the mouth, the ventral surface of tongue and the alveolar mucosa. Masticatory mucosa is keratinised and occurs on the hard palate and gingivae. Specialised mucosa is present on the dorsum of the tongue.

Because of their entirely different functions as a distensible lining and as a tissue which resists compression and shearing forces, lining mucosa and masticatory mucosa are farther apart in the degree of keratinisation than the extremes found in skin.

No epidermal region is non-keratinised and, even in the sole of the foot, despite the thick stratum corneum, keratinisation is not advanced to the same degree as in the hard palate (Alvares and Meyer, 1971).

The lamina propria in masticatory mucosa is thick and dense and is bound tightly to the underlying bone. The submucosa varies in different regions of masticatory mucosa, being absent in gingiva but present in most areas of the palate where it contributes to a tightly bound but resilient tissue comparable to palmar and plantar skin (Squier et al, 1975). Provenza (1964) was of the opinion, however, that the recognition of a true submucosa was unjustified because the oral mucosa lacks the muscularis mucosa which is present in the gastrointestinal tract, where it separates the lamina propria from the underlying connective tissue of the submucosa.

The lamina propria in lining mucosa is thin and elastic and variously bound to underlying structures by a submucosa, so that there are differences in texture and elasticity.

The lamina propria in the specialised mucosa of the dorsum of the tongue is such that it firmly attaches the epithelium to the underlying muscle by a thin but densely fibrous layer. There is no distinct submucosa.

1.2.5 Regional Differences in Oral Mucosa

In the oral cavity with its complement of teeth, oral mucosa is subdivided into several regions: alveolar, gingival, palatal, floor of the mouth, lip, cheek and tongue (Orban, 1976).

The mucosa of the lip and cheek is a lining mucosa with non-keratinised epithelium and has a lamina propria of dense connective tissue. The submucous layer connects the lamina propria to the fascia of the muscles.

The mucosa of the lip and cheek reflects from the vestibular fornix to the alveolar mucosa covering the alveolar bone. The mucous membrane covering the outer surface of the alveolar process is loosely attached to the periosteum and its epithelium is non-keratinised. The alveolar mucosa is separated from the attached gingiva by the mucogingival junction.

The gingivae extend from the mucogingival junction to the dentogingival junction and are subjected to friction and pressure in the process of mastication. The morphology of the epithelium, which is keratinised, and the lamina propria, which is dense, indicates that the gingival tissue is adapted to these forces. On the inner surface of the lower jaw a line of demarcation is found between the gingivae and mucosa of the floor of the mouth. On the palate the demarcation between the

gingivae and the palatal mucosa is less distinct.

The gingivae can be divided into the attached gingiva, the free gingiva, the interdental papilla and the dentogingival junction. The attached gingiva is characterised by a surface that appears stippled due to the firm attachment of the epithelium to the deeper connective tissue. This results in shallow depressions with portions of epithelium which appear to be elevated. The epithelium is keratinised. The attached gingiva is separated from the free gingiva by the free gingival groove which runs parallel to the gingival margin at a distance of about 1mm. The free gingiva extends from the free gingival groove to the gingival margin. The inderdental papilla is the part of the gingiva that fills the space between two adjacent teeth. The dentogingival junction is the region of epithelium between the gingival margin and the tooth. From the gingival margin to the bottom of the gingival sulcus it is lined by sulcular or crevicular epithelium. The epithelium from the base of the sulcus to its most apical extension is called junctional epithelium (Osborn and Ten Cate, 1976).

On the palatal aspect of the upper teeth the gingiva blends with the palatal mucosa. The palatal mucosa is tightly fixed to the underlying periosteum and the epithelium has a keratinised surface. Two regions of the hard palate can be recognised; the palatine raphe

extending posteriorly from the incisive papilla and the lateral zone between the raphe and the free gingiva (Orban, 1976).

The gingiva and palatine raphe have no submucosa, with only lamina propria and periosteum under the epithelium. A submucosa is present in the lateral zone of the hard palate. Despite this submucosa, the mucous membrane is immovably attached to the palatal periosteum. This attachment is formed by dense bands or trabeculae of fibrous connective tissue that join the lamina propria to the periosteum. The submucous space is thus subdivided into intercommunicating compartments of various sizes. These are filled with adipose tissue in the anterior part, and with glandular tissue in the posterior part of the hard palate. The glandular layers of the hard and soft palates are continuous.

At the junction between the alveolar process and the horizontal part of the hard palate, the anterior palatine vessels and nerves course, surrounded by loose connective tissue. This wedge-shaped area is large in the posterior part of the palate and smaller in size in the anterior part.

The mucous membrane on the oral surface of the soft palate is highly vascularised and reddish in colour, noticeably differing from the pale colour of the hard palate. The papillae of the connective tissue are

few and short. The stratified squamous epithelium is non-keratinised. The lamina propria shows a distinct layer of elastic fibres separating it from the submucosa. The latter is relatively loose and contains an almost continuous layer of mucous glands. It also contains taste buds. Typical oral mucosa continues around the free border of the soft palate for a variable distance and is then replaced by nasal mucosa which has a pseudo-stratified ciliated columnar epithelium.

The mucosa of the floor of the mouth and the ventral surface of the tongue is thin, non-keratinised and loosely attached to the underlying structures to allow free mobility. The dorsal surface of the tongue has a highly specialised keratinised mucosa with papillae and taste buds.

1.3 THE EFFECT OF EXTRACTION OF TEETH

1.3.1 Initial Effects

The immediate effects of the extraction of a tooth and the healing of the wound during the first twenty-one post-operative days are well described by Shafer et al (1974). They pointed out that the healing of an extraction wound does not differ from the healing of other wounds of the body except that it is modified by the peculiar anatomical situation which exists after

the extraction of a tooth. They described how the immediate result of extraction is the coagulation of the blood which fills the socket. Within the first twenty-four to forty-eight hours, the surface of the blood clot is covered with a thick layer of fibrin and there is vasodilatation of the blood vessels in the remnants of the periodontal membrane. Within the first week, the epithelium at the periphery of the wound exhibits evidence of proliferation in the form of mild mitotic activity and the crest of the alveolar bone shows osteoclastic activity. At seven days there is extensive proliferation of epithelium over the surface of the wound, although the wound is not completely covered. By fourteen days the surface of the wound has become completely epithelialised.

1.3.2 Long Term Effects

The long term effects of the extraction of teeth have been reviewed by Watt and MacGregor (1976). The rate of change of ridge contour was described by Watt and Likeman (1974) and by Likeman and Watt (1974) in a longitudinal study of casts of edentulous patients. They pointed out that 72% of the change in shape of the ridge occurred in the first year after extraction, that 8% occurred between one and two and a half years and that a further 20% occurred between two and a half and seventeen years. They also showed that two and a half years after the extraction of the maxillary teeth

the only part of the denture-bearing area unaffected by change was an area in the centre of the palate. They stated that this area was roughly bounded by a line drawn on the surface of the palate at a horizontal distance of about one centimetre from the palatal gingival margins of the teeth.

1.4 SOURCES OF MATERIAL FOR EXAMINATION

1.4.1 Introduction

Human oral mucosa may be obtained for examination from necropsy, biopsy or from smears taken for cytological examination. Oral mucosa may also be obtained from animals for analysis. Each of these sources has its advantages and disadvantages.

1.4.2 Post-mortem Material

The advantage of post-mortem material is that it can be obtained in large sections so that the relationship between different areas can be established. The main disadvantage of this method is that when the interval between death and the fixation of the material is long, post-mortem changes take place, rendering the results inaccurate. These changes occur particularly in the epithelium. Another difficulty with this method is that detailed information on the previous dental history is often unavailable. In addition, the terminating illness, particularly if prolonged, may have affected the oral mucosa.

Pendleton, in a series of papers between 1931 and 1951, described the microscopic anatomy of the edentulous maxilla and mandible. His studies were based mainly on post-mortem material. He obtained palates from necropsies and produced sagittal and coronal sections. As a prosthodontist, he was able to apply clinical considerations to his detailed anatomical work.

Frohlich (1958) reported on alveolar bone changes in a post-mortem histological investigation, but made no mention of epithelial changes. Fish (1962) carried out a combined biopsy and post-mortem study but made no comment on any differences between the groups.

Van Scotter and Boucher (1965), in a post-mortem study, examined palatal mucosa and, in particular the stratum corneum. They did not discuss the difficulties of post-mortem changes which were likely to affect the stratum corneum but did note that the specimens were obtained "as soon after death as authorised".

1.4.3 Cytological Material

The advantage of cytology is that the method of collecting the material by smear is simple, easy to use, and not unpleasant for the patient. In addition, repeated sampling from the same site can be carried out. One of the disadvantages of the method is that it does not demonstrate the material in its anatomical relationship. It is also difficult to quantify the

the results, and comparative results may vary according to the precise method of sampling and staining.

Exfoliated oral epithelial cells were studied by Miller in 1890. Cytology was used by Weinmann (1940) to study the keratinisation of oral mucosa from the palate, gingiva, tongue and cheek and by Trott (1958), Miller et al (1951) and Derbyshire and Mankodi (1964) to study gingival keratinisation. Cytology has also been used by Al-Ani et al (1966), Markov (1968) and by McMillan (1971) to assess the response of palatal and buccal mucosa to the presence of dentures.

1.4.4 Biopsy Material

The biopsy is the best method since it gives the anatomical relationships of the elements of vital tissue which can be fixed immediately after the biopsy has been taken. The disadvantages of this method are that the same site cannot be re-examined, that only relatively small pieces of tissue can be examined and that the method is uncomfortable for the patient. These disadvantages, however, can be overcome to a large extent, or at least minimised. It is possible to examine the other side of the mouth at a later time to obtain comparative information. If handled correctly and carefully, small specimens can give the required information and the specimens can be kept sufficiently small so that the patients suffer little post-operative discomfort. This method was used by

Orban (1930) and is the technique preferred by most authors.

1.4.5 Animal Material

The advantages of using animals for experiments are that the experiments can be controlled more effectively, large amounts of tissue can be available and the timing of the experiments can be conveniently arranged. The disadvantage of using animals is that the information obtained cannot be applied directly to the human situation because of differences between animal mucosa and human mucosa. In relation to dentures, there is the added disadvantage that it is almost impossible to construct for an animal a denture which will be tolerated and used in the same way as a denture is used by a human.

Alvares and Meyer (1971) examined the extent of keratinisation of palatal and buccal mucosa in the rat and pointed out that the buccal mucosa in the rat is keratinised, whereas human buccal mucosa is non-keratinised.

Cleaton-Jones and Fleish (1973) carried out a comparative study of the extent of keratinisation in different intra-oral sites in the vervet monkey. They found that the crests of the rugae in the hard palate had an orthokeratinised epithelium and that the valleys between the rugae had a parakeratinised epithelium. Fejerskov (1973) found that the palatal epithelium in guinea pigs

was orthokeratinised. Appleton and Heaney (1977) found that the palatal epithelium in pigs was also orthokeratinised.

McHugh (1964) carried out a comparative study on gingival keratinisation in man, rat, hamster and monkey. He found that in the rat and hamster the keratinisation of attached gingival epithelium and skin epithelium appeared identical in type and degree, whereas in the monkey and man the process of keratinisation in the attached gingival epithelium was similar in many respects to that of skin but was less complete. He also found that the crevicular epithelium in the rat and hamster was normally keratinised but in the monkey and man it was not.

1.5 VARIABLE FACTORS WHICH AFFECT ORAL MUCOSA

There are many factors which may affect the condition and appearance of oral mucosa. Among the most important are the particular site being examined, the age and sex of the patient, his smoking habits and the presence of intra-oral or systemic disease.

1.5.1 Site

As described in Section 1.2.5, there are regional differences in oral mucosa. Meyer and Gerson (1964) carried out a comparison of human palatal and buccal mucosa and Alvares and Meyer (1971) described the

variable features and regional differences in the oral epithelium of the dentate mouth. Regional differences persist in the edentulous mouth as shown by Pendleton (1934) but no quantitative study has been carried out in the edentulous mouth.

1.5.2 Age

All tissues undergo gradual and cumulative changes with age. Jamieson (1958) observed that aging begins at birth. It is believed that the epidermis becomes thinner with advancing age, but detailed studies of age-related changes in the oral epithelium are few and contradictory.

Massler (1956) found a tendency to hyperkeratosis in oral epithelium with age but Wentz et al (1952) found that there was no significant change in keratinisation with age. Zimmermann and Zimmermann (1965) stated that age brought about a decrease in keratinisation.

In a group of patients over the age of sixty years, Shklar (1966) found that the epithelium was thinner in all areas of the mouth and that the rete ridges were shorter than in his group of younger patients. He also observed that the palatal keratinisation was hyperkeratotic. He could usually predict the patient's age group by examination of histological sections if

the patient were over sixty or under twenty-five. In the intervening age group the tissue patterns varied considerably and could resemble those of either youth or old age, including, in some instances, features of both groups.

Kydd et al (1974) found that age had no effect on the extent to which tissue could be compressed but that age did have a significant effect on tissue recovery from compression such that, in older people, recovery took longer. Lammie (1956) observed that the oral epithelium showed a reduction in the number of cells with age but did not give any data to support this conclusion.

1.5.3 Sex

Cyclical changes due to variations in the oestrogen concentrations occur in the keratinisation of the vaginal epithelium to the extent that the phases of the menstrual cycle can be studied by changes in keratinisation of the vaginal epithelium (Papanicalaou, 1933). Since structural similarities exist between oral and vaginal epithelia it has been suggested that the influence of sex hormones on both should be similar. However, Trott (1957) in a histological study, and Montgomery (1951) and Trott (1958) in cytological studies, found no relationship between the menstrual cycle and keratinisation in the oral epithelium.

Shklar (1966) and Montgomery (1951) found no sex-related differences in oral mucosa. Wentz et al (1952) found differences in basement membrane shape between males and females in the age group fifteen to twenty-nine years but this was not evident in the age group thirty to forty-four years.

Jani and Bhargava (1976) excluded female subjects from their study on the effects of complete dentures on oral mucosa because they were of the opinion that there were "changes in the mucous membrane associated with the menstrual cycle, pregnancy and the menopause".

Markov (1968), in a cytological study of keratinisation under dentures, found the degree of keratinisation to be higher in males than in females but this result was not statistically significant. However McMillan (1972), also in a cytological study, found that the degree of keratinisation of palatal epithelium covered by dentures was significantly less in females than in males.

1.5.4 Smoking

There is now little doubt that the most important factor in the dramatic rise in the incidence of bronchial carcinoma is the habit of smoking, particularly of cigarettes (Muir, 1976). The relationship between smoking and oral cancer is less clear, but from the evidence available it seems that there is an association between oral cancer and pipe smoking, and possibly cigar

smoking. There is, however, conflicting opinion with regard to cigarette smoking (Binnie, 1975).

Nicotine stomatitis was described by Thoma in 1941. Some authors consider it merely as a type of leukoplakia but several authors, including Pindborg et al (1971), have shown that it is directly related to smoking. Ramula et al (1973) have shown that nicotine stomatitis is common in individuals practicing reverse smoking, in which the lighted end of a cigarette is held within the mouth.

The effects of smoking on normal oral mucosa have been examined, in a cytological study, by Zimmermann and Zimmermann (1965) who found that smoking increased keratinisation in the gingiva, hard palate, tongue and buccal mucosa. Wrubel and Scopp (1961) were unable to detect any cytological changes in the keratinisation of palatal or buccal mucosa following cessation of smoking in previous smokers.

Al-Ani et al (1966) found no significant differences in the palatal mucosa of smokers and non-smokers, neither of which group wore dentures. They did not comment on the effect of smoking on the palatal mucosa of their denture-wearing patients. Markov (1968) found that edentulous patients with and without denture experience, showed higher keratinisation

values in smokers than in non-smokers.

1.5.5 Oral Disease

There are many diseases which specifically, or to some extent, affect the oral mucosa. Candida albicans may occur as a commensal organism in the mouth but the host defence mechanism normally prevents invasion of the tissues. In the presence of a "local disturbance" or systemic illness the organism can become pathogenic (Walker, 1975).

Herpes simplex and viruses of the coxsackie type are examples of viruses which have specific effects on the oral mucosa. Among the bacteria which can infect the oral tissue are the tubercle bacillus and Treponema pallidum.

Lichen planus, aphthous ulceration, pemphigus and squamous cell carcinoma are other conditions which can affect oral mucosa.

1.5.6 Systemic Disease

Many diseases involving the whole body can produce, at some stage in their development, oral changes, albeit often slight and overshadowed by the major manifestations of these diseases. The oral changes may be part of the primary disease process, or they may be a complication of it (Jones, 1975). In

particular, diseases of the blood and haematopoietic system can produce intra-oral changes.

1.5.7 Combined Variables

It is evident that there are many factors which may affect oral mucosa, sometimes in different directions. It is, therefore, difficult to investigate each factor separately. When the effect of a denture is added to the factors mentioned above, the situation becomes even more complex.

1.6 DENTURES AND ORAL MUCOSA

Oral mucosa is not normally covered by dentures. When covered by a denture it is sandwiched between the denture and the underlying bone. Although protected from the direct effects of stimulation by food it is subjected to the traumatic effect of the denture. This varies considerably depending on the circumstances of each case.

It is not surprising that there is no general agreement on the effects of dentures on oral mucosa since there are many factors which may alter the effects. Among the factors which vary are the type of denture, the type of denture base material, the denture hygiene, the number of hours of denture-wearing each day, the length of denture experience, the condition of the

denture and the dentition in the opposing arch.

In addition to all these variable factors, the site selected for examination is likely to affect the results. It is largely for this reason that the results from the work on the effects of dentures on oral mucosa are difficult to interpret.

1.6.1 Type of Denture

Since partial dentures and complete dentures cover varying amounts of tissue, and are supported by the tissues in different ways, it is likely that the effects of these types of denture on oral mucosa will differ. It is not surprising, therefore, to find that, whereas Turck (1965) found an increase in the thickness of the stratum corneum under partial dentures, Hedegard (1962) found a reduction in keratinisation under complete dentures. Kapur and Shklar (1963) found that their immediate complete dentures increased the thickness of the stratum corneum whereas Ostlund (1958) found that complete dentures reduced the thickness of this layer.

1.6.2 Denture Base Material

Stansbery (1928) was of the opinion that vulcanite, when improperly vulcanised, had a rough surface which would damage oral mucosa to a greater extent than completely processed vulcanite. Hedegard (1955) examined the

effect of dentures made in self-curing acrylic on oral mucosa and concluded that self-curing acrylic was no more harmful than heat-cured acrylic.

Van Scotter and Boucher (1965) compared the effects of vulcanite and of methyl methacrylate on oral epithelium and found that vulcanite was more damaging. Van Huysen et al (1954) drew attention to the very damaging effect of a rubber suction disc on oral mucosa.

1.6.3 Denture Hygiene

Love et al (1967) found that poor denture hygiene had a greater effect on the oral mucosa in younger patients than in older patients and was more likely to produce clinically inflamed mucosa. Turck (1965) noted the oral and dental hygiene standards of his patients but he did not relate these to his results.

1.6.4 Hours of Denture-wearing per Day

Markov (1968) found that if dentures were regularly removed at night, keratinisation was increased. He believed that rest made it possible for the oral mucosa to recover from the wear and tear of dentures. This view was also held by Wannenmacher (1958).

Love et al (1967), in a subjective study, found that the removal of dentures at night considerably reduced the incidence of clinically inflamed mucosa.

Bergman et al (1971) examined two groups, each of thirty patients. One group wore their dentures both during the day and at night, and the other wore them during the day only. Clinical examination revealed no significant differences between the groups. Histological differences between the groups were not recorded.

1.6.5 Length of Denture Experience

Ostlund (1958) found that within the first six months of denture experience most complete denture patients showed clinically normal mucosa. Despite this appearance, however, histological changes were pronounced. He found a progressive decrease in the thickness of the stratum corneum and a tendency towards parakeratosis.

In cytological studies Markov (1968) found that the degree of keratinisation decreased with the length of denture experience, but McMillan (1971) found no relationship between keratinisation and the length of denture experience.

1.6.6 Condition of the Denture

Nyqvist (1952) was of the opinion that "the potential traumatising properties of dentures, such as poor fit, instability and poor occlusion and articulation were important contributory causes of stomatitis prosthetica".

This being the case, it is likely that less severe denture faults will give rise to less marked, though significant, histological changes.

Love et al (1967) were of the opinion that the "fit" of a denture had more influence on the condition of the mucosa than any other factor. Kapur and Shklar (1963) stated that a "well adapted" denture stimulated the underlying mucosa and was not irritant. Jani and Bhargava (1976) concluded that the oral mucosa responded favourably to well-constructed dentures.

1.6.7 Opposing Arch

Since the forces applied to a denture depend to some extent on the opposing dentition, it is likely that a complete upper denture opposed by a complete lower denture will have a different effect on the palatal mucosa from that of a complete upper denture opposed by lower natural teeth.

Pendleton (1937) described a case of a complete upper denture opposed by five teeth in the lower jaw and stated that the bone, connective tissue, and epithelium of the upper jaw had reacted "favourably" to the forces applied to them.

Fish (1962) found that, in patients who had a complete upper denture opposed by natural teeth, the epithelial surface covered by the denture was almost completely

"denuded of keratinised cells". He suggested that "the physiological limit for the epithelium had been exceeded".

1.6.8 Sites Examined

Since the work of Pendleton in 1940 it has been known that there is a considerable regional variation in the structure of the oral mucosa of the edentulous maxilla. It is not surprising, therefore, that since different authors have examined different sites their results are not in agreement.

Turck (1965) took two biopsies from partially edentulous patients; one from the tissue of the edentulous ridge, the other from tissue surrounding the natural teeth. Each biopsy was obtained as a narrow strip of tissue extending from the height of the gingival crest or edentulous ridge to the alveolar mucosa. Most of his biopsies were taken in the canine and premolar areas, some in the upper jaw and some in the lower jaw. Hedegard (1962) and Carlsson et al (1967) examined the upper labial gingiva, attached mucosa and alveolar mucosa.

Fish (1962) examined crestal mucosa from edentulous ridges and from smaller edentulous areas bounded by natural teeth. Kapur and Shklar (1962 and 1963) examined mucosa from the crests of alveolar ridges in partially edentulous patients and followed the progress

of these areas after the patients had been wearing complete immediate dentures for three months. Nedelman et al (1970) examined the alveolar ridge mucosa in denture-wearing and non-denture-wearing patients.

Most of the work which has been carried out using exfoliative cytology has been in relation to palatal mucosa but the authors seldom stated from which area of the palate the smears were taken.

The palate has been examined by histological means by Jani and Bhargava (1976), Pendleton (1930), Ostlund (1958), Van Mens et al (1975), Van Scotter and Boucher (1965) and Sillevis Smitt (1973). However, since these authors did not examine the same sites, direct comparisons between these results should be made only with caution.

Ostlund (1958) examined mucosa just anterior to the vibrating line between the hard and soft palates, as near to the midline as possible, because he believed that this was a region damaged by dentures. Van Mens et al (1975) obtained their biopsies from the hard palate, about halfway between the position of the first molar and the midline. The reason for selecting this area was that it was felt that, because of the distribution of the nerves and blood vessels, it was an area which remained in good condition even

after a considerable period of denture-wearing. It is clear that Ostlund and Van Mens et al chose their sites for directly opposite reasons.

Jani and Bhargava (1976) examined the same site as Van Mens et al (1975).

Van Scotter and Boucher (1965) obtained tissue 1 cm x 2 cm from the premolar area. Sillevis Smitt (1973) selected as his biopsy site an area 1 cm away from the midline in the first molar area.

1.7 KERATINISATION

1.7.1 Type of Keratinisation

As described in Section 1.2.2, three types of surface specialisation of oral epithelia have been described, namely orthokeratinisation, parakeratinisation and non-keratinisation.

1.7.2 Degree of Keratinisation

The concept of degree of keratinisation was described by Alvares and Meyer in 1971. They pointed out that the concept of keratinisation of stratified squamous epithelium, varying by degree or steps rather than as an all-or-none event, was first proposed by Weinmann in 1940. They stated that the stratum corneum of different regions of oral mucosa, whether parakeratotic

or orthokeratotic, showed one of two kinds of staining behaviour when treated with Mallory's connective tissue stain or with a number of other stains which stain keratin differently from other layers. The stratum corneum either stained a uniform red or orange throughout its thickness or a layered red or orange, and blue. In non-keratinising regions the whole epithelium stained blue like the cellular layers of keratinising regions.

The uniform keratin staining was described as complete orthokeratinisation or complete parakeratinisation, and the non-uniform staining as incomplete orthokeratinisation or incomplete parakeratinisation. This gave four degrees of keratinisation, while non-keratinisation was the fifth and lowest degree. Since the inner keratin layer of the incomplete variant may, in different regions, vary from a single row of cells to a large proportion of the whole stratum corneum, a continuous spectrum of degrees was thought to exist. Since, in the complete variant, the staining of the stratum corneum was identical to the staining of hair shafts, Alvares and Meyer (1971) suggested that this variant represented a higher degree of keratinisation than the incomplete types. They also suggested that, in regions of incomplete keratinisation, an unstable type of keratin was formed which, under the action of saliva,

resumed the staining behaviour of the cellular layers, whereas in regions showing uniform staining the keratin was resistant to this influence. Evidence supporting this interpretation was seen in: (1) the fact that in conditions known to inhibit keratinisation in the gingiva, for example inflammation, the degree of keratinisation reduced with the degree of inhibiting conditions; (2) the fact that the incomplete variant was commonly seen intervening between the complete variant and non-keratinisation; (3) the fact that in vitamin A deficiency all regions of rabbit oral mucosa are converted from being non-keratinised or incompletely keratinised to being completely keratinised.

1.7.3 Dentures and Keratinisation

The published work on the effects of dentures on the oral epithelial surface has, for the most part, not used the five degrees of keratinisation described above, but has used only the three types: namely, orthokeratinisation, parakeratinisation and non-keratinisation. Over the years there have been a number of attempts to assess the effects of dentures on the oral epithelial surface. A few of these are well-documented experimental projects but many are little more than hypotheses backed up by little evidence. Most of this literature deals to a large extent with keratinisation and is therefore reviewed in detail in this section. The following sections

deal with other aspects of the literature on oral mucosa in relation to dentures.

Stansbery (1928) commented that "the change of tissue character which takes place in the edentulous mouth is natural and normal to this condition and one must not expect conformity with the condition of a mouth containing its complement of teeth". Wright (1929) noted that the pressure applied to dentures was distributed to the epithelial tissues, which reacted proportionately to the amount of pressure and the manner in which it was applied.

Pendleton (1931, 1932, 1934, 1935, 1940, 1946, and 1951) carried out detailed post-mortem studies of the edentulous mouth. His contribution to the literature on the subject of the anatomy of the edentulous mouth is outstanding because he was able to describe the microscopic and macroscopic anatomy from the viewpoint of both an anatomist and a prosthetist. He said, "In the science of dentistry as well as medicine, a knowledge of normal structure is necessary to the successful termination of any clinical procedure". His work did much to increase the knowledge of anatomy and to influence clinical procedures.

In his first three papers, Pendleton described the microscopic anatomy of the edentulous maxilla. He

pointed out that, since he had studied a relatively small number of cases, they would not represent all of the various structural developments to be found in the edentulous jaw.

In 1940 Pendleton commented that a general examination of the epithelium under dentures revealed a keratinised surface layer at the residual ridge and in the region of the hard palate. In 1951 he reported a study of biopsy material obtained from 126 edentulous individuals. The regions from which the biopsy specimens were taken were the maxillary tuberosity and the labio-buccal surfaces of the crest of the maxillary and mandibular ridges. All the individuals were in good health at the time when the biopsies were taken. The dentures were worn under varying conditions; some of the patients wore partial dentures, some wore complete upper dentures opposed by six or more lower teeth or complete mandibular dentures. All three types of stratified squamous epithelium were found. Parakeratotic epithelium was usually present at the residual alveolar ridge and in the denture-bearing area of the vestibule in the edentulous mouth. Orthokeratinised zones often appeared to be limited to the crest of the residual alveolar ridge. However, orthokeratinised and parakeratinised epithelium were often found alternately in the same field of a given section.

Nyqvist (1952) pointed out that normal mucosa in an edentulous denture-wearing patient had a smooth surface, a keratinised layer and a slight sub-epithelial lymphocytic infiltration. In the type of denture sore mouth which he described as having a clinically smooth surface, the layer of keratin was present and the sub-epithelial lymphocytic infiltration was greater. He described histologically, but not clinically, a type of denture sore mouth in which the epithelium lacked a layer of keratin, the papillae of the lamina propria were nearer the surface than normal and there was marked lymphocytic infiltration.

Van Huysen et al (1954), describing the histological appearance in cases of palatal hyperplasia under dentures, said that the rete ridges were elongated, the surface epithelium between the rete ridges was thin and, in some areas, almost non-existent. There was no ulceration and the stratum corneum was absent. These papers by Nyqvist and Van Huysen et al suggested that, in mucosa severely damaged under dentures, the thickness of the stratum corneum reduced and was eventually lost.

Ostlund (1958) carried out a study to relate the histological appearance of mucosa covered by dentures to its clinical appearance. The biopsy site was just anterior to the "vibrating line" as near the midline as possible because this was a region which he thought

was commonly damaged by wearing dentures. He graded the mucosa as clinically normal, locally inflamed, diffusely red or granulated.

Ostlund examined biopsies from 118 patients with clinically normal palatal mucosa who had been wearing dentures for between six months and fifteen years. It was found that 23% had a stratum corneum similar in appearance and thickness to that of the non-denture wearers, 34% had a thinner stratum corneum than the non-denture wearers and 43% had a parakeratotic epithelium. Females seemed to be more predisposed to such injury than males. He felt it remarkable that, of biopsies taken from patients who had worn dentures for about six months, only 39% could be characterised as clinically and histologically normal. 77% of denture-wearing patients with clinically normal mucosa showed more or less pronounced histological changes in the mucosa and that, in reality, the frequency of such changes was much higher, because only about 40% of denture-wearers accounted for in this sample showed clinically normal mucosa. The other 60% showed more serious histopathological changes.

Ostlund summarised this part of his paper by noting that, in the epithelium under dentures, the thickness of the stratum corneum was firstly reduced in

thickness, then the orthokeratotic epithelium was replaced by a parakeratotic epithelium. Finally in more damaged epithelium, all the keratin disappeared. This paper drew attention to the size of the problem, because over 90% of the complete denture-wearers in this sample showed some histopathological changes related to wearing dentures.

Fish (1962) examined fifty specimens of epithelium from the edentulous alveolar ridge. Forty-two specimens were biopsies and eight were necropsies. The cases were divided into five groups. The first group consisted of biopsies from small bounded saddles, opposed by natural teeth, no denture having been worn. This group in general showed a parakeratotic epithelium with a poorly developed stratum granulosum. The second group consisted of only one specimen and was from the crest of a completely edentulous alveolar ridge, no denture having been worn in either jaw. The keratinised layer was 80 µm thick and the stratum granulosum was more marked. The third group was from the edentulous ridge, complete dentures having been worn and having been satisfactory. The histology of this group suggested that the first response to dentures was the enhancement of the degree of keratinisation. The keratinised layer was not thickened but nuclei were visible only in the deeper layers. The fourth group was from the edentulous ridge, dentures having

been worn for a long time and being in need of replacement. Fish concluded that the normal parakeratinised structure of the edentulous alveolar ridge responded well to the pressure of dentures, although the keratinising process was rarely complete. The fifth group was from the edentulous alveolar ridges of patients in whom upper dentures were opposed by natural teeth. These cases showed a marked variation from those in the other groups. The epithelial surface was almost completely denuded of keratinised cells and, furthermore, an intra-epithelial lymphocytosis was present. These variations were very striking and suggested that "the physiological limit for the epithelium had been exceeded". These features were seen in all cases in this group and in no other group.

The study by Fish (1962) was useful in that it attempted to compare the effects of different types of denture and occlusion on the epithelium. It did not, however, describe the size of the biopsies, the method by which they were removed, or the precise location of the biopsies.

Hedegard (1962) carried out a histological study on the effect of immediate dentures on the attached gingiva. The material consisted of fifteen patients,

six of whom had no denture during the observation period of three months after the extraction of all the remaining teeth, and nine of whom wore immediate dentures. At the time of the extractions, the "midsection" of the labial wall of the upper right central incisor socket was removed and prepared for histological examination. Three months later the corresponding "midsection" of the labial wall of the healing tooth socket of the left central incisor was excised and prepared in the same manner. The group of patients without dentures showed a normal orthokeratinised layer both before and three months after the extractions. In eight of the nine cases in the immediate denture group, the orthokeratotic stratum corneum was altered to a parakeratotic layer. The difference between these groups was statistically significant and the injury to the epithelium was ascribed to the action of the denture. Apart from these changes, the epithelium did not show any marked downgrowth or other changes. Hedegard pointed out that the results related to one area only and, although it was likely that similar changes occurred in other areas of the mucosa, it could not be stated that this was definitely so.

Kapur and Shklar (1962) carried out an investigation to determine the effects of stimulation of the edentulous ridge and mucosa with an automatic toothbrush.

They studied twelve patients, three of whom were completely edentulous and nine of whom had six maxillary anterior teeth and required immediate complete dentures. Stimulation was applied with an automatic toothbrush to each experimental area for fifteen seconds, six days per week for four weeks. The other side of the mouth was not stimulated and was used as a control. Twenty-four hours after the final stimulation, biopsy specimens were taken from identical premolar areas of the control and experimental sides. In each of the twelve patients there was an increased amount of keratin in the experimental edentulous tissue compared with the control tissue. It was thought that the increased keratinisation might serve the mucosal tissues as a means of increased resistance to irritation or injury from various local traumatic influences.

Kapur and Shklar (1963) followed up the nine immediate denture patients for twelve weeks and took biopsies from locations similar to the previous ones in the premolar regions on both sides of the dental arch. The microscopic examination of the specimens before insertion of dentures revealed, in most instances, a distinct stratum corneum. Microscopic examination after the denture had been worn for twelve weeks showed a marked increase in the thickness of the

stratum corneum. The keratin layer was generally orthokeratotic but zones of parakeratosis were occasionally seen. There was evidence of lack of keratinisation of the mucosa in only one patient. There was equal keratinisation in both experimental and control sites, indicating that stimulation prior to the insertion of dentures had no obvious relationship to tissue reaction under dentures.

Kapur and Shklar (1963) pointed out that, for a given period of time, wearing dentures resulted in excellent tissue response with an increase in thickness of the stratum corneum. They commented that their findings were at variance with those of Ostlund (1958), but noted that Ostlund's biopsies had been taken from the "posterior palatal seal area", whereas their biopsies of the ridge presented a more accurate picture of mucosal reactions to well-adapted dentures.

These papers by Kapur and Shklar contained many comments but little detail and no figures for the results. The method of measuring the thickness of the keratin layer was not stated nor was the technique for assessing the amount of inflammation described. Quantitation was expressed in terms of "slight", "moderate", "marked", "greater" and "less" without explanation and definition of these terms. Kapur and Shklar (1963) stated that all their immediate dentures were rated (by them) as

good to excellent for stability, retention, occlusion and denture extension and implied that if dentures constructed by others did not stimulate keratinisation the dentures must be at fault.

Kapur and Shklar (1963) stated that this was an initial survey and that further studies were required and would be carried out. A search of the literature, however, has failed to reveal published reports of such studies.

Turck (1965) investigated thirty-two biopsies taken from the oral mucosa of sixteen patients over the age of fifty years. Each patient was partially edentulous. Eight patients had worn removable partial dentures for at least three years and eight patients had never worn partial dentures. Two biopsies were taken from each patient; one from the tissue covering the edentulous ridge and the other from tissue surrounding the natural teeth. Each biopsy was obtained as a narrow strip of tissue extending from the height of the gingival crest or edentulous ridge to below the mucogingival junction. Most of the biopsies were taken from the canine or premolar regions of the upper or lower jaw. The biopsies were examined in three regions: (1) the alveolar mucosa; (2) the attached gingiva from the mucogingival junction to the free gingival groove from tooth-bearing areas, or the corresponding area of

residual attached mucosa from edentulous ridges; (3) the region over the crest of the edentulous ridge.

No keratin was found in the alveolar mucosa. The mucogingival junction, in most cases, was possible to locate due to the distinct histological differences above and below the junction. However, in the tissue from the edentulous ridge, there was occasionally a gradual transition from one type of epithelium into the other and the mucogingival junction was less well-defined.

Where natural teeth were present, the attached gingiva was usually orthokeratinised but parakeratosis and incomplete parakeratosis often occurred near the mucogingival junction. Variation in keratinisation was characteristic of the residual attached gingiva and crest of the edentulous ridge in non-denture-bearing areas. Orthokeratosis was usually found in the residual attached gingiva of denture-bearing areas and the thickness of the stratum corneum was slightly greater than in the attached gingiva, where natural teeth were present. However, in two patients with ill-fitting dentures keratinisation was entirely absent. Orthokeratosis and parakeratosis occurred at the crest of denture-bearing ridges.

Turck (1965) was of the opinion that "a well-fitting denture could be considered as a protection to the underlying soft tissues." His findings that dentures tended to increase keratinisation in the residual buccal attached gingiva agreed with the findings of Kapur and Shklar (1963), although they all described cases with loss of keratinisation after wearing dentures. Turck was of the opinion that dentures protected the underlying mucosa, while Kapur and Shklar were of the opinion that dentures stimulated keratinisation.

Al-Ani et al (1966), in their cytological study found that denture-wearing resulted in a decrease in keratinisation of the palatal mucosa but the buccal mucosa showed no significant changes. They stated that a denture was a "foreign body" and that the tissue with which it was in contact was subjected to irritation and mechanical trauma. It is interesting to note that Shklar was a co-author of this paper and also the co-author of the papers by Kapur and Shklar (1962 and 1963) described earlier. Presumably Shklar changed his view on the effect of dentures on the keratinisation of oral mucosa between the first papers and this one. One possible explanation for this could be that the Kapur and Shklar papers dealt with gingival mucosa while this paper dealt with palatal mucosa.

Thomson (1967) was of the opinion that, under complete dentures, there was a greater degree of keratinisation on the crest of the alveolar ridge than on the lateral aspect of the ridge. He felt that the direct load-bearing areas of the ridge and palate suffered less than the tissues on the lateral aspect of the ridge which were subjected to trauma during tilting and lateral movements of dentures.

Carlsson et al (1967) investigated thirty patients who were having their remaining upper teeth extracted; seventeen received complete upper immediate dentures and thirteen patients were without dentures during the observation period. At the time of the extractions, the "midsection" of the labial wall of the upper right central incisor socket was removed and prepared for histological examination. After an interval of between three and 210 days, the corresponding "midsection" of the region where the upper left central incisor had been present, was excised and prepared in the same way. The attached gingiva was selected for detailed examination. The type of keratinisation of the epithelium often differed within the same specimen and they termed this "local keratinisation".

Of the first specimens, eight exhibited general keratosis, ten "local keratosis" and eleven para-keratosis, while in one patient the surface layer

consisted only of flattened cells and showed complete absence of keratinisation. In the group not wearing dentures during the observation period one changed from parakeratosis to keratosis, one changed from "local keratosis" to keratosis, and two changed in the other direction from keratosis to parakeratosis. The other nine cases showed no change and the mean change was not significant. In the immediate denture group, one changed from parakeratosis to keratosis; in three cases there was a change from general or "local keratosis" to parakeratosis and in two other cases the changes were so pronounced that practically all evidence of keratinisation had disappeared. The mean change was in the direction of decreased keratinisation.

Carlsson et al summarised their paper by stating that in the group of patients not wearing dentures there was no change in keratinisation, whereas in the patients wearing immediate dentures there was a significant reduction in keratinisation.

1.8 EPITHELIAL THICKNESS

1.8.1 Methods of Analysis

Histological analysis may be descriptive or quantitative. In descriptive analysis the examiner comments on what he observes. It is a poor way of

assessing epithelial thickness because it is entirely subjective. Quantitative analysis is a better method of evaluating epithelial thickness since it enables the examiner to arrive at numerical values. The results obtained by quantitative analysis are only useful if the methods used are valid and if they have been correctly applied. In order that a paper may be properly assessed, it is necessary for its author to present in detail the method of analysis of the material, the results and the way in which the results are analysed. This is not always done.

In some studies the technique used is not adequately described and the validity of the method is uncertain. Turck (1965) used an ocular micrometer but did not say how he used it or how many measurements he made on each section. The validity of the results is uncertain since he described "average thickness of epithelium," and "average length of rete pegs" without explaining how he calculated the average values.

In other studies the techniques used are described well and the methods of calculating the results are fully explained but the validity of the technique is suspect. Jani and Bhargava (1976) described how three sections from each biopsy of palatal mucosa were mounted on microscope slides. The rete ridge length and inter-rete ridge thickness were measured

once on each section using an eyepiece micrometer which had been calibrated with a microscope slide micrometer. The mean of the three measurements from each of the biopsies was calculated to obtain what they called the mean thickness of the rete ridge and inter-rete ridge regions. They did not say how they decided which particular area to measure on each section,and in the histological sections shown in their paper there was a considerable difference in rete ridge length. In view of this and the fact that only three sections from each biopsy were examined it is likely that the results are not representative of the epithelium as a whole.

In some instances the techniques used are well described and the methods of calculating the results are accurate but the techniques are slow and laborious. Van Mens et al (1975) used a photomicrographic method to measure epithelial thickness in their investigation of palatal biopsies from forty patients. The subjects were ten male and ten female denture wearers of about sixty years of age, and ten male and ten female non-denture wearers of about forty years of age. Each of the denture wearers had worn complete dentures for longer than four years. Only patients who were in good general health and had clinically normal mucosa were selected. The biopsies were taken from the hard palate, about halfway between the position of the first

molar and the midline, with a trephine-type biopsy instrument, having a diameter of 1.5 mm. Five sections from each specimen were mapped out photomicrographically at a magnification of 900. Sections of the photographs, 100 mm x 150 mm, were then traced on drawing paper, the stratum corneum was cut off and the pieces weighed. The weight of the pieces of paper gave an indication of the mean thickness of the epithelium being examined. They expressed their results as weight of drawing paper but did not convert these figures into thickness of epithelium. This is an accurate but tedious method of analysis and their results cannot be used in comparison with other work.

Another accurate method of measuring mean epithelial thickness is to use a planimeter to measure the area and to divide this by the width of the section in order to calculate the thickness. This is only valid, however, if all the procedures are carried out correctly. This method was used by Meyer and Gerson (1964) and by Ostlund (1958). Ostlund, however, divided the area obtained by the planimeter by the surface length. This is not strictly accurate since the surface length is not the same as the width of the section, particularly where the surface has an undulating shape.

Meyer and Schroeder (1975) used a stereological method to measure the thickness of the epithelium.

1.8.2 Results of the Studies of Epithelial Thickness

Meyer and Schroeder (1975) obtained ten biopsies from female patients between the ages of nine and sixteen years. The biopsy site was halfway between the gingival margin and the palatine raphe level with the mesial surface of the upper first molar. The mean thickness of the epithelium was 0.25 mm, the mean length of the rete ridges was 0.31 mm and the mean thickness of the epithelium between the rete ridges was 0.12 mm.

Meyer and Gerson (1964) examined fifteen biopsy specimens of normal buccal mucosa and five biopsy specimens of normal palatal mucosa. They found that the thickness of the palatal epithelium was 0.27 mm and the buccal epithelium was 0.45 mm. They did not, however, state the precise location of biopsies or whether they were all taken from the same sites.

Turck (1965) observed that the epithelium of alveolar mucosa in partially edentulous patients was a broad band with sparse rete ridges. This epithelium was consistently thicker in areas adjacent to edentulous ridges (average 0.25 mm)

in denture-wearing and non-denture-wearing patients as compared with the epithelium from regions adjacent to natural teeth (average 0.17 mm). This was evident for each patient and for the group as a whole, but no differences were noted between the maxilla and the mandible. In contrast, the attached gingiva showed no differences between edentulous and tooth-bearing regions of the same patient. The average length of rete ridges was 0.35 mm and the average thickness of the epithelium was 0.16 mm. There was no significant difference in the residual attached gingiva in patients who wore dentures and those who did not. It appeared from these results that the increase in the thickness of the epithelium of the alveolar mucosa was due to the loss of teeth and altered function of this area, and not to dentures.

Turck (1965) also found that in the region of the crest of the edentulous ridge, the average length of the rete ridges (0.43 mm), and the average thickness of the epithelium (0.23 mm) did not differ in non-denture and denture patients but was statistically significantly thicker when compared with the attached gingiva. An increase in thickness of the epithelium was marked when the outer layer was damaged.

No significant difference in epithelial thickness between the group of complete denture wearers and

the group of non-denture wearers was found by Van Mens et al (1975). The stratum corneum was not included because they said that its superficial layers could have been damaged by the denture, or that some layers could have been lost during histological processing of the specimens. However, since the aim of the study was to find differences between the epithelium of denture-bearing mucosa and non-denture-bearing mucosa, they should not have excluded the stratum corneum from the measurements, since any damage of the tissue during processing would have been a factor common to both groups. As described in Section 1.8.1, the results were expressed as weight of drawing paper and not in absolute units which could be compared with other work.

Jani and Bhargava (1976) found that the mean length of rete ridges in non-denture wearers was 0.222 mm, and 0.251 mm after wearing complete dentures for three months. The corresponding results for inter-rete ridge epithelial thicknesses were 0.129 mm and 0.132 mm. The increase in length of rete ridges was 13%, a result which was the opposite of the result found by Van Mens et al (1975).

In patients who had never worn dentures Ostlund (1958) found that the thickness of the palatal epithelium

was 0.18 mm and that the epithelium was 6.2% of the thickness of the total mucosa. In patients with "denture sore mouth" the thickness of the epithelium was 0.28 mm which was 10.5% of the thickness of the total mucosa. The increase in thickness of the epithelium in "denture sore mouth" was statistically significant but he did not comment on the thickness of epithelium of "normal" mucosa under dentures.

It is difficult to summarise the results of the different studies on epithelial thickness because different sites have been examined by different methods and the results have been expressed in different ways. However, the concensus appeared to be that the mean thickness of palatal epithelium was of the order of 0.25 mm. There was no agreement on the effect of dentures on epithelial thickness: Turck (1965) and Van Mens et al (1975) were of the opinion that dentures did not alter the epithelial thickness, whereas Jani and Bhargava (1976) found that dentures increased the thickness of the epithelium.

1.9 STRATUM CORNEUM THICKNESS

1.9.1 Methods of Analysis

The stratum corneum is the region of epithelium

which is most difficult to study since it is liable to damage unless great care is taken. The damage can be done either at the stage of obtaining the biopsy or at any time during processing. Van Mens et al (1975) were of the opinion that the stratum corneum was not suitable for accurate measurements because of this possibility of damage. However, several authors have been able to examine it. If care is taken at all stages then this layer can be preserved and examined to provide useful quantitative data.

The methods described in Section 1.8.1 to measure epithelial thickness by Turck (1965), Meyer and Gerson (1964), Ostlund (1958) and Jani and Bhargava (1976) were used by these authors to calculate the thickness of the stratum corneum.

Nedelman et al (1970) carried out a descriptive study on the mucosa of the alveolar ridge in denture and non-denture wearers. He took biopsies from the ridge mucosa of a group of sixty-two patients whose ages ranged from thirty-one to eighty years. Forty-two of these patients had not previously worn dentures; twenty had worn partial dentures and all were in need of complete dentures.

Nedelman et al (1970) and Kapur and Shklar (1962 and 1963) used the terms "thicker" and "thinner" in relation

to the stratum corneum but did not quantify these terms or describe how they measured them. Fish (1962) quoted figures for the thickness of the stratum corneum but gave no indication of how the calculations were carried out.

Van Scotter and Boucher examined only the stratum corneum in their study in 1965. They measured the thickness of the stratum corneum with an eyepiece micrometer in a microscope. Measurements were made at 2 mm intervals over the surface of the section at a magnification of 430. These sections were numbered at random in a blind study to eliminate any possibility of bias. Their method of analysis was well-documented but they did not discuss the potential damage to the layer which, since their study was on post-mortem cases, was likely to be greater than in a biopsy study because of autolytic changes.

1.9.2 Results of the Studies of Stratum Corneum Thickness

Van Scotter and Boucher (1965) found that the thickness of the stratum corneum of the palatal mucosa was 14.25 µm in the second premolar area when teeth were present. The thickness of the stratum corneum when no teeth were present and no denture was worn, was 20.24 µm. The natural teeth were thought to provide some protection to the palatal tissues and the increase of almost 6 µm in the thickness of the stratum corneum was considered

to be due in part to the loss of this protection after the teeth were extracted. The thickness of the stratum corneum under acrylic dentures was 17.80 µm. It was suggested that an acrylic resin denture was a stimulating factor since it was not perfectly adapted to the supporting tissues and could move during function. The thickness of the stratum corneum under vulcanite dentures was 5.83 µm. The reason for this reduction in thickness was not discussed.

Turck (1965) observed that the thickness of the stratum corneum in the attached gingiva ranged from 6 µm to 15 µm but did not comment on these findings in any greater detail.

Thilander (1968) noted that the stratum corneum of palatal mucosa was from five to eight cells thick and that no nucleus or cytoplasmic organelles were evident.

Nedelman et al (1970) found that sections of alveolar ridge mucosa from non-denture-wearing edentulous patients exhibited a thickened stratum corneum. The age, sex and length of the edentulous state were not related to the morphologic appearance of the mucosa. The wearing of a denture apparently did alter the structural appearance of the mucosa in

that the stratum corneum was thinner than in the non-denture-bearing mucosa and prolonged wearing of a denture produced a further thinning of the stratum corneum.

Ostlund (1953) found that the average thickness of the stratum corneum in palatal mucosa was 13 µm. In a later paper Ostlund (1958) noted that the first observable sign of tissue damage associated with wearing a denture was a thinning of the stratum corneum.

Fish (1962), examining mucosa from the crest of the edentulous ridge, found that in small areas flanked by standing teeth, the thickness of the stratum corneum was 20-30 µm; that in one completely edentulous case where no denture had been worn, the thickness was 80 µm, and that in cases where complete dentures had been satisfactory and worn "for a long time" the thickness was 25 µm.

Jani and Bhargava (1976) found that the mean thickness of the stratum corneum in palatal mucosa before wearing dentures was 17 µm and after wearing dentures for three months was 20 µm.

1.10 EPITHELIAL - CONNECTIVE TISSUE BOUNDARY

The three-dimensional nature of the basement membrane and the epithelial-connective tissue boundary in gingivae was discussed by Loe and Karring (1967 and 1969) and by Karring and Loe (1970). Most specimens showed conical connective tissue papillae projecting into the epithelium, although more or less continuous ridges also occurred. Epithelial pegs were rarely seen.

The shape of the basement membrane is of importance because it is through this that the epithelium receives nutrition. The greater the length and complexity of the rete ridges, the greater is the total area of basement membrane across which the transfer of nutrients can occur. Bullough (1972) was of the opinion that the shape of the basement membrane was related to the epithelial thickness and the mitotic index of the epithelium.

The configuration of the basement membrane varies from region to region of the oral mucosa (Squier et al, 1975). Turck (1965) found that in the alveolar mucosa, from regions with natural teeth, the basement membrane was found to be a relatively straight line with little irregularity and the mucogingival junction was well-defined. In the edentulous regions the shape

of the basement membrane was more irregular and the mucogingival junction was less well-defined. Attached gingiva from tooth-bearing areas and residual attached gingiva from edentulous regions showed only minor differences and the irregularity in the shape of the basement membrane was a constant feature in both regions. The correlation between the degree of keratinisation and the irregularity of the basement membrane was particularly evident in the residual attached gingiva under dentures such that when the stratum corneum in denture-bearing areas was missing, the irregularity was considerably increased. In the edentulous mucosa with or without dentures the irregularity was marked at the crest of the alveolar ridge, even when the epithelium was fully keratinised and when the underlying connective tissues were not inflamed.

From Turck's (1965) comments it appeared that the shape of the basement membrane was dependent on the site and the extent of keratinisation rather than on the effect of the denture.

Van Mens et al (1975), on the other hand, found that dentures had a profound effect on the shape of the basement membrane. The basement membrane shape was mapped out photomicrographically and the length of

the basement membrane was measured with a curvimeter. It was found that the epithelial-connective tissue interface in denture wearers was more regular than in non-denture wearers, i.e. the basement membrane shape in denture wearers had shallower rete ridges.

It has been suggested that stimulation of the epithelium would produce an increase in the length of the rete ridges. Kapur and Shklar (1963) observed that in mucosa stimulated by an electric toothbrush, the rete ridges showed, in some instances, greater downward extensions than in unstimulated mucosa. No comment was made on the effect of dentures on this feature.

Wentz et al (1952), in a paper on age changes and sex differences in clinically normal gingiva, classified epithelia according to epithelial thickness and length of rete ridges. The distance between the surface of the epithelium and the epithelial-connective tissue junction at the tip of the connective tissue papilla was termed the "suprapapillary width". This width could be thin, medium or thick. The lengths of the rete ridges were either short, medium or long. By combining these features, nine possible "histiotypes" were obtained. The two most common were "medium width-medium ridge length", and "thin width-long ridge length". This study was based on the histological

examination of biopsy material from thirty-eight female and sixty-four male patients between four and eighty years of age. Their findings were in relation to attached gingiva in dentate areas. They observed sex differences in the fifteen to twenty-nine years group, in that among the females there was a tendency for medium suprapapillary width and medium long ridges, whereas in the males the narrow suprapapillary width of epithelium and long ridges were more common.

1.11 CELL RENEWAL IN ORAL EPITHELIUM

1.11.1 Basic Concepts

MacDonald (1971 a) summarised the knowledge on cell renewal of normal oral epithelium and discussed the methods of calculating the indices of cell production and tissue turnover times.

Oral epithelium is not a homogeneous structure but consists of several subdivisions or compartments of cells with similar functions. Cell division occurs in a proportion of cells located in the deeper layers of the epithelium, constituting the progenitor cell compartment. Superficial to this are the maturation compartment and the stratum corneum. On comparing these layers with the histological strata normally described, the stratum basale, or basal cell layer

consists only of those cells in contact with the basement membrane; superficial to the stratum basale lie the stratum spinosum, the stratum granulosum and the stratum corneum. These last two are not present in all oral epithelia. The cornified cell compartment is clearly identical to the stratum corneum. The relationship of the progenitor cell compartment and the maturation compartment with the histological strata varies in different regions and is not well-defined in many epithelia.

The cell cycle of progenitor cells can be divided into four stages (Figure 1.1). Of these, mitosis is the only readily distinguishable stage in conventional light microscope sections. Before cell division occurs, the DNA content of the nucleus is duplicated during the synthesis phase (S phase). Between the synthesis and mitosis phases there is a gap which is designated as G_2, or the postsynthetic-premitotic gap. In oral epithelium there is a prolonged gap after mitosis before the cell again enters the synthesis phase. This is known as G_1, or the postmitotic-presynthetic gap. There is also an exit pathway from the cell cycle. The presence of a steady state implies that the size of the progenitor cell compartment remains the same and thus for every cell division one cell must migrate from

the progenitor cell compartment to the maturation compartment. This migration occurs after mitosis while the cell is in the G_1 phase.

1.11.2 Factors affecting Cell Kinetic Activity

The usual method of assessing cell kinetic activity is to count the number of cells in the mitotic phase of the cell cycle in histological sections. This is then expressed as a mitotic index by relating the number of mitoses to another parameter, for example a unit of surface length or a fixed number of cells.

It is now generally accepted that mitosis is largely under the control of local tissue hormones termed chalones (Bullough and Lawrence, 1964). These substances have a dual role of stimulating cell maturation and inhibiting mitosis. Cell division is highest in regions of low chalone concentration (Bullough, 1962).

It is difficult to evaluate site differences in cell kinetic activity from the literature because of variations in experimental method. It is, however, clear that the non-keratinised lining mucosa of the cheek in primates is renewed much faster than the keratinised masticatory mucosa of the attached gingiva (Squier et al, 1975). Dhawan and Toto (1965) also found site variation in that the duration of the S phase

for palatal mucosa of mice was eight hours and for the ventral surface of tongue was eight and a half hours.

Alvares and Meyer (1971), describing work done by Gigoux on rabbits in 1962, showed that the mitotic index, given as the number of dividing cells per millimetre of surface length, varied from 6.5 in the cheek molar region, 2.6 over palatal rugae in the molar region, 2.3 in palatal valleys in the molar region to 1.5 on the ventral surface of the tongue. The rate of cell renewal varied in direct proportion to the thickness of the epithelium. Alvares and Meyer commented that, although one might suppose that in a thin epithelium the basal cells must be replaced more often than in a thick one where they are more protected, the fact is that in the thick region replacement is several times faster.

Alvares and Meyer (1971) speculated that greater distensibility does not by itself lead to rapid wearing out of cells, but does so only if coupled with exposure to injury. In the cheek opposite the occlusal plane where the mucosa is exposed to trauma, rapid cell replacement is required for maintenance of epithelial integrity and a thick epithelium is necessary for the protection of the underlying parts. On the palatal epithelium resistance to the impact of masticatory

forces is built into the structure of the epithelium and a moderate rate of cell replacement suffices to maintain the steady state of the epithelial cell population.

Karring and Loe (1973) showed that the duration of the process of cell division in the oral epithelium of rats tends to increase with age. This study suggested that mitotic activity decreased with age.

MacKenzie and Miles (1973) studied the effects of frictional stimulation on skin and the epithelium of hamster cheek pouch mucosa and found increased mitotic activity accompanied by an accelerated passage of cells to the surface and an increased epithelial thickness due to an increase both in the number and the size of cells.

The majority of the work discussed above has been carried out in animals, particularly rodents. Care should therefore be taken in applying these results to human oral mucosa.

1.11.3 Cell Renewal in Epithelium Under Dentures

Knowledge of the change in mitotic activity due to dentures would help greatly in assessing the effect of dentures on oral mucosa. Little work has been done on this subject.

Van Mens et al (1975), however, did make a contribution by calculating the mitotic index of palatal epithelium from the photomicrographs which were used for measurements of mean epithelial thickness. All mitotic figures from early prophase to late telophase were counted and mapped on the enlarged prints. Every third section was examined to avoid counting the same mitotic figure twice. The frequency of cells in mitosis in the epithelium was expressed as mitotic figures per 1000 nuclei. In denture wearers there were 5.1 mitoses per 1000 nuclei and in non-denture wearers the corresponding figure was 1.6. Thus the denture wearers investigated showed a significantly greater mitotic index in their palatal epithelium than the non-denture wearers.

This result must be interpreted with caution, since no reference is made to the criteria used to determine when a cell was regarded as being in mitosis. In addition, since identification of mitotic figures is difficult under oil immersion on a microscope, it must be even more difficult to identify and count mitoses on photomicrographs with any degree of accuracy.

Ostlund (1958) stated that the initial reaction of the mucosa to a denture is an increase in the number of mitoses, probably to compensate for the increased strain placed on the surface epithelium by the denture. In

severely damaged epithelium the number of mitoses decreased. The method of calculating the frequency of mitoses was simply to count the number in each section and compare it with other sections.

Turck (1965) found that mitoses were more frequent when the outer layer of the epithelium was injured and showed a decrease or absence of keratinisation. It was suggested that the denture did not affect the rate of mitosis directly, but only indirectly when the denture had damaged the surface. No figures were quoted in his paper for the number of mitoses counted. This, together with the random method and the lack of detail in this part of the paper means that one must view the results with great caution.

In 1965, Turck made the observation that there was no agreement as to whether the mitotic activity of the epithelium is increased or decreased under dentures. This is still the case.

1.12 CONCLUSION

It was evident from this review of the literature that there was considerable doubt about the effect of dentures on oral mucosa and for this reason the present investigation was undertaken.

CHAPTER TWO

PRELIMINARY POST-MORTEM STUDY

2.1 INTRODUCTION

The principal aim of the investigations reported in this work was to study the oral mucosa of patients attending for prosthodontic treatment. It was felt, however, that before investigations of patients were undertaken, several preliminary studies should be made.

It was considered that these preliminary studies might involve either animal tissue or post-mortem human material. It was decided that work with animals would not be relevant because the present investigations were to be concerned essentially with the edentulous state and no realistic edentulous animal model has been developed.

One of the problems of histological studies involving tissues from patients is that the biopsy procedures and the healing of the biopsy site should be as free from discomfort as possible. This requires that the size of the specimen should be as small as possible consistent with obtaining sufficient material for reliable

evaluation. It was felt that the post-mortem study would provide data to allow the method of taking specimens to be tested. As the overall aims of the study included subjective description of parts of the oral mucosa and objective quantitation of selected histological features, it was felt that post-mortem specimens would also provide suitable material upon which preliminary evaluation of the histological and quantitative techniques could be made.

2.2 AIMS OF PRELIMINARY POST-MORTEM STUDY

There were four aims of the preliminary study reported in this Chapter:

(i) to assess the feasibility of using post-mortem material to study oral mucosa;

(ii) to evaluate a technique for obtaining specimens for histological examination;

(iii) to provide descriptive histological data on edentulous oral mucosa from upper and lower jaws;

(iv) to develop techniques for the objective quantitation of histological features of oral mucosa at the light microscopic level.

2.3 MATERIALS AND METHODS

2.3.1 Selection of Cases

It was considered that many factors might affect post-

mortem material and alter the oral tissues from the normal healthy living state. The nature of the disease causing death, or other conditions, such as chronic debilitating diseases, might cause mucosal changes. Accordingly, only those cases with a short history of illness and a sudden death, such as by myocardial infarction, were selected.

Arrangements were made for post-mortem tissue to be available from the Pathology Department of Glasgow Royal Infirmary. Only cases wearing complete upper and lower dentures at the time of post-mortem were selected. A detailed dental history was not readily available and this was not sought.

The age and time interval between death and autopsy were recorded and are shown in Table 2.1.

2.3.2 Specimen Collection

As noted in Section 2.1, any biopsy technique to be used in the clinical situation should cause the patient minimal discomfort. A small specimen size is desirable and it is preferable if suturing of the wound is not required.

The technique described by Warnakulasuriya (1976) appeared suitable. A 3mm diameter trephine-type punch (Figure 2.1) was used to make a circular incision

through the epithelium into the underlying connective tissue. The base of the incised mucosa was then cut with a pair of fine scissors to free the 3mm diameter cylinder of mucosa consisting of epithelium and some 2-4mm thickness of mucosa (Figure 2.2).

Autopsy tissue was obtained from the crest of the ridge in the upper first molar region and from the crest of the ridge in the lower anterior region. The specimens were immediately placed in a buffered neutral 10% formalin solution.

2.3.3 Trimming, Processing, Embedding and Section Cutting

The specimens were trimmed into blocks at right angles to the epithelial surface under a dissecting microscope so that, after embedding, the plane of section to be cut could be as nearly perpendicular to the surface as possible. The processing was carried out in a Histokine automatic tissue processor. The tissue was then embedded in paraffin.

Sections, 5 μm thick, were cut at right angles to the epithelial surface with all the blocks presented to the microtome knife edge at the same orientation, keratinised surface first, in order that the compression effects during cutting should be the same in each case.

2.3.4 Staining

The sections were stained with haematoxylin and eosin (Figure 2.3), and with the Crooke-Russell modification of Mallory's stain (Figure 2.4).

Haematoxylin is a blue dye which is most commonly used as a nuclear stain. Eosin is a red dye which stains connective tissue and cytoplasm. The combination of these two dyes in a haematoxylin and eosin stain is the most widely used histological stain (Drury and Wallington, 1967). Nuclei are stained blue-black, cytoplasm pink, collagen light pink and keratin an orange-red colour. It does not, however, demonstrate the keratin layer as well as some other stains, nor does it distinguish fully between different degrees of keratinisation. As discussed in Section 1.7.2, keratinisation of stratified squamous epithelium is not an all-or-none event but varies by degrees or steps. It was noted by Weinmann and Meyer (1959) that the stratum corneum, when treated with Mallory's connective tissue stain, could be classified into degrees of keratinisation. The Crooke-Russell modification of Mallory's stain (Culling, 1974) was used to demonstrate the different degrees of keratinisation. This method stains complete keratinisation a uniform red or orange (Figure 2.5), and incomplete keratinisation a layered red or orange with areas of blue (Figure 2.6).

2.3.5 Methods of Analysis

There are two principal methods of analysing the microscopic features of epithelium; namely descriptive and quantitative. Descriptive analysis is a subjective method which enables the examiner to comment upon what is observed. Quantitative analysis is an objective method which enables the examiner to arrive at numerical values for the parameters examined.

2.3.6 Descriptive Analysis

Descriptive analysis is the method which has most often been used to examine epithelium under dentures. This method was used in this study to assess the type and degree of keratinisation.

The type of keratinisation was examined on haematoxylin and eosin sections and was classified as orthokeratinisation, parakeratinisation or non-keratinisation.

The degree of keratinisation was examined on sections stained with the Crooke-Russell modification of Mallory's stain as described by Alvares and Meyer (1971) which allows classification of the stratum corneum into five varieties. These were complete orthokeratosis which is the most highly keratinised, incomplete orthokeratosis, complete parakeratosis, incomplete parakeratosis and non-keratinisation.

2.3.7 Quantitative Analysis

Morphometry is the science which allows the use of quantitative data to describe structural features. Stereology is the branch of morphometry which permits the derivation of information from two dimensional sections on the basis of geometrico-statistical reasoning. The principles of stereology were described by Weibel (1969) and more recently by Rohr et al (1976). Stereological methods have been applied to oral mucosa by MacDonald (1971b, 1973 and 1974) in relation to oral neoplasia, and by Warnakulasuriya (1976) as part of his study on cell proliferation in human oral mucosa. Meyer and Schroeder (1975) presented a stereological study of the electron microscopic features of normal human hard palatal epithelium.

2.3.8 Principles of Stereology

The stereological principle of area estimation by point counting was described by Glagoleff in 1933. He described how areas could be estimated by superimposing a lattice with marked points on a tissue and counting the number of points falling on the tissue. The area is proportional to the number of points over the tissue being measured. The theoretical basis of this method had already been worked out by Blichfeldt in 1914 and was described in detail by Weibel in 1969. The diagram

in Figure 2.7 shows a lattice with small squares superimposed on a shape to be examined. The area of the shape can be derived from a count of the number of squares covered. The squares cut by the perimeter of the shape have to be counted as fractions in proportion to the fraction inside the perimeter. The degree of precision can be increased by making the squares smaller. The problem of rounding off can be made easier if the centre point of each square is marked. If the centre point of a square is inside the profile, the square is counted; if it is outside it is not counted. It is evident that the point marked need not be the centre point but may be one of the corners of the squares.

Thus one point is equivalent to the area of one small square of length d:

$$1 \text{ point} = d^2 \qquad \text{Formula 1}$$

The stereological principle employed in the estimation of the length of a curved line using a grid of parallel lines was described by Smith and Guttman in 1953 (Figure 2.8). The mathematical principle of this method was explained by Weibel in 1969. The formula derived by this method is:

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

where d = the distance between the lines on the grid.

L = the length being measured.

i = the number of intersections.

2.3.9 Principles of Sampling in Stereology

The validity of stereology is dependent on the random selection of areas to be quantified, and consideration of sampling procedures is required at all levels of the study. Sampling should be carried out in a bias-free random manner (Weibel, 1969).

In a tissue demonstrating random distribution of elements a random sampling procedure is carried out at all stages, from the selection of tissue blocks to the selection of the precise area of tissue to be used for quantitation. In certain tissues the cellular components are orientated in preferential directions. This feature is known as anisotropy. Oral epithelium is an anisotropic structure in which the cells and other elements are orientated with respect to the surface. Weibel (1969) recommended that with such tissues, the sections examined should not be random, but that the plane of section should include the axis of anisotropy. In lining epithelia,

such as oral epithelium this axis is perpendicular to the surface.

Sample size is an important consideration in any stereological study. The larger the sample size the more likely it is that reliable information will be obtained. However, the selection of the sample size for a particular study depends on the degree of accuracy required, the time available for sampling and the characteristics of the tissue (Weibel, 1969). One of these characteristics is the proportion of components forming the tissue and, where one component is proportionately very much smaller than the rest of the components, a larger sample size may be required for the provision of accurate data.

2.3.10 Area and Thickness Measurements Used in This Study

The general principles described above were applied to the sections obtained from the specimens of the post-mortem mucosa. Point counting stereology was used to calculate the mean epithelial thickness. The point counts were made on the projected image of the section upon a Leitz Ortholux microscope with a back projecting teaching head (Figure 2.9).

Two vertical lines were marked on the projection screen and the section orientated such that a column of known

width was visualised running through the epithelium at right angles to the surface (Figure 2.10). A 10mm point counting grid was superimposed upon the projected image and the number of points falling upon the column of epithelium was counted (Figure 2.11). In an initial trial, it was found that the cumulative mean of two adjacent fields differed by less than 10% from the cumulative mean of three adjacent fields. It was, therefore, decided that two adjacent columns of epithelium should be examined to obtain values representative of the specimen. In order to prevent bias, the sections were examined without knowledge of the group to which they belonged, and the counting started half a microscope field from the left edge of each section.

In order to overcome the effects of anisotropy in the test system and in the tissue and to increase the point count to a statistically acceptable number, the grid was rotated to two positions at 60^{o} to the first position and repeat counts were made for both columns examined.

When calculating the thickness of the epithelium, the objective magnification (O_{mag}) was 25 and the Leitz Ortholux screen magnification (E_{mag}) was 12.5. The distance (d) between the lines on the grid was 10mm or 10,000 μm.

Formula 1 derived earlier (Section 2.3.8) applies when the grid is superimposed directly on the tissue. In this case the grid was superimposed on a magnified image of the tissue and the formula was modified thus:

$$1 \text{ point} = \left(\frac{d}{O_{mag} \times E_{mag}} \right)^2 \qquad \text{Formula 3}$$

Substituting these values into this Formula:

$$1 \text{ point} = \left(\frac{10{,}000}{25 \times 12.5} \right)^2$$

$$= 1{,}024 \ \mu m^2$$

When one field is examined and counted once, the area is calculated by multiplying the number of points (p_e) by the above value:

$$\text{Area} = p_e \times 1{,}024 \ \mu m^2$$

In the study reported in this Chapter, two columns were examined and counted three times each. Thus the mean point count value was the total number of points counted (P_e) divided by six:

$$\text{Mean count } (p_e) = \frac{P_e}{6}$$

$$\text{Mean area} = p_e \times 1{,}024 \ \mu m^2$$

The mean thickness was then calculated by division of the mean area by the column width:

$$\text{Mean thickness} = \frac{\text{mean area}}{\text{column width}} \qquad \text{Formula 4}$$

The column width in this case, at a magnification of 25 was 270 µm.

$$\text{Mean thickness} = \frac{p_e \times 1{,}024}{270}$$

$$= p_e \times 3.792 \text{ µm.}$$

Since the stratum corneum was considerably thinner than the entire epithelium, greater magnification was required to obtain a sufficient degree of accuracy. The objective magnification used was 40. In addition, to ensure greater accuracy the distance between the lines on the grid was 5,000 µm. The column width in this case was 170 µm. Substituting these values into Formulae 3 and 4:

$$\text{Mean thickness} = p_k \times 0.588$$

where p_k = Mean number of points in the keratin layer.

2.3.11 Length Measurements Used in This Study

The lengths of the epithelial surface and of the basement membrane were measured by intercept point counting using a grid of parallel lines superimposed on the projected image of the epithelium (Figure 2.12). The objective magnification used was 25 and the width between the grid lines was 10,000 μm.

Substituting these values into Formula 2 and considering the magnification:

$$\text{Mean length} = \frac{\pi}{2} \times \frac{d}{O_{mag} \times E_{mag}} \times i$$

$$= \frac{\pi}{2} \times \frac{10{,}000}{25 \times 12.5} \times i$$

$$= 50.4 \times i \ \mu m$$

where i = Mean number of intercept points.

The absolute lengths can be calculated in this way. It was felt that the ratio of the length of the basement membrane to the length of the surface would be more meaningful since it indicates the length of basement membrane which subtends a unit length of surface epithelium. This ratio gives information on the length of the rete ridges and describes the parameter which is designated in this work as epithelial morphology.

The ratio of basement membrane length to surface length can be obtained by dividing the corresponding values of absolute lengths or by dividing the total number of intercept points on the basement membrane (I_{bm}) by the total number of intercept points on the surface (I_s):

$$\text{Epithelial morphology} = \frac{I_{bm}}{I_s} \qquad \text{Formula 5.}$$

2.3.12 Statistical Methods of Analysis Used in This Study

The number of specimens examined in this preliminary post-mortem study was small and analysis was therefore undertaken using nonparametric statistical methods. The Mann-Whitney U test was used to evaluate the differences between the groups of specimens in relation to epithelial thickness, epithelial morphology and stratum corneum thickness.

2.4 RESULTS

Of the specimens taken, fourteen were suitable for detailed analysis; six from the upper jaw and eight from the lower jaw. There were five cases (numbered 1 to 5) where the specimens from both the maxilla and the mandible were suitable for analysis.

2.4.1 Epithelial Thickness

Table 2.2 shows the epithelial thickness in each of the upper and lower specimens. The Mann-Whitney U test indicated that there was a significant difference ($P = 0.02$) between these two groups (Table 2.3). The thickness of the epithelium at the crest of the ridge in the upper molar region was greater than that at the crest of the ridge in the lower anterior region.

The same test was applied to the five cases from which specimens were obtained from both the upper and the lower jaws. The difference between the thickness of the epithelium was not found to be significant.

2.4.2 Epithelial Morphology

Table 2.4 shows the values for the ratio of basement membrane length to surface length in the upper and lower specimens.

The Mann-Whitney U test was applied to the total numbers in each group and to the five pairs of biopsies and in neither instance was the difference significant.

2.4.3 Stratum Corneum Thickness and Degree of Keratinisation

Table 2.5 shows the thickness of the stratum corneum in each of the upper and lower specimens. The Mann-Whitney U test was applied to the total numbers in each group

and to the five pairs of specimens and in neither case was the difference significant.

Table 2.6 shows the degree of keratinisation found in each case. Parakeratosis was found to be present in all the lower specimens, whereas in the upper specimens half showed orthokeratosis. The number of specimens of each degree of keratinisation are shown in Table 2.7. The small sample number and multiple categories of keratinisation precluded statistical analysis. However, inspection of the results suggested that incomplete orthokeratosis was more common in the upper specimens, whereas complete parakeratosis was more common in the lower specimens.

2.5 DISCUSSION

Autolysis is a problem which can be encountered when dealing with any post-mortem material. The greater the time interval between death and fixation of the material the greater is the degree of autolysis in the histological sections. Another factor which affects the degree of autolysis is the temperature at which the body is stored before autopsy; the lower the temperature the lesser the degree of autolysis.

Several of the first biopsies which were taken showed autolytic changes to such an extent that they were not suitable for using in the quantitative studies (Figure 2.13). The reason for this was that the time interval between death and autopsy was too long. Thereafter the cases selected were those where the interval between the times of death and autopsy was less than 36 hours and when the body had been stored at a low temperature.

In spite of these precautions and the fact that only cases where the cause of death was of sudden onset were selected, a few cases showed autolytic changes which prevented their use for quantitation.

The stratum corneum was particularly prone to damage. This was partly due to autolysis and partly because the keratin layer, being the most superficial layer, was more likely to be damaged during the taking of the specimen or at any stage during preparation of the sections.

The epithelial thickness calculated from histo-pathological material was the thickness of epithelium on the microscope slide and not that in vivo, due to the shrinkage of the tissue during specimen preparation. The shrinkage in each case in the present study was,

however, likely to be of the same degree since the tissue was handled in the same way each time.

The results showed that the epithelium was thicker in the specimens obtained from the upper molar area than in those obtained from the lower anterior area when all the specimens were considered. It was surprising, therefore, to find, when the five paired biopsies were examined, that this difference was not significant. This was probably due to the fact the numbers were small and that, although in four of the cases the thickness of the epithelium was greater in the upper than in the lower specimens, the fifth case had thicker epithelium in the lower specimen.

The results showed that the degree of keratinisation appeared to be less in the lower specimens than in the upper. If the assumption is made that the mucosa on the upper and lower edentulous ridges are the same if no denture is worn, then these results suggest that the lower denture-bearing mucosa responds less well to the presence of a denture.

2.6 CONCLUSIONS

The histological appearance of the mucosa from the crest of the maxillary ridge in the first molar area is shown

in Figure 2.14 and from the crest of the mandibular ridge in the anterior area in Figure 2.15. These Figures give a descriptive picture for a summary of the results. The mean thickness of the epithelium in the maxillary specimens (247.9 μm) was significantly greater than the mean thickness of the epithelium in the mandibular specimens (200.6 μm). There was no significant difference between the mean epithelial morphology in the maxillary specimens (2.92) and in the mandibular specimens (2.87). The mean stratum corneum thickness in the maxillary specimens (11.45 μm) was not significantly greater than the mean stratum corneum thickness in the mandibular specimens (9.54 μm).

This study showed that it is possible to use post-mortem material to examine oral mucosa if care is taken at all stages when handling the material. Autolytic changes present the main problem, but these can be avoided if the tissue is obtained soon after death and is fixed immediately after it is removed.

The size of the biopsy punch used was large enough to provide sufficient material to examine the selected parameters. The methods of stereology used were appropriate for the types of analysis employed, provided that all the necessary assumptions of the method were met.

This initial study, by demonstrating the use of post-mortem material to study oral mucosa, justified the second study; namely the use of intact post-mortem palates to examine regional variations in palatal mucosa.

CHAPTER THREE

POST-MORTEM STUDY OF INTACT PALATES

3.1 INTRODUCTION

In a series of articles, the first of which was published in 1931, Pendleton showed histological cross-sections of intact edentulous palates and described their morphologic features at low power magnification. A search of the literature did not reveal any published work which described a quantitative study of the regional variations in intact edentulous palates.

The feasibility of using post-mortem material to make a quantitative examination of palatal epithelium has been shown in Chapter Two. It was felt that, in order to obtain information on regional variations in palatal epithelium, it would be of value if intact palates could be obtained at autopsy. This would also enable studies to be made of the connective tissues of the palate. Arrangements were made to obtain these specimens from the Pathology Department of Glasgow Royal Infirmary.

3.2 AIMS OF THIS STUDY

There were three main aims of the post-mortem study of intact palates:

(i) to examine and quantify regional variations in edentulous palatal epithelium and related connective tissues;

(ii) to assess any differences in the palatal mucosa between males and females;

(iii) to assess any differences in the palatal mucosa associated with aging.

3.3 MATERIALS AND METHODS

3.3.1 Specimen Collection

Intact palates were obtained at autopsy from eight cases who had been wearing complete upper and lower dentures until the time of death. The only cases selected were those where the cause of death was of sudden onset. The age and sex of the cases are shown in Table 3.1. It was not possible to obtain information about the dental histories of the cases.

The palates were obtained by incising the mucosa in the depth of the buccal sulcus from tuberosity to tuberosity. An incision was also made through the soft palate. An osteotome was placed on the bone in the region of the nasal spine and pressure applied to fracture the palate

away from the skull as in a Le Forte $\overline{1}$ osteotomy. At this stage, and all subsequent stages, the palates were carefully handled to minimise damage to the tissues.

Each intact palate (Figure 3.1) was placed in a buffered neutral 10% formalin solution and left for at least forty-eight hours to ensure complete fixation.

After fixation, specimens were obtained from four sites using a 3mm diameter trephine-type biopsy punch as described in Chapter Two. These specimens were taken before the palates were decalcified so that the stratum corneum could be examined without any alteration due to the decalcifying process. The sites, in a line across the palate in the first molar region (Figure 3.2), were:

(i) 5mm buccal to the crest of the ridge;

(ii) the crest of the ridge;

(iii) halfway between the crest of the ridge and the mucosa covering the palatal blood vessels;

(iv) the midline.

3.3.2 Decalcification and Sectioning of Palates

In order to cut histological sections from the palates, it was necessary to decalcify the bone. The longer the tissue is in the decalcification solution the poorer is the quality of the soft tissue in the resultant

histological sections. It was therefore felt that the palates should be cut into blocks before decalcification since this would allow easier penetration of the decalcifying solution and faster decalcification.

The first palate was cut into transverse blocks using a water-cooled circular saw (Figure 3.3). The blocks were then decalcified in a solution of 15% formic acid. This method was not entirely successful due mainly to difficulties in cutting with the circular saw, and also to the face that the palate had to be held firmly in a clamp during sawing which resulted in damage to the epithelium. This method was not used for the remaining palates and this palate was not used in the subsequent analyses.

The remaining palates, after complete fixation, were placed in the decalcifying solution intact and radiographs were taken at intervals to assess decalcification. When the palates were completely decalcified, each was cut transversely with a dermatome knife into blocks at right angles to the midline of the palate (Figure 3.4). The length of time required for decalcification was between six and eight days, and the soft tissues were not noticeably damaged by the decalcifying solution.

3.3.3 Processing, Embedding and Section Cutting

Two sections from each of the four specimens obtained with the biopsy punch were prepared in the same way as described in Section 2.3.3.

The blocks from the intact palates were also processed in the Histokine automatic tissue processor and embedded in paraffin wax (Figure 3.5). The excess wax was then trimmed to reduce the wax blocks to a size suitable for mounting on a microtome chuck (Figure 3.6). The block in the first molar area was selected and two sections 6 μm thick, were then cut through the entire width of each palate (Figure 3.7).

3.3.4 Staining

One section from each of the specimens obtained with the biopsy punch was stained with haematoxylin and eosin stain and the other section with the Crooke-Russell modification of Mallory's stain. The cross-sections obtained from the palates were stained with haematoxylin and eosin stain.

3.3.5 Descriptive Analysis

This method of analysis was used to determine the type and degree of keratinisation found in the stratum corneum of the specimens obtained from the palates.

3.3.6 Quantitative Analysis

The stereological principles described in Section 2.3.8 were used for quantitative analysis of the intact palates. As in the previous Chapter, stereological point counts and intercept point counts were made on columns of epithelium projected on to the back projecting teaching head of the Leitz Ortholux Microscope.

Alternate columns of epithelium were examined from one side to the other side of one cross-section of each palate. This allowed a continuous assessment of the palatal epithelium. The midline and the highest point of the crest of the ridge on each side were marked on the section before starting and were noted at the corresponding part of the results.

When calculating the thickness of the epithelium the objective magnification was 16 and the Leitz Ortholux screen magnification was 12.5. The distance between the lines on the grid was 10mm or 10,000 μm.

Substituting these values into Formula 3:

$$1 \text{ point} = \left(\frac{10{,}000}{16 \times 12.5} \right)^2$$

$$= 2{,}500 \ \mu m^2$$

If the mean number of points in one column is p_e then the mean area = $p_e \times 2{,}500 \ \mu m^2$.

The column width in this case was 425 μm. Using Formula 4:

$$\text{Mean thickness} = \frac{p_e \times 2{,}500}{425}$$

$$= p_e \times 5.882\ \mu m$$

The mean value of epithelial thickness was then calculated from each group of three columns which were counted. Each group of three columns was termed a "field of epithelium".

At the same magnification, with a grid of parallel lines 10,000 μm apart, the ratio of basement membrane length to surface length was calculated for the same columns which were examined for epithelial thickness. The mean value for the ratio of basement membrane to surface length was calculated for each field of epithelium.

The thickness of the tissue between the epithelium and the palatal bone, including connective tissue and salivary gland tissue, was measured in the same manner as for epithelial thickness. In this case, however, the objective magnification was 2.5 and all the columns were counted. The column width was 2,700 μm.

Substituting these values into Formulae 3 and 4:

Mean thickness = p_c x 37.925 μm

where p_c = mean number of points on column of connective tissue.

Using this method, the mean thickness of the tissue across the full width of the palate was calculated. In addition, where present, the mean thickness of salivary gland tissue in each of the columns was calculated separately.

The thickness of the stratum corneum was assessed on the sections obtained from the specimens which were taken from the palates before decalcification. The method used was that described in Section 2.3.10 for measuring the thickness of the stratum corneum.

3.3.7 Sites Examined

In order that comparisons could be made within the eight cases, the following sites were selected for detailed analysis (Figure 3.8):

(i) the crest of one ridge (C);

(ii) one quarter of the way between the crest and the midline (B);

(iii) halfway between the crest of the ridge and the midline (H);

(iv) one field before the midline (P_m);

(v) the midline (M);

(vi) one field after the midline (P_m);

(vii) halfway between the midline and the opposite crest (H);

(viii) three quarters of the way between the midline and the opposite crest (B);

(ix) the opposite crest (C).

Sites (ii) and (viii) were selected since these were the areas chosen for the biopsy in the studies on patients described later in Chapters Four and Five. For this reason, sites (ii) and (viii) were termed the "biopsy" sites. The crest and midline sites were the ones marked on the slides before analysis. One field away from the midline was termed the paramedian site. The halfway sites were taken as the fields halfway between the crest and the midline. If there were an even number of fields between the crest and the midline then the mean value of the two adjacent fields was taken as the halfway site value. The biopsy sites were the fields midway between the halfway site and the crest. The values of the parameters at the biopsy sites were calculated in a similar manner to the halfway sites.

3.3.8 Statistical Methods of Analysis Used in This Study

The Wilcoxon matched-pairs signed-ranks test was used to evaluate the differences between those results which

could be examined as matched pairs, for example between the crest and paramedian sites of the eight cases. The Mann-Whitney U test was used to evaluate the differences between those results which could not be examined as matched pairs, for example between the two crestal sites and the one midline site in each palate.

The Spearman rank correlation coefficient was used to determine if any relationship existed between the ages of the cases and each of the parameters examined. The Mann-Whitney U test was used to assess the relationship between the sex of the cases and each of the parameters examined.

3.4 RESULTS

The age and sex of the cases analysed are shown in Table 3.1.

The general distribution of the tissues is shown in Figure 3.7. This shows the extent of the cross-sections from the depth of one buccal sulcus to the other and from the oral epithelium through the connective tissue to the palatal bone and the mucosa of the floor of the nasal cavity. The position of the palatal salivary gland tissue can also be seen and this is shown in greater detail at a higher magnification in Figure 3.9.

Figure 3.10 shows the size of the palatal blood vessels and the extent of the nerve tissue in this part of the palate.

3.4.1 Regional Variation in Epithelial Thickness

A graph of the epithelial thickness at alternate fields across one palate is shown in Figure 3.11. The thickness increased from the depth of the sulcus and reached a peak at the crest of the ridge. The thickness then reduced markedly until it approached the midline where it rose to a second peak which was smaller than the one at the crest of the ridge.

The pattern of the graph from the midline to the other side is similar, but the two sides are not identical in either shape or size. This general shape of graph was found for all the cases examined.

3.4.2 Variation in Epithelial Thickness at Selected Sites

In order that comparisons could be made within the eight cases, the sites described in Section 3.3.7 were used for detailed analysis. The histological appearance of the epithelium at each of these sites is shown in Figure 3.12.

The values of the epithelial thickness found at these sites in each of the cases are shown in Table 3.2.

The histogram in Figure 3.13 shows the mean values and the range of values of the eight cases obtained at each of the sites. The general pattern of the graph in Figure 3.11 is evident in this histogram.

The statistical analyses of the results relating to the crest, the biopsy, the halfway and the paramedian sites were evaluated using the Wilcoxon matched-pairs signed-ranks test (Table 3.3). The statistical analyses of the results relating these four sites to the midline site were carried out using the Mann-Whitney U test.

The epithelium at the crest of the ridge was found to be significantly thicker than the epithelium at the halfway site ($P = 0.02$), the paramedian site ($P < 0.01$) and the midline ($P < 0.05$). The epithelium at the crest of the ridge was found to be not significantly thicker than the epithelium at the biopsy site.

The epithelium at the biopsy site was found to be significantly thicker than the epithelium at the halfway site ($P = 0.02$) and the paramedian site ($P < 0.01$) but not significantly thicker than at the midline. The epithelium at the halfway site was found to be significantly thicker than the epithelium at the paramedian site ($P < 0.01$) but not significantly thicker than at the midline. The epithelium at the midline was

found to be significantly thicker than at the paramedian site ($P < 0.05$).

3.4.3 Regional Variation in Epithelial Morphology

The ratio of basement membrane length to surface length was defined as epithelial morphology (Section 2.3.11). The graph of epithelial morphology found at alternate fields across one palate is shown in Figure 3.14. The ratio of basement membrane length to surface length increased from the sulcus to reach a peak in the region of the crest of the ridge. The ratio then reduced quickly and continued to do so until the midline was approached, where it rose to a second peak, which was smaller than the first one. This graph has a similar shape between the midline and the opposite crest but is not symmetrical in shape or size. This general shape was found in most of the other cases but one failed to show the peak at the midline.

3.4.4 Variation in Epithelial Morphology at Selected Sites

In order to compare the epithelial morphology of the eight cases, the same sites used to examine the epithelial thickness were selected for detailed analysis. The values of the ratio of basement membrane length to surface length are shown in Table 3.4. The histogram in Figure 3.15 shows the mean values and the range of values of the eight cases obtained at each of the sites.

The statistical analyses were carried out using the Wilcoxon matched-pairs signed-ranks test or the Mann-Whitney U test according to the criteria described in Section 3.4.2.

The ratio of basement membrane length to surface length was found to be significantly greater at the crest of the ridge than at the biopsy site, the halfway site, the paramedian site and the midline ($P < 0.01$ in each case). The ratio of basement membrane length to surface length was found to be significantly greater at the biopsy site than at the halfway site ($P < 0.01$) and the paramedian site ($P < 0.01$) but not significantly greater than at the midline. The ratio of basement membrane length to surface length was found to be not significantly greater at the halfway site than at the paramedian site or at the midline. The ratio of basement membrane length to surface length was found to be significantly greater at the midline than at the paramedian site ($P < 0.01$).

3.4.5 Epithelial Thickness and Epithelial Morphology

Since the shapes of the graphs in Figure 3.11 and Figure 3.14 and the histograms in Figure 3.13 and Figure 3.15 are similar, the correlation between epithelial thickness and morphology was evaluated using the Spearman rank correlation coefficient (Table 3.5). This was done for individual pairs of values for each field of epithelium.

The results (Table 3.6) showed that in all but one case there was a highly significant correlation between the epithelial thickness and the epithelial morphology such that, as the thickness increased, the ratio of basement membrane length to surface length increased.

Reinspection of the results of the case which did not show this correlation revealed that it had the highest mean epithelial thickness and the lowest range in the ratio of basement membrane length to surface length.

3.4.6 Regional Variation in Connective Tissue Thickness

The connective tissue thickness was regarded as the total thickness of tissue between the epithelium and the palatal bone, including salivary gland tissue. Every column of tissue from one side of the palate across to the other side was counted. The graph in Figure 3.16 shows the pattern obtained from one case. The point of greatest thickness was approximately halfway between the crest of the ridge and the midline. The thinnest region was at the midline. This general shape of graph was found in all cases examined but none of the graphs was completely symmetrical.

3.4.7 Variation in Connective Tissue Thickness at Selected Sites

The sites described in Section 3.3.7 were used for detailed analysis of the connective tissue thickness. The

connective tissue thicknesses are shown in Table 3.7 and the position and amount of salivary gland tissue are shown in Table 3.8. The mean values and range of values of the eight cases are shown in the histogram in Figure 3.17.

The statistical analyses of the connective tissue thicknesses were carried out using the Wilcoxon matched-pairs signed-ranks test and the Mann-Whitney U test as described in Section 3.4.2. Statistical analysis of the amount of salivary gland tissue was not possible because it was not found to be present at a sufficient number of sites.

The connective tissue thickness at the halfway site was found to be significantly thicker than at the biopsy site ($P < 0.05$) and the crest, the midline and the paramedian site ($P < 0.01$ in each case). The connective tissue at the biopsy site was found to be significantly thicker than at the crest, the midline and the paramedian site ($P < 0.01$ in each case). The connective tissue thickness at the crest was found to be significantly thicker than at the midline and the paramedian site ($P < 0.01$) in both cases. There was found to be no statistical difference between the connective tissue thickness at the midline and the paramedian sites.

3.4.8 Epithelial and Connective Tissue Thickness

The Spearman rank correlation coefficient was used to assess the relationship between the epithelial thickness and the connective tissue thicknesses at each of the sites. No correlation was found at any of the sites.

3.4.9 The Effects of Age

The Spearman rank correlation coefficient was used to assess the relationship between the age of the individual and each of the parameters.

The correlation was carried out between the ages and the total mean epithelial thicknesses of the cases (Table 3.9) and no correlation was found. This was also done at each of the sites and again no correlation was evident. The epithelial thickness in these cases, therefore, was not age-related.

The Spearman rank correlation coefficient was used to assess the relationship between the age of the individuals and the epithelial morphology. This was done for the total mean value of epithelial morphology for each of the cases shown in Table 3.9 and no correlation was found. This was also done at each of the sites and no correlation was found at eight of the nine sites. A correlation ($P < 0.05$) was found at one of the paramedian sites. When this test was applied

to both the paramedian values at the same time no correlation with age was found. It was evident from these figures that there was no significant correlation between epithelial morphology and the age of the patient.

The correlation coefficient was also used to determine the relationship between the age of the individuals and the connective tissue thickness. There was no correlation between the total mean thickness and the age of the individuals. When the sites were examined separately, eight of the nine sites showed no correlation. A correlation ($P < 0.05$) was found at one crest site but, when both crests were considered together, no correlation was found. There was therefore no correlation between the connective tissue thickness and the age of the individual.

No correlation was found between the total amount of salivary gland tissue present and the age of the individuals.

3.4.10 Sex Differences

The Mann-Whitney U test was used to assess the differences in the parameters between males and females.

This was carried out for the total mean epithelial thickness of the cases (Table 3.9) and there was found to be a significant relationship ($P = 0.02$), such that the

epithelium was thicker in males. This was also carried out at each of the sites but the relationship described above was found only at three of the nine sites. The reason for these apparently conflicting results was that the sex-related difference at each site was fairly small and was only clearly evident when the total mean epithelial thicknesses were considered.

No correlation between the sex of the individual and the epithelial morphology or the connective tissue thickness was evident.

3.4.11 Stratum Corneum Thickness at Selected Sites

Because it was felt that the decalcifying solution might affect the stratum corneum, the specimens used to measure the thickness of the stratum corneum were obtained from the palates prior to decalcification.

The thickness of the stratum corneum in each of the cases at each site selected for this study are shown in Table 3.10. The Wilcoxon matched-pairs signed-ranks test was used to assess the differences between the thicknesses of the stratum corneum at each of the sites. The thickness at the biopsy site was significantly greater ($P = 0.02$) than the thickness buccal to the crest of the ridge. No other differences between sites were significant.

The relationship between the age of the individual and the thickness of the stratum corneum was assessed by the Spearman rank correlation coefficient test. A significant correlation was found at the crest of the ridge ($P < 0.05$) such that the thickness of the stratum corneum reduced with age. No other correlation was significant.

No correlation between the sex of the individuals and the thickness of the stratum corneum was found when analysed by the Mann-Whitney U test.

3.4.12 Type and Degree of Keratinisation at Selected Sites

The same specimens used to measure the thickness of the stratum corneum were used to assess the type and degree of keratinisation at the selected sites.

The type and degree of keratinisation found at each site are shown in Tables 3.11 and 3.12 respectively. The complexity of the results did not permit statistical analysis. Inspection of Table 3.11 shows that parakeratosis was present, at least to some extent, in all the specimens obtained from the area buccal to the crest of the ridge. Orthokeratosis was the type of keratinisation most often seen at the biopsy site. Inspection of Table 3.12 shows that the degree of keratinisation was least in the region buccal to the crest of the ridge and greatest at the biopsy site.

3.5 DISCUSSION

The results showed that, although there was a wide range in the thickness of the epithelium among the cases, there was a striking similarity in the pattern of the variation of the epithelial thickness. This was also found in the results for epithelial morphology.

The region where the epithelium was thickest and the rete ridges were longest was at the crest of the ridges. A possible reason for this was that following extraction of teeth, the crest was covered by epithelium growing in from the sides and the fusion of the epithelium might have produced a region of thicker epithelium with longer rete ridges. Another factor which might have contributed to the shape of the epithelium at the crest of the edentulous ridge was that the crevicular epithelium from one side of the socket might have fused with the crevicular epithelium from the other side at a deeper level.

A similar anatomical reason for the increase in epithelial thickness and the ratio of basement membrane length to surface length at the midline may be that embryologically the midline is formed by the fusion of two palatal shelves and the joining of the two separate epithelial areas might have produced a region of thicker epithelium with longer rete ridges.

The distribution of connective tissue from an anatomical point of view was not surprising. The mean thickness at the halfway site was 3.7mm, which compared with the value of 3.1mm obtained in dentate patients by Kydd et al (1971). They observed that the thickness of palatal mucosa was greater in edentulous patients than in dentate patients.

The stratum corneum at the biopsy site was significantly thicker than at the site buccal to the crest of the ridge. In addition, the degree of keratinisation was greatest at the biopsy site and was least buccal to the crest of the ridge. These findings suggested that there was a direct relationship between the thickness of the stratum corneum and the degree of keratinisation.

There was a significant correlation between the thickness of the stratum corneum and the age of the cases only at the crest of the ridge, such that the thickness decreased with age. No other parameter was affected by age. Since no detailed dental history was available it was not possible to assess the effects of the length of denture experience on the parameters.

3.6 CONCLUSIONS

The epithelium was thickest at the crest of the ridge (mean thickness 251.6 μm) and thinnest at the paramedian

sites (mean thickness 163.5 μm). There was a considerable range in the thickness of the epithelium from one case to another but the general pattern in all cases was similar.

The rete ridges were longest at the crest of the ridge where the mean value of epithelial morphology was 3.90. The epithelial morphology was directly related to epithelial thickness.

The connective tissue thickness was greatest in the region halfway between the crest of the ridge and the midline.

The age of the cases had no effect on the parameters with the exception of the thickness of the stratum corneum at the crest of the ridge which reduced with age.

The sex of the cases had no effect on the parameters except that the epithelial thickness was greater in males.

The mean thickness of the stratum corneum at the crest of the ridge was 14.3 μm and at the biopsy site was 17.9 μm.

This study showed that there was a considerable regional variation in the edentulous palatal mucosa. It was not possible to determine the extent to which dentures were responsible for the results obtained.

In order to assess the effects of complete dentures on palatal mucosa a clinical investigation, reported in the following Chapters, was undertaken.

CHAPTER FOUR

NORMAL HUMAN PALATAL MUCOSA

4.1 INTRODUCTION

Having established in the post-mortem study in Chapter Two that a biopsy with a diameter of 3mm provided sufficient material for histological analysis, it was decided to extend the study to include patients. Since there was little quantitative information available in the literature about normal oral mucosa, this was first sought before studying mucosa related to dentures.

Because it was proposed to examine the effects of dentures on oral mucosa, the site selected had to be one which did not change greatly after the extraction of teeth. The effects of age changes and smoking habits, both of which might alter the oral mucosa, were also studied.

Van Mens et al (1975) found that the presence of a denture significantly altered the mitotic index in palatal epithelium and it was decided to examine the cell kinetics of the epithelium in addition to the parameters examined in Chapter Two.

4.1.1 Cell Kinetics

It is customary to divide the life span of progenitor cells into a number of phases (Figure 1.1). A period of mitotic division, M, is followed by a postmitotic-presynthetic gap, G_1. In the synthesis phase, S, the cell prepares for further division by replicating the DNA. This phase is separated from the period of mitosis by the postsynthetic-premitotic gap, G_2. A dicophase is sometimes postulated between M and G_1, this being a period of indeterminate length during which each daughter cell makes the decision to remain part of the progenitor population or to begin differentiation (Squier et al, 1975). The period of mitosis is subdivided into a prophase, a metaphase, an anaphase and a telophase.

The standard methods of examining the cell cycle are to calculate the mitotic index or to study the synthesis phase of the cell cycle using radioactive labelling indices.

4.1.2 Mitotic Indices

The usual method of measuring the mitotic index is to count the number of cells in mitosis in histological sections. The major difficulty with this method is the relative infrequency of mitotic figures. It is therefore necessary to examine a large number of sections in order to make a reliable estimate. Another difficulty

is the subjective error in detecting cells in mitosis. Various workers have used different criteria to identify cells in mitosis. Depending on the criteria employed for recognition of prophases the mitotic count will vary. It is sometimes difficult to distinguish an early prophase from clear cells, such as melanocytes, which may be present in the area (Warnakulasuriya, 1976). Another possible source of error is that the nuclei of dividing cells are often larger in size than the nuclei of cells in other stages of the cell cycle and therefore the count of cells is likely to be biased in favour of mitotic figures (Aherne, 1970).

In order to express meaningfully the number of mitoses counted in a histological section it is necessary to relate this count to a reference unit in the epithelium. The method which is commonly used relates the mitotic count to one hundred or one thousand nucleated cells in the epithelium. This involves counting the total number of viable or nucleated cells in the epithelium. The section thickness can considerably influence the nuclear count and therefore the estimated mitotic index. Abercrombie (1946) suggested a formula to correct the error which arises from including nuclear fragments in the count, thus causing an over-estimation of observed cell numbers within the volume of tissue section examined. The correction is a function of nuclear diameter and section thickness.

As well as expressing the mitotic index related to a fixed number of viable cells as described earlier, it is also possible to express the mitotic index per unit length of surface or of basement membrane. Karring and Loe (1973) compared the various methods of measuring mitotic indices and found a varying degree of correlation. They believed that the differences were due to variations in epithelial cell density and in the ratio of basement membrane length to surface length.

The ideal mitotic index for expressing the mitotic activity of human oral epithelium has not yet been established. Each investigator, therefore, must select the reference unit most appropriate to the nature of his study.

4.1.3 Radioactive Labelling Indices

These methods of investigation of the cell cycle use radioactive isotopes to label DNA. The method most frequently used on oral epithelia is pulse labelling with tritiated thymidine, in which a single injection of the isotope is given. Thymidine is incorporated into DNA and the radioactive thymidine labels the cells which are in the synthesis phase when the isotope is available. The labelled nuclei may be demonstrated by autoradiography.

A double labelling method using two isotope pulses

separated by a known time interval (t_i) was proposed by Wimber and Quastler in 1963. Galand et al (1968) used a double labelling method with two pulses of tritiated thymidine in vitro. In the autoradiographs prepared from such double labelled material two groups of labelled cells can be identified by grain density over their nuclei. Counts of the heavily labelled cells (H.L.C.) and the lightly labelled cells (L.L.C.) can be made and, using the formula given below, the length of the synthesis phase (T_s) calculated:

$$\frac{T_s}{t_i} = \frac{\text{H.L.C.}}{\text{L.L.C.}}$$

4.2 AIMS OF THE STUDY OF NORMAL PALATAL MUCOSA

There were three main aims of the study of normal palatal mucosa:

(i) to quantify light microscopic morphological parameters of normal palatal mucosa;

(ii) to assess the effects of age on these parameters;

(iii) to assess the effects of smoking on the same parameters.

4.3 MATERIALS AND METHODS

4.3.1 Selection of Cases

The main study was restricted to male patients in order

to avoid any influences due to sex hormone related variations in the oral epithelium. A small number of female patients were, however, included to provide a comparison with the male cases, and because these same female cases were to be used in the study described in Chapter Six after they had been wearing dentures.

All patients were in good general health with normal, healthy oral mucosa and had never worn a partial or a complete denture. They were all attending the Department of Oral Surgery of Glasgow Dental Hospital and School for the extraction of one or more upper teeth. Some were attending only for extractions but the majority were having extractions carried out as part of a treatment plan involving the construction of immediate dentures. Those in the latter group were to be followed up after they had been wearing dentures.

There were twenty-one male patients and four female patients. The ages and smoking habits of the patients were recorded.

The project was approved by the Ethics Committee of Glasgow Dental School and the procedures complied with the Declaration of Helsinki (1964) in letter and in spirit. Each patient was informed of the nature of the project and his permission was obtained.

4.3.2 Selection of The Biopsy Site

As mentioned in Section 4.1, a site was required which does not change significantly after the extraction of teeth. There is a considerable change in shape of the alveolar bone and mucosa in the lower jaw which makes it unsuitable for this type of comparative study. The work of Watt and Likeman (1974), discussed in Section 1.3.2, showed that there was a considerable change in shape of the tissues on the buccal side of the crest of the ridge after the extraction of a tooth. Their work also showed that the change in shape was less marked on the palatal aspect, particularly in the area remote from the palatal gingival margin.

From the work described in Section 3.4.7, it was found that the mucosa near the midline was fairly thin and that it was difficult to obtain a biopsy from this area. It was also felt that a biopsy in this area would leave a painful wound which would heal slowly owing to the thinness of the mucosa and the relatively poor blood supply. It was also shown in Figure 3.10 that the palatal blood vessels in the edentulous mouth are large and fairly close to the surface. It was therefore felt that it would be unwise to take a biopsy from the mucosa covering these vessels.

The anterior part of the palatal mucosa is unsuitable for a comparative study using a small biopsy because

the rugae are likely to lead to inaccuracies in the results, depending on whether the biopsy includes the rugae or not.

In view of these factors, the region described as the biopsy site in Section 3.3.7 and illustrated in Figure 3.8 was selected for analysis.

4.3.3 Specimen Collection

The biopsies were carried out using adrenaline-free local anaesthesia remote from the biopsy site (Figure 4.1). A 3mm diameter trephine-type biopsy punch was used to make a circular incision by pushing it into the palatal mucosa with a slight rotary movement (Figure 4.2). The base of the biopsy was initially cut using a pair of suture scissors but later it was found that ophthalmic scissors were more suitable. The biopsy was then carefully removed from the mouth with a spatula such that the tissue was not traumatised. Pressure was applied to the wound for two minutes.

It was not found necessary to suture the wound and no patient had any post-biopsy complication. The initial appearance of the wound is shown in Figure 4.3. The appearance of a wound after five days in a different patient is shown in Figure 4.4.

4.3.4 Trimming, Processing, Embedding and Section Cutting

The biopsy specimens were trimmed into blocks, cut at right angles to the epithelial surface, and fixed in Bouin's fluid. The processing and embedding were carried out as described in Section 2.3.3.

Two sections, 5 μm thick, were cut and placed on separate slides for quantitative analysis. Thirty consecutive sections were then cut at 5 μm and placed with five sections on six separate slides and numbered one to thirty.

4.3.5 Staining

One of the first two sections was stained with haematoxylin and eosin and the other with the Crooke-Russell modification of Mallory's stain. The thirty serial sections were all stained with haematoxylin and eosin.

4.3.6 Methods of Analysis

The same methods of stereology described in Chapter Two were used for the biopsies in this study. The degree of keratinisation was assessed as described in Chapter Two. In addition the mitotic index of each biopsy was estimated as described in the next Section.

The initial biopsies were also labelled in vitro with a double pulse of tritiated thymidine in order to study

the S phase of the cell cycle using the technique described by Warnakulasuriya (1976). This, however was not successful because the tissue produced a negative chemographic effect on the emulsion layer of the autoradiograph. It was not possible to overcome this difficulty and this method had to be abandoned.

4.3.7 Mitotic Index Used in This Study

The mitotic index was calculated by counting the number of cells in mitosis (Figure 4.5). As stated earlier there are certain difficulties in the identification of mitosis and the following criteria were laid down for the identification of a mitosis and for the particular stage of mitosis in which the cell was:

Prophase	(i) loss of nuclear membrane
	(ii) hyperchromatism
	(iii) condensation of chromatin threads
	(iv) relative increase in nuclear size and spherical form
Metaphase/Anaphase	separation of chromatids
Telophase	two daughter nuclei until the stage of cytoplasmic separation.

The mitotic counting was carried out at an objective

magnification of one hundred under oil immersion. Every fifth section, the middle one on each slide, of the thirty serial sections was counted in order that no mitotic figure would be counted more than once. The microscope was fitted with an eyepiece graticule to facilitate the counting. The stage of mitosis was recorded. The length of the epithelial surface was measured using the eyepiece graticule which was calibrated by using a microscope stage micrometer. This enabled the mitotic index to be expressed in relation to a unit length of surface.

4.3.8 Number of Cells per Unit Surface Length

The number of cells per unit surface length (cell density) was calculated by counting the number of viable keratinocyte nuclei. Squames in the stratum corneum were not included in the total cell count and degenerating cells were also excluded from this count by identifying degenerating cell nuclei using the cytological characteristics described by Warnakulasuriya (1976):

(i) pyknosis - shrinkage of the nucleus into a hyperchromatic mass;

(ii) flattening of the nucleus to the extent that no nuclear material was able to be seen within the condensed nuclear membrane;

(iii) fragmentation of the nuclear membrane.

The total number of nucleated cells in a column running through the full thickness of the epithelium at right angles to the surface was counted using a magnification of 400. Twenty-five smaller squares in an eyepiece graticule were used to delineate small areas of epithelium in order to facilitate counting of the nuclei. Where the thickness of the epithelium was greater than the height of the graticule, the section was moved up to a second position, without overlap, in order to complete the counting of the column of cells through the full epithelial thickness. It was necessary to change the focal plane up and down to ensure that no nucleus was missed.

4.3.9 Statistical Methods of Analysis Used in This Study

The correlations between epithelial thickness, epithelial morphology, stratum corneum thickness, mitotic index, cell density and age were assessed by linear regression analysis.

The degree of keratinisation was ranked on an ordinal scale to permit nonparametric statistical analysis. The Fisher exact probability test was used to determine the relationship between the degree of keratinisation and smoking. The relationship between the degree of keratinisation and each of the other parameters was assessed using the Spearman rank correlation test.

The Mann-Whitney U test was used to determine the relationship between smoking and each of the parameters.

In order to compare the results obtained from the four female cases with the results obtained from the male cases, four male cases, whose ages and smoking habits were similar to the four females, were selected. The comparisons were made using the Mann-Whitney U test.

4.4 RESULTS

The age, smoking habits, epithelial thickness, epithelial morphology, stratum corneum thickness, mitotic index, cell density and the degree of keratinisation found in the male cases are shown in Table 4.1. In one case the smoking habit was not recorded. The corresponding results for the four female cases are shown in Table 4.2.

4.4.1 Epithelial Thickness

The mean value of epithelial thickness was 268.9 μm and the standard deviation was 58.1.

A scatter diagram was plotted between the epithelial thickness and the epithelial morphology (Figure 4.6). Linear regression analysis showed that there was no tendency for a linear relationship and the correlation coefficient was low ($r=+0.179$). There was therefore no correlation between the epithelial morphology and

the epithelial thickness. A scatter diagram was plotted between the epithelial thickness and the stratum corneum thickness (Figure 4.7). Linear regression analysis showed that there was a tendency for a linear relationship and the correlation coefficient was +0.464. This gave a P value of < 0.05, indicating that there was a significant positive correlation between epithelial thickness and stratum corneum thickness.

The same correlation test showed that there was a significant relationship ($P < 0.01$) between the epithelial thickness and the cell density such that as the thickness increased so did the number of cells (Figure 4.8).

There was no significant correlation between the epithelial thickness and the mitotic index.

4.4.2 Epithelial Morphology

The mean value of epithelial morphology was 2.68 and the standard deviation was 0.50.

There was found to be a significant correlation ($P < 0.01$) between the epithelial morphology and the cell density, such that as the epithelial morphology increased so did the cell density (Figure 4.9).

No significant correlation was found between the epithelial morphology and any of the other parameters.

4.4.3 Stratum Corneum Thickness

The mean value of stratum corneum thickness was 20.4 μm and the standard deviation was 8.4.

As stated in Section 4.4.1, there was a significant correlation found between the thickness of the stratum corneum and the epithelial thickness. There was no significant correlation between the thickness of the stratum corneum and the epithelial morphology, the mitotic index, the cell density or the degree of keratinisation.

4.4.4 Mitotic Index

The mean mitotic index, expressed as the number of mitoses per millimetre of surface length, was 0.72 and the standard deviation was 0.29.

There were no significant correlations between the mitotic index and any of the other parameters.

4.4.5 Cell Density

The mean cell density, expressed as the number of viable cells per millimetre of surface length, was 1602.7 and the standard deviation was 307.8.

There was a significant correlation between cell density and epithelial thickness (Section 4.4.1),

and between cell density and epithelial morphology (Section 4.4.2). No other significant correlations with cell density were found.

4.4.6 Degree of Keratinisation

The degree of keratinisation of each of the cases is shown in Table 4.1. There were nine cases of complete orthokeratosis (Figure 4.10), five cases of incomplete orthokeratosis (Figure 4.11) and seven cases of a mixture of complete and incomplete orthokeratosis. The Spearman rank correlation coefficient was used to assess the relationship between the degree of keratinisation and each of the parameters. In order to use this test it was necessary to rank the degrees of keratinisation on an ordinal scale (Table 4.3). No significant correlations were found between the degree of keratinisation and any of the other parameters.

4.4.7 Age

The relationship between the ages of the cases and each of the parameters was calculated by linear regression analysis.

The scatter diagram plotted between age and the thickness of the stratum corneum is shown in Figure 4.12. The correlation coefficient ($r=+0.417$) was almost high enough to reach the five per cent level of significance. Inspection of this scatter diagram showed that one point

was markedly higher than the others on the y axis. No reason was evident for the particularly thick stratum corneum in this case. Linear regression analysis was, however, carried out omitting this point (Figure 4.13) and the correlation coefficient was found to be +0.527 ($P < 0.02$). These results suggested that there was probably a real correlation between age and stratum corneum thickness such that with increasing age the thickness of the stratum corneum also increased.

No other correlations were found to be significant.

4.4.8 Smoking

The cases were classified as being smokers or non-smokers. The numbers of smokers and non-smokers showing the different degrees of keratinisation were cast into a 2 x 3 table (Table 4.4). The cells of the table were combined in various ways and the relationship between smoking and the degree of keratinisation analysed by the Fisher exact probability test. The relationship between smoking and each of the other parameters was evaluated using the Mann-Whitney U test (Table 4.5). No significant relationship between smoking and any of the parameters examined was found to exist.

4.4.9 Female Non-denture Wearers

The results obtained from the four female non-denture wearers are shown in Table 4.2. The main reason for

including these cases in this study is because they were used for the investigation described in Chapter Six. The results are given in this Chapter because they provide some comparison with male non-denture wearers.

Four male cases (numbered 8,10,14 and 15 in Table 4.1) with the same smoking habits and similar ages were chosen from the male group to assess any sex differences in the parameters examined. The statistical test used was the Mann-Whitney U test.

A statistically significant difference ($P < 0.05$) was found between the cell density of the males and females such that the cell density was less in females. No other significant differences were found to be present.

4.5 DISCUSSION

The mean value of epithelial thickness found in this study was 268.9 μm. This is remarkably similar to the value of 270 μm obtained for palatal epithelium by Meyer and Gerson (1964). Meyer and Schroeder (1975) found that the mean thickness of palatal epithelium in the rete ridge regions was 310 μm and 120 μm between the ridges. Jani and Bhargava (1976), however, found a lower value of 222 μm in the rete ridge regions and a value of 129 μm in the inter-rete ridge regions.

The finding that the number of cells per unit surface length increased with increasing epithelial thickness was to be expected. The significant correlation between the number of cells per unit surface length and the epithelial morphology was due to the fact that the increase in length of the basement membrane produced an increase in the cell dense progenitor cell compartment.

The mean value of thickness of the stratum corneum found in this study was 20.4 µm. The corresponding value found by Van Scotter and Boucher (1965) in their group of dentate patients was 14.25 µm. Jani and Bhargava (1976) found the thickness of the stratum corneum to be 17 µm and Meyer and Gerson (1964) found it to be 32 µm.

The finding that the thickness of the stratum corneum increased with age is in agreement with the opinion of Massler (1956). Van Scotter and Boucher (1965) however found no direct correlation between age and the thickness of the stratum corneum.

It was surprising that there was no correlation between smoking habits and any of the parameters examined. The reason for this may be that it was necessary to categorise each case as a smoker or non-smoker and it was therefore not possible to differentiate between light and heavy smokers.

4.6 CONCLUSIONS

This study provided baseline data for normal epithelium at one particular site in the palate. This data enabled a comparison to be made with mucosa under complete dentures.

CHAPTER FIVE

PALATAL MUCOSA UNDER COMPLETE DENTURES

5.1 INTRODUCTION

Having established in Chapter Four the values of the parameters of normal palatal mucosa, palatal mucosa which had been covered by dentures was examined. This Chapter reports this study of mucosa under complete dentures. Chapter Six describes the comparisons between normal mucosa and that covered by complete dentures.

In addition to examining all the parameters described in the previous Chapter, the effects of the length of time dentures had been worn and the number of hours per day the dentures were worn are discussed in this Chapter.

5.2 AIMS OF THE STUDY OF PALATAL MUCOSA UNDER DENTURES

The aims of the study of palatal mucosa under dentures were:

(i) to quantify light microscopic morphological parameters of clinically normal palatal mucosa under dentures;

(ii) to assess the effects of age on these parameters;

(iii) to assess the effects of smoking on these parameters;

(iv) to assess the effects of the length of denture experience on these parameters;

(v) to assess the effects on these parameters of the number of hours per day that the denture was worn.

5.3 MATERIALS AND METHODS

5.3.1 Selection of Cases

For the reasons stated in the previous Chapter (Section 4.3.1) the main study was concerned mainly with male patients. The small group of female patients were again included since they were to be used in the follow-up studies of individual patients described in Chapter Six.

All patients were in good general health and were wearing complete upper and lower dentures. They were all attending the Prosthodontic Department of Glasgow Dental Hospital for the provision of replacement complete dentures. The dentures were being replaced because of inadequate tissue contact of the fitting surface and lack of satisfactory occlusal balance. No detailed assessment of the dentures was carried out.

In all cases the oral mucosa was normal in colour and texture and no apparent intra-oral lesions were present.

There were twenty-seven male patients and four female patients. A note was made of the age of the patient, his smoking habits, the length of denture experience and the number of hours per day that the dentures were worn. This project was approved by the Ethics Committee of Glasgow Dental School. Each patient was informed of the nature of the project and his permission was obtained.

5.3.2 Selection of The Biopsy Site

The site described in the previous Chapter was again selected, namely midway between the alveolar crest and the mucosa covering the blood vessels and nerves. In patients who were having a second biopsy taken, the opposite side of the palate was used.

5.3.3 Specimen Collection

The method described in the previous Chapter was used to obtain the biopsies. The denture was replaced immediately after removal of the biopsy and the patient examined after five minutes to check that haemorrhage had stopped. No patient reported any post-biopsy complication.

5.3.4 Section Preparation

The biopsy specimens were trimmed into blocks cut at right angles to the epithelial surface and fixed in Bouin's fluid. The processing and embedding were carried out as described in Section 2.3.3. The sections were cut as described in Section 4.3.4 and stained by haematoxylin and eosin stain and by the Crooke-Russell modification of Mallory's stain as described in Section 4.3.5.

5.3.5 Methods of Analysis

The methods of analysis described in Sections 4.3.6 to 4.3.8 were also used on the material examined in this Chapter.

The statistical methods of analysing the results described in Section 4.3.9 were used to evaluate the results obtained in this study. In addition, linear regression analysis was used to assess the relationship between the parameters examined and the length of time the patient had been wearing dentures. The Mann-Whitney U test was used to determine the relationship between the parameters examined and the number of hours per day that the patients wore their dentures.

5.4 RESULTS

The age, smoking habits, epithelial thickness, epithelial morphology, stratum corneum thickness, mitotic index,

cell density, degree of keratinisation, length of denture experience and the number of hours of denture-wearing per day are shown in Table 5.1. In three cases it was not possible to calculate the mitotic index accurately because insufficient material was available. The corresponding results for the four female cases are shown in Table 5.2.

5.4.1 Epithelial Thickness

The mean value of epithelial thickness was 240.4 μm and the standard deviation was 43.8.

Linear regression analysis showed that there was a significant relationship ($P < 0.02$) between the epithelial thickness and the cell density such that as the thickness increased so also did the cell density (Figure 5.1). There was also a significant correlation ($P < 0.05$) found between the epithelial thickness and the mitotic index such that as the thickness increased so also did the mitotic index (Figure 5.2).

There was no significant correlation found between the epithelial thickness and the epithelial morphology, the stratum corneum thickness or the length of denture experience.

5.4.2 Epithelial Morphology

The mean value of epithelial morphology was 2.22 and the standard deviation was 0.56.

There was found to be a significant correlation between the epithelial morphology and the cell density, such that as the epithelial morphology increased so also did the cell density (Figure 5.3).

No significant correlation was found between the epithelial morphology and the epithelial thickness, the stratum corneum thickness, the mitotic index or the length of denture experience.

5.4.3 Stratum Corneum Thickness

The mean value of the stratum corneum thickness was 13.8 μm and the standard deviation was 5.7.

No significant correlation was found between the stratum corneum thickness and the epithelial thickness, the epithelial morphology, the mitotic index, the cell density or the length of denture experience. A significant correlation between the stratum corneum thickness and the degree of keratinisation was found (Section 5.4.6).

5.4.4 Mitotic Index

The mean mitotic index, expressed as the number of mitoses per millimetre of surface length, was 0.77 and the standard deviation was 0.69.

As stated in Section 5.4.1 a significant positive correlation ($P < 0.05$) was found between the mitotic

index and the epithelial thickness. There was no significant correlation found between the mitotic index and the epithelial morphology, the stratum corneum thickness, the cell density or the length of denture experience.

5.4.5 Cell Density

The mean cell density, expressed as the number of viable cells per millimetre of surface length was 1225.74 and the standard deviation was 282.60.

There was a significant correlation between the cell density and the epithelial thickness (Section 5.4.1) and between the cell density and the epithelial morphology (Section 5.4.2). No significant correlation was found between the cell density and the stratum corneum thickness, the mitotic index or the length of denture experience.

5.4.6 Degree of Keratinisation

The degree of keratinisation of each of the cases is shown in Table 5.1. In order that the degree of keratinisation could be statistically evaluated in relation to the other parameters, an ordinal scale of the degree of keratinisation was established (Table 5.3). The Spearman rank correlation coefficient was used to assess the relationship between the degree of keratinisation and the epithelial thickness, the

epithelial morphology, the stratum corneum thickness, the mitotic index, the cell density, the length of denture experience and age.

A significant correlation ($P < 0.01$) was found between the degree of keratinisation and the thickness of the stratum corneum such that, as the degree of keratinisation reduced the thickness of the stratum corneum also reduced.

No other significant correlation was found between the degree of keratinisation and any of the other parameters.

5.4.7 Age

The relationship between the age of the cases and each of the parameters was assessed by linear regression analysis.

A significant correlation ($P < 0.01$) was found between age and the epithelial morphology such that, as age increased the epithelial morphology decreased (Figure 5.4). A significant correlation ($P < 0.001$) was also found between age and the cell density such that, as age increased the cell density decreased (Figure 5.5).

No significant correlation was found between age and the epithelial thickness, the stratum corneum thickness or the mitotic index.

5.4.8 Smoking

As in Section 4.4.8 the cases were classified as either being smokers or non-smokers.

The degree of keratinisation and smoking were cast into a 6 x 2 table (Table 5.4). Because of the number of cells in this Table with low values the columns were combined in various ways to provide 2 x 2 tables amenable to analysis by the Fisher exact probability test. No correlation was found to exist between smoking and the degree of keratinisation.

The relationship between the smoking habits and each of the other parameters was evaluated using the Mann-Whitney U test and no significant relationships were found to exist.

5.4.9 Number of Hours of Denture-wearing per Day

The cases were classified as either wearing their dentures for twenty-four hours per day or as not wearing their dentures for twenty-four hours per day.

The degree of keratinisation and the number of hours of denture-wearing per day were cast into a 6 x 2 table (Table 5.5). The Fisher exact probability test was used to assess the relationship between these parameters by combining adjacent columns as described in Section 5.4.8. No correlation was found.

The relationship between the number of hours per day that the dentures were worn and each of the other parameters was evaluated using the Mann-Whitney U test.

The study of the relationship between the length of time that the denture was worn per day and the thickness of the stratum corneum produced a value by the Mann-Whitney U test of 59.5. The critical value of U in this test was 55. The correlation between the length of time that the denture was worn per day and the thickness of the stratum corneum, therefore, approached the level of significance such that if the denture was worn for twenty-four hours per day, the stratum corneum was thinner.

A significant correlation ($P < 0.02$) was found between the length of time that the denture was worn per day and the mitotic index, such that the mitotic index was lower in individuals who wore their dentures for twenty-four hours per day.

No significant relationship was found between the length of time that the denture was worn per day and the epithelial thickness, the epithelial morphology or the cell density.

5.4.10 Length of Denture Experience

No significant correlation was found between the length of denture experience and any other parameter.

5.4.11 Female Denture-wearers

The results obtained from the four female denture-wearers are shown in Table 5.2.

Four male cases (numbered 1,5,8 and 9 in Table 5.1) with the same smoking habits, similar ages, similar lengths of denture experience and denture-wearing habits were chosen from the male group to allow an assessment of any sex differences in the parameters examined. The statistical test used was the Mann-Whitney U test.

No statistically significant differences were found for the parameters examined between males and females.

5.5 DISCUSSION

The mean value of epithelial thickness found in this study was 240.4 μm. Jani and Bhargava (1976), in their group of denture-wearing patients, found a value of 251 μm for epithelial thickness in the rete ridge regions and a value of 132 μm in the inter-rete ridge regions. Comparison of the results in this study with those obtained by Jani and Bhargava must only be made with caution, bearing in mind the factors discussed in Sections 1.6.8 and 1.8.1. Nevertheless, the results were of the same order of magnitude.

The mean value of the stratum corneum thickness found

in this study was 13.8 μm. Van Scotter and Boucher (1965) found a mean value of 17.8 μm for the stratum corneum thickness under acrylic dentures. Jani and Bhargava (1976) obtained a value of 20 μm in their edentulous group of patients who had been wearing acrylic dentures for three months.

There was a significant positive correlation between the cell density and both the epithelial thickness and the epithelial morphology. This result was also noted in the previous Chapter for mucosa not covered by dentures. This meant that the number of cells per unit surface length increased either as the epithelium increased in thickness or the length of basement membrane increased.

The correlation between the degree of keratinisation and the thickness of the stratum corneum suggested that there was a relationship between the quality and the quantity of the keratin present.

The positive correlation between the epithelial thickness and the stratum corneum thickness described in the previous Chapter was not found in this study.

The positive correlation between the epithelial thickness and the mitotic index found in this study was not found in the previous study for mucosa not covered by dentures.

The significant negative correlation between age and both the epithelial morphology and the cell density was surprising, since neither was found in mucosa not covered by a denture. It was likely therefore that the combined effects of age and the wearing of a denture produced these results.

It has been suggested by MacKenzie (1972, and 1973) that frictional stimulation of gingival mucosa increased the mitotic index of the epithelium and increased the thickness of the stratum corneum. In this study, if a denture was worn for twenty-four hours per day, the mitotic index was lower and there was a tendency for the stratum corneum to be thinner. These findings suggested that in the group of patients wearing their denture for twenty-four hours per day, the palate was not subjected to frictional stimulation.

Since there was no similar correlation with the length of denture experience, this would suggest that wearing a denture for twenty-four hours per day was more damaging than denture-wearing over many years.

5.6 CONCLUSIONS

This study produced baseline data for epithelium under complete dentures and provided the information required for the comparative assessment of normal mucosa and mucosa covered by dentures described in Chapter Six.

CHAPTER SIX

THE EFFECTS OF COMPLETE DENTURES ON ORAL MUCOSA

6.1 INTRODUCTION

The values of the parameters for normal mucosa described in Chapter Four and the values of the same parameters described in Chapter Five for mucosa covered by complete dentures enabled conclusions to be drawn about the effects of complete dentures on palatal mucosa.

6.2 MATERIALS AND METHODS

The comparison was undertaken in two ways. Firstly, the results from the entire group of male non-denture wearers described in Chapter Four were compared with those from the entire group of male denture wearers described in Chapter Five. Secondly, the results of the ten male patients who were present in both groups were analysed together with the four female patients discussed in Chapters Four and Five, all of whom had immediate complete dentures constructed. In this direct follow-up group, the second biopsy was taken from the same site as the first biopsy, but from the opposite side of the palate.

In addition to comparing the mitotic indices expressed as mitoses per millimetre surface length, the mitotic indices were calculated as mitoses per 1,000 viable cells. The mitotic index per 1,000 viable cells was calculated from the mitotic index per millimetre surface length and the number of cells per millimetre surface length (Tables 4.1, 4.2, 5.1 and 5.2).

6.2.1 Correction to Cell Counts

In order to compare mitotic indices expressed as mitoses per 1,000 cells it was necessary to consider the need for correcting the cell counts. As cell counts were carried out by counting nuclei in histological sections, the required correction was to allow for nuclear fragments which could result in overestimation of cell numbers. The method described by Abercrombie in 1946 was appropriate and applicable for the correction of light microscopic cell counts in oral epithelium. Abercrombie's Correction Factor is a function of the section thickness and the nuclear projection ("d" in Figure 6.1).

All the sections used in these studies were cut on the same microtome at the same setting of 5 μm. This microtome was used exclusively for research purposes and had previously been checked for accuracy of section thickness.

For measurement of nuclear projection, Abercrombie suggested that the block should be recut at right angles

to the original axis in order to estimate the nuclear projection at right angles to the plane of section utilised for cell counting. This was, however, not practicable in the present study because of the small size of the biopsy. For the purposes of this study it was assumed that the nuclear projection would be the same in the plane of section and at right angles to this plane.

Five cases from the non-denture group and five from the denture group were chosen at random to determine if there was any difference in the mean nuclear projection of the two groups. The nuclear projection of each nucleus in three separate columns of epithelium, 25 μm wide, was measured at an objective magnification of one hundred under oil immersion. The mean nuclear projection of each of the cases was then calculated. The variation in the mean nuclear projection within each group was small and the Mann-Whitney U test showed that there was no significant difference in the mean nuclear projection between the groups.

Since the studies discussed in this Chapter were direct comparative studies and there were no differences in section thickness and mean nuclear projection between the groups, it was not necessary to apply Abercrombie's Correction Factor. The mitotic indices per 1,000 cells of the groups could therefore be compared without correcting

the cell counts. The same argument applied to the comparison of the results for the number of cells per millimetre of surface length.

6.3 ENTIRE GROUP OF MALES

There were twenty-one cases in the non-denture-wearing group and twenty-seven in the denture-wearing group. The statistical test used to evaluate the results of this study was the Student's t test.

The mean values, the standard deviations and the number of observations for each of the parameters examined are shown in Table 6.1.

6.3.1 Age

The statistical test showed that there was no significant difference between the ages of the groups. This meant that a direct comparison could be made between the groups without considering the effects of aging.

6.3.2 Smoking

Since there was no difference between the parameters due to smoking in the non-denture group (Section 4.4.8) and no difference between the parameters due to smoking in the denture group (Section 5.4.8), a direct comparison could be made between the groups without reference to their smoking habits.

6.3.3 Epithelial Thickness

The mean value of epithelial thickness in the non-denture group was 268.9 μm and in the denture group was 240.4 μm. The t value was 1.872, which was below the level required for a significant difference at the five per cent level.

6.3.4 Epithelial Morphology

The mean value of epithelial morphology in the non-denture group was 2.68 and in the denture group was 2.22. The difference between the two groups was statistically significant ($P < 0.01$). This meant that in the denture group, the basement membrane had a more regular contour and the rete ridges were shorter than in the non-denture group.

6.3.5 Stratum Corneum Thickness

The mean value of stratum corneum thickness in the non-denture group was 20.4 μm and in the denture group was 13.8 μm. The difference between the two groups was statistically significant ($P < 0.01$), such that the stratum corneum was thinner in the mucosa under complete dentures.

6.3.6 Mitotic Indices

The mean value of the mitotic index in the non-denture group was 0.72 mitoses per millimetre of surface length and the corresponding value in the denture group was 0.77.

The mean value of the mitotic index in the non-denture group was 0.48 mitoses per 1,000 viable cells and the corresponding value in the denture group was 0.65. In both cases, the differences between the groups were not statistically significant using the Student's t test. In view of the large standard deviations it was felt that this test might not be reliable and the results were also evaluated using the Mann-Whitney U test. Again it was found that the differences between the groups were not significant.

6.3.7 Cell Density

The mean value of the cell density in the non-denture group was 1,602.73 cells per millimetre of surface length and the corresponding value in the denture group was 1,225.74. The difference between the groups was statistically significant ($P < 0.001$).

6.3.8 Degree of Keratinisation

The number of cases of each degree of keratinisation in each of the groups is shown in Table 6.2. The number of cases of each type of keratinisation is shown in Table 6.3. By combining the categories and applying the Fisher exact probability test it was found that orthokeratinisation was significantly more common in the non-denture group ($P < 0.01$).

6.4 DIRECT FOLLOW-UP STUDY

There were ten male and four female cases in this study. The ten males were part of the larger groups discussed in Chapters Four and Five. The four females were those discussed in Sections 4.4.9 and 5.4.11. The results are presented in Table 6.4.

The statistical test used to evaluate these results was the paired t test (Tables 6.5 and 6.6). Since in this test the cases acted as their own controls, the male and female cases were combined for analysis.

6.4.1 Age

The time interval between the first and second biopsies ranged from five to twenty-eight months. Since the cases acted as their own controls the denture-wearing group were older by the length of time between the first and second biopsies. The mean age difference was 13.8 months.

6.4.2 Smoking

There was no significant change in the smoking habits of the cases.

6.4.3 Epithelial Thickness

The mean reduction in the epithelial thickness in the denture group was 20.75 μm. The t value was 2.03 which

was below the level required ($t = 2.16$) for a significant difference at the five per cent level (Table 6.5).

6.4.4 Epithelial Morphology

The mean reduction in the value of epithelial morphology in the denture group was 0.40. The difference between the groups was statistically significant ($P < 0.02$).

6.4.5 Stratum Corneum Thickness

The mean reduction in the stratum corneum thickness in the denture group was 4.19 µm. The difference between the groups was statistically significant ($P < 0.05$).

6.4.6 Mitotic Indices

The mean increase in the mitotic index expressed as mitoses per millimetre surface length was 0.06. The mean decrease in the mitotic index expressed as mitoses per 1,000 viable cells was 0.04. In neither case was the change in the mitotic index significant.

6.4.7 Cell Density

The mean reduction in the cell density in the denture group was 219.4. The difference between the groups was statistically significant ($P < 0.05$).

6.4.8 Degree of Keratinisation

The change in the degree of keratinisation in each case is shown in Table 6.7. This table also shows the sign test applied to these results. These results showed that there was a significant reduction ($P = 0.02$) in the degree of keratinisation under dentures.

6.5 DISCUSSION

In the entire group of male patients and in the follow-up group there was a reduction in the epithelial thickness under dentures, but this did not reach the level of significance. In both of the groups there was a significant reduction in the epithelial morphology. It would appear from these results that the epithelium undergoes an adaptive change as a result of surface loading to produce a more uniform epithelium.

The degree of keratinisation was less and the stratum corneum was thinner in the epithelium under dentures in both groups. The complete dentures in these studies seemed to reduce the quantity and quality of the keratin layer.

6.6 CONCLUSION

These studies showed that the presence of a denture produced a more regular epithelium with fewer rete ridges, fewer cells, and a thinner, less highly keratinised stratum corneum.

CHAPTER SEVEN

CONCLUSIONS

7.1 INTRODUCTION

The purposes of this Chapter are:

(i) to compare the values of the parameters obtained in the post-mortem studies with those obtained in the clinical studies;

(ii) to summarise the effect of complete dentures on each of the parameters studied:

(iii) to discuss the overall effect of complete dentures on oral mucosa;

(iv) to comment on further work and future applications of the techniques described.

7.2 COMPARISON OF RESULTS OF CLINICAL AND POST-MORTEM STUDIES

7.2.1 Crest of the Maxillary Ridge

The two studies in which the crest of the maxillary ridge was examined were the preliminary post-mortem study and the post-mortem study of intact palates. In both studies complete dentures had been worn.

The mean epithelial thickness at the crest of the maxillary ridge in the preliminary post-mortem study was

247.9 μm and in the main post-mortem study it was 251.6 μm. These results are remarkably similar. There was less consistency in relation to epithelial morphology. The mean value of epithelial morphology at the crest of the maxillary ridge in the preliminary post-mortem study was 2.92 and in the main post-mortem study it was 3.90. The reason for the lower value in the preliminary post-mortem study may be that, because of difficulty of access, some of the specimens may not have been taken precisely on the ridge. This would result in the area of longer rete ridges at the crest of the ridge being omitted in the specimen and the value of epithelial morphology being lower. The mean value of stratum corneum thickness at the crest of the maxillary ridge in the preliminary post-mortem study was 11.5 μm and, in the main post-mortem study it was 14.3 μm. This difference may be a true reflection of the range of stratum corneum thickness among individuals, or alternatively it may be due to the fact that the specimens obtained for the preliminary post-mortem study were more liable to surface trauma.

7.2.2 Biopsy Site

The biopsy site (Figure 3.8) was examined in the post-mortem study of intact palates and in the clinical study of denture-wearing patients.

The mean epithelial thickness at the biopsy site in male patients in the main post-mortem study was 249.3 μm

and in the clinical study on male denture-wearing patients was 240.4 μm. The value obtained for epithelial morphology in the main post-mortem study was 2.54 and in the clinical study on denture-wearing patients this was 2.22. There was again greater variability in epithelial morphology than in epithelial thickness. The mean stratum corneum thickness at the biopsy site in male patients in the main post-mortem study was 17.8 μm, and 13.8 μm in the clinical study on male denture-wearing patients. This result is difficult to explain since it is to be expected that there might have been more damage to the surface layer in the post-mortem study.

7.2.3 The Effect of Age

Age was found to have no effect on the thickness of the epithelium in any of the studies. The only study in which age had any effect on the epithelial morphology was in the clinical study in denture-wearers where increasing age brought about a reduction in epithelial morphology. The finding that, in this study, age also brought about a reduction in the cell density is in accordance with the reduction in epithelial morphology with age since the length of the basement membrane is directly related to the quantity of the cell-dense progenitor cell compartment.

The thickness of the stratum corneum was the parameter in which the results differed most widely. In the

clinical study on non-denture-wearing patients the thickness of the stratum corneum increased with age. This finding is in agreement with those of Massler (1956) and Shklar (1966). In the main post-mortem study, at the crest of the ridge, the stratum corneum thickness reduced with age and in the clinical study on denture-wearing patients there was a tendency for the thickness of the stratum corneum to reduce with age, although this was not significant.

7.2.4 The Effect of the Sex of the Cases

The epithelial thickness in males in the main post-mortem study was greater than in females. In the clinical studies the only difference between males and females was that in the non-denture group the cell density was less in females.

7.3 THE EFFECT OF COMPLETE DENTURES ON THE PARAMETERS STUDIED

The purpose of this Section is to summarise the effect of complete dentures on each of the parameters individually. This summary discusses the results obtained from both the entire group of males and from the direct follow-up group.

7.3.1 Epithelial Thickness

Although it was shown in Section 6.3.3 and Section 6.4.3 that there was a reduction in the thickness of epithelium

under complete dentures compared with epithelium not covered by dentures, the reduction was not statistically significant. Van Mens et al (1975) also found a slight, but not significant reduction in epithelial thickness in their group of complete denture wearers.

7.3.2 Epithelial Morphology

In Section 6.3.4 and Section 6.4.4 it was shown that there was a significant reduction in epithelial morphology under complete dentures. This means that the basement membrane was more regular and the rete ridges were shorter. This finding is in agreement with the findings of Van Mens et al (1975) in relation to basement membrane shape.

7.3.3 Stratum Corneum Thickness

There was a significant reduction in the stratum corneum thickness under dentures (Sections 6.3.5 and 6.4.5).

7.3.4 Degree of Keratinisation

The degree of keratinisation was reduced by the presence of complete dentures (Sections 6.3.8 and 6.4.8).

7.3.5 Cell Density and Mitotic Indices

There was a significant reduction in the cell density of epithelium under dentures (Sections 6.3.7 and 6.4.7) but no significant change was found in the mitotic indices of epithelium under dentures (Sections 6.3.6 and 6.4.6).

The only comparable study found in the literature was that carried out by Van Mens et al (1975). Van Mens et al, in their group of non-denture wearers, found 1.6 mitoses per 1000 nuclei. They also quoted the mitotic index in this group of patients as 1.5 mitoses per 15 cm width of photomicrograph at a magnification of 900. If these figures of Van Mens et al are translated into results appropriate to the present study, this would give values of 9 mitoses and 5625 nuclei per millimetre surface length if it is assumed that the epithelial surface was straight. These values are to be compared with the values in the present study of 0.48 mitoses and 1602 nuclei per millimetre surface length reported in Sections 6.3.6 and 6.3.7. Van Mens et al found a significant increase in mitotic index under complete dentures.

It is evident that there is a considerable discrepancy between the results of the studies in this work and those of Van Mens et al (1975). One possible explanation for the difference in the number of cells per millimetre surface length is that in both cases the values quoted refer to uncorrected cell counts. However, since there was a difference of only 1 μm in the thickness of the sections between the two studies, this could not explain the large differences. A more likely explanation is an arithmetical error related to the magnification factor on the part of Van Mens et al.

The reason for the difference in mitotic indices between the studies may be differences in the criteria for identifying mitoses. No criteria for identification of mitoses were given by Van Mens et al. Another possible reason for the differences in mitotic indices may be the difficulty in identifying mitoses on photomicrographs.

7.4 THE OVERALL EFFECT OF COMPLETE DENTURES

It is difficult to combine all the factors discussed in Section 7.3 and to describe the overall changes which occur in oral mucosa covered by a denture. It is particularly difficult to evaluate the effects on the stratum corneum and the degree of keratinisation. This is partly because the stratum corneum, being the most superficial layer is most liable to damage and partly because the process of keratinisation, and the factors which influence it, are not fully understood. In order to discuss the effect of dentures on keratinisation, consideration must be given to some general factors which may affect the process of keratinisation.

7.4.1 Keratinisation

The role of stimulation of the epithelial surface in the process of keratinisation has been discussed by several authors (Robinson and Kitchin, 1948; Stahl et al, 1953; Carter, 1956; Kapur and Shklar, 1962;

MacKenzie, 1972 and 1973; and MacKenzie and Ettinger, 1975). Stahl et al (1953), in a cytological study, showed that toothbrushing increased the degree of gingival keratinisation. In a biopsy study Robinson and Kitchin (1948) found that toothbrushing increased keratinisation of the gingival epithelium. Their definition of increased keratinisation was a change in keratinisation from non-keratinisation to para-keratinisation or from parakeratinisation to ortho-keratinisation. Carter (1956) found, in an experiment with the Merion rat, that brushing of the gingival epithelium produced an increase in the thickness of the stratum corneum.

MacKenzie (1972 and 1973) discussed the effect of toothbrushing on gingival epithelium in greater detail. He stated that toothbrushing appeared to have two separate effects on gingival keratinisation. Firstly, the direct effect of frictional stimulation led to an increased mitotic rate and greater thickness of both the epithelium as a whole and of the stratum corneum. The second effect which he described was an indirect effect of the more efficient removal of dental plaque which led to a reduction in inflammation and a consequently higher degree of keratinisation of the gingival epithelium.

The conclusions which could be drawn from these studies were that toothbrushing increased the thickness of the

stratum corneum and that inflammation reduced the degree of keratinisation. Alvares and Meyer (1971) were also of the opinion that inflammation reduced the degree of keratinisation.

The results of these studies allowed a more meaningful assessment to be made of the effect of dentures on the stratum corneum.

7.4.2 The Effect of Dentures on the Stratum Corneum

The studies reported in this work (Sections 6.4.5 and 6.3.5) showed that the stratum corneum thickness was reduced under dentures. Since it has been shown in Section 7.4.1 that stimulation increases the thickness of the stratum corneum, this reduction in thickness under dentures may be due to a lack of frictional stimulation. In other words, a denture prevents the normal stimulation of the palatal epithelium and does not itself act as a stimulating factor.

Since inflammation has been shown to reduce the degree of keratinisation, it is possible that the reduction in the degree of keratinisation under dentures is due to mild inflammation.

7.4.3 The Effect of Dentures on Palatal Epithelium

The typical histological appearance of normal mucosa from the biopsy site is shown in Figure 7.1. The typical

appearance of clinically normal mucosa under a denture at the same site is shown in Figure 7.2. These Figures give a descriptive picture for a summary of the results. Complete dentures in this study produced a more regular epithelium with fewer rete ridges, fewer cells per unit surface length, and a thinner, less keratinised stratum corneum. The complete dentures did not alter the epithelial thickness or the mitotic index of the epithelium.

7.5 FURTHER WORK AND FUTURE APPLICATIONS

7.5.1 Post-mortem Studies

The intact palates used in Chapter Three could be used to examine oral mucosa in other parts of the palate. The material may also be used to examine other related structures: the bone in the residual alveolar ridge, the tissues of the soft palate and the palatal salivary gland tissue.

7.5.2 Clinical Studies

If the problem of negative chemography discussed in Section 4.3.6 were overcome, then use could be made of the material which has been labelled with the double pulse of tritiated thymidine to measure the length of the S phase of the cell cycle. This would shed more light on cell kinetics.

Having established quantitative values for parameters

of normal palatal epithelium and of clinically normal epithelium under dentures it should be possible to undertake studies on clinically abnormal epithelium under dentures.

Perhaps the most interesting study from a clinical point of view would be to trace the change in the parameters of patients who have been wearing the same complete dentures for a number of years and for whom replacement complete dentures are constructed.

ABBREVIATIONS

B	-	biopsy site
C	-	crest of the ridge
C.O.	-	complete orthokeratinisation
C.P.	-	complete parakeratinisation
d	-	distance
di	-	difference in ranks
E_{mag}	-	eyepiece magnification
G_1	-	postmitotic-presynthetic gap
G_2	-	postsynthetic-premitotic gap
H	-	halfway site
H.L.C.	-	heavily labelled cells
i	-	number of intersections
I_{bm}	-	total number of intersections on the basement membrane
I_s	-	total number of intersections on the surface
I.O.	-	incomplete orthokeratosis
I./C.O.	-	showing both incomplete orthokeratosis and complete orthokeratosis
L	-	length
L.L.S.	-	lightly labelled cells
M	-	mitotic phase
M	-	midline site
n	-	number
N	-	non-smoker
N.K.	-	non-keratinised

O_{mag}	-	objective magnification
p_c	-	mean point count over connective tissue
p_e	-	mean point count over epithelium
p_k	-	mean point count over stratum corneum
p_n	-	palatal nerve tissue
P	-	probability
P_e	-	total point count over epithelium
P_m	-	paramedian site
r	-	correlation coefficient
r_s	-	Spearman rank correlation coefficient
R	-	sum of ranks
s	-	salivary gland tissue
S	-	synthesis phase
t_i	-	time interval
T	-	sum of ranks with less sign
T_s	-	length of synthesis phase
v	-	blood vessel
Y	-	smoker

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ORAL MUCOSA

With Particular Reference to the Edentulous Mouth

Volume 2 of Two Volumes

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THESIS

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Glasgow Dental Hospital and School

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Figures and Tables arranged in sequence as they are referred to in Volume 1.

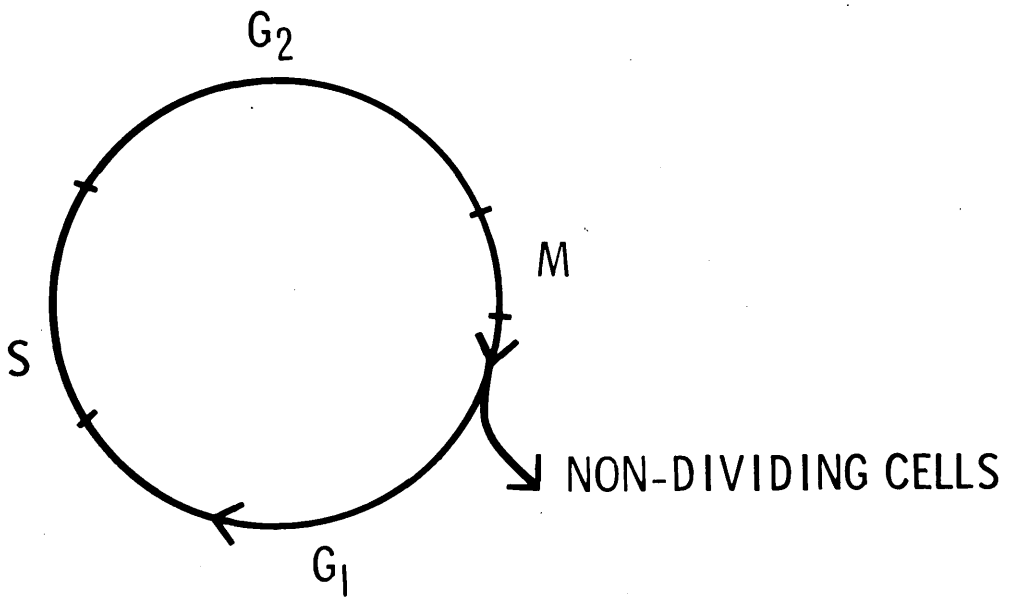


Figure 1.1 Diagramatic illustration of the cell cycle of progenitor cells.

M = Mitotic phase.

G_1 = Postmitotic - presynthetic gap.

S = Synthesis phase.

G_2 = Postsynthetic - premitotic gap.

Case Number	Age (Years)	Time Interval (Hours)
1	75	17
2	70	26
3	53	-
4	62	48
5	70	21
6	53	36
7	54	24
8	66	28
9	54	27

Table 2.1 Age and time interval between death and autopsy in the preliminary post-mortem study.

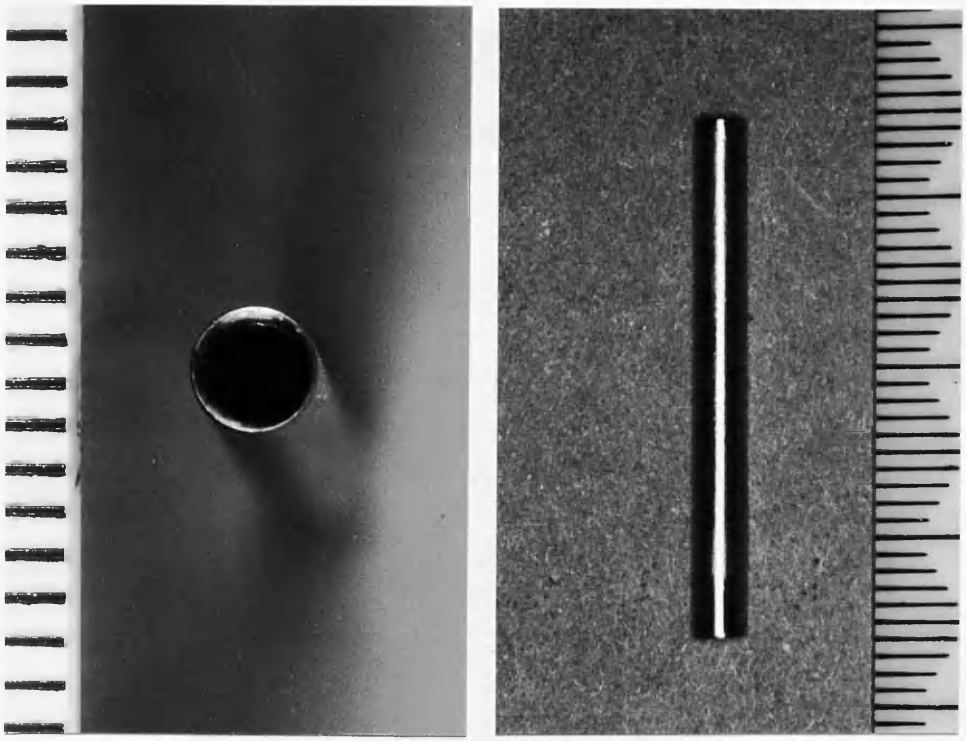


Figure 2.1 3mm diameter trephine-type biopsy punch.

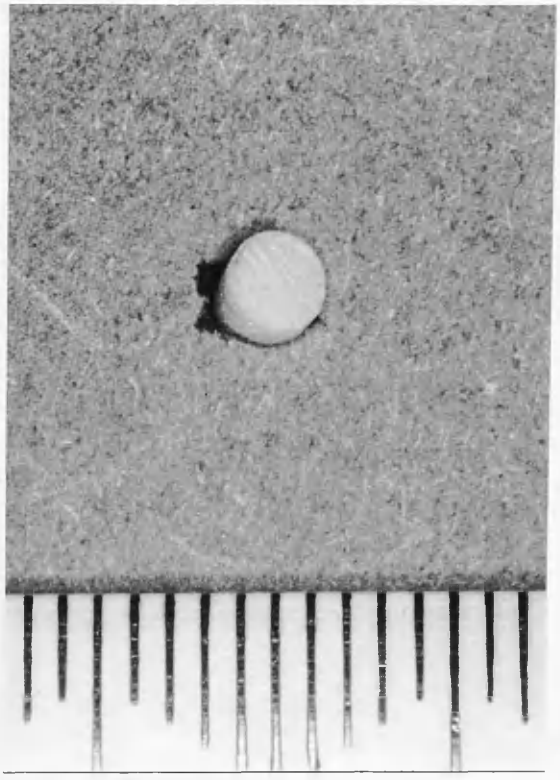


Figure 2.2 3mm diameter specimen.

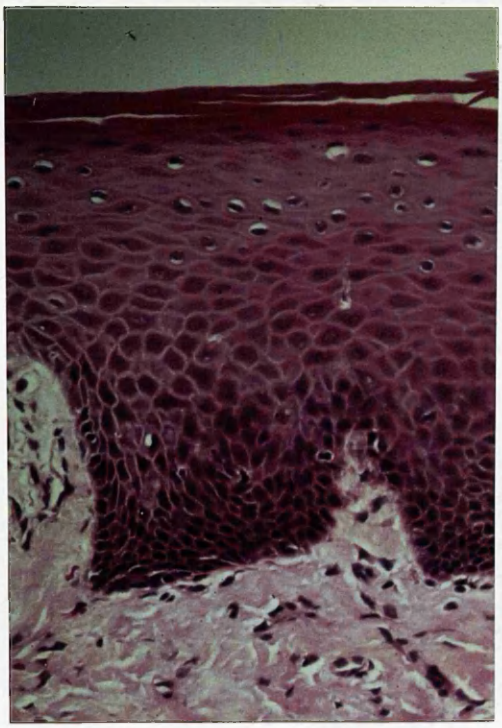


Figure 2.3 Palatal epithelium stained with haematoxylin and eosin x 250.

Figure 2.4 Palatal epithelium stained with the Crooke-Russell modification of Mallory's stain x 250.

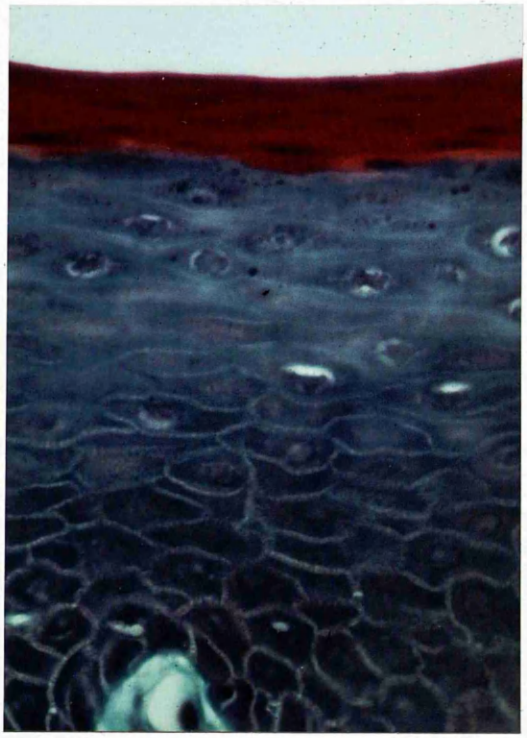


Figure 2.5 Crooke-Russell modification of Mallory's stain showing complete keratinisation x 500.

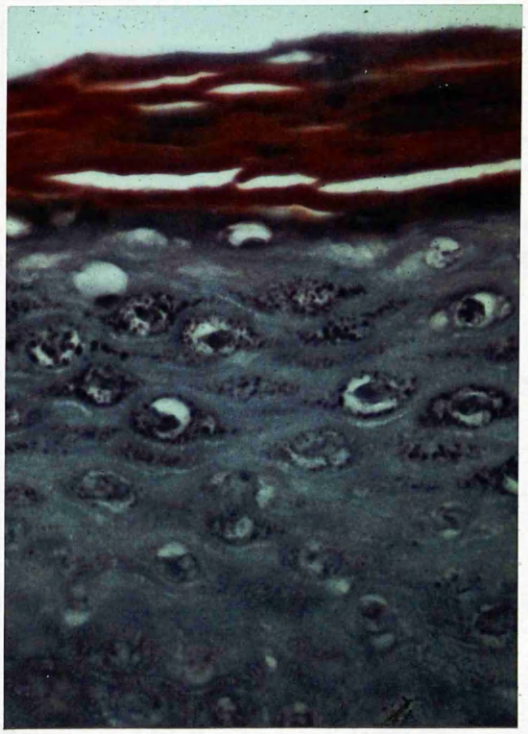


Figure 2.6 Crooke-Russell modification of Mallory's stain showing incomplete keratinisation x 600.

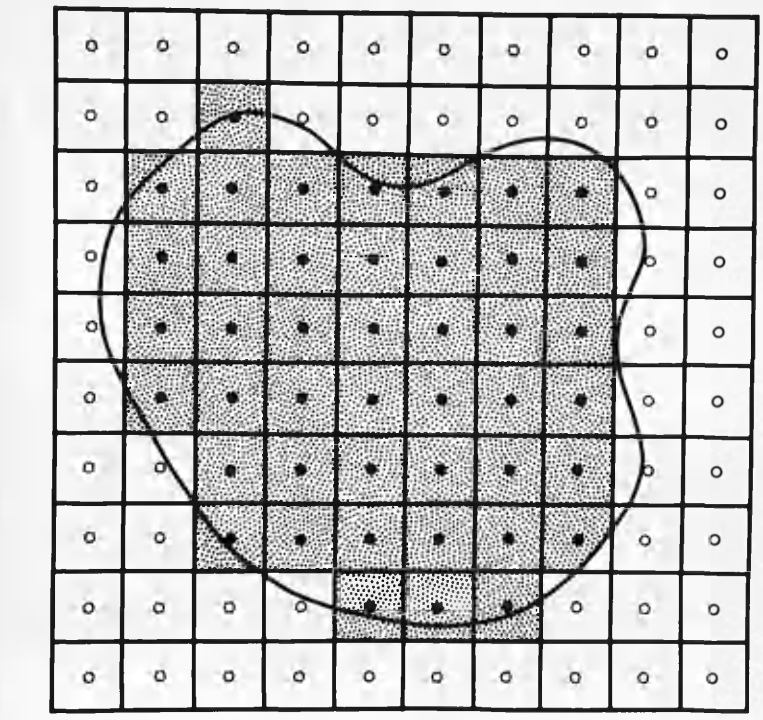


Figure 2.7 Lattice of squares with marked centre points superimposed on the shape to be examined.

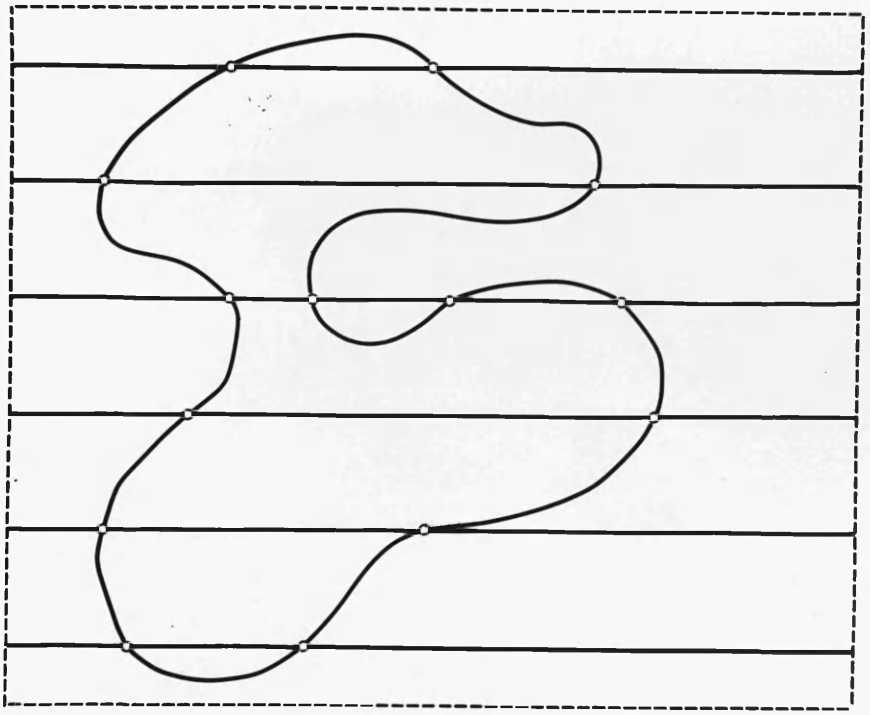


Figure 2.8 Grid of parallel lines superimposed on shape to be examined.

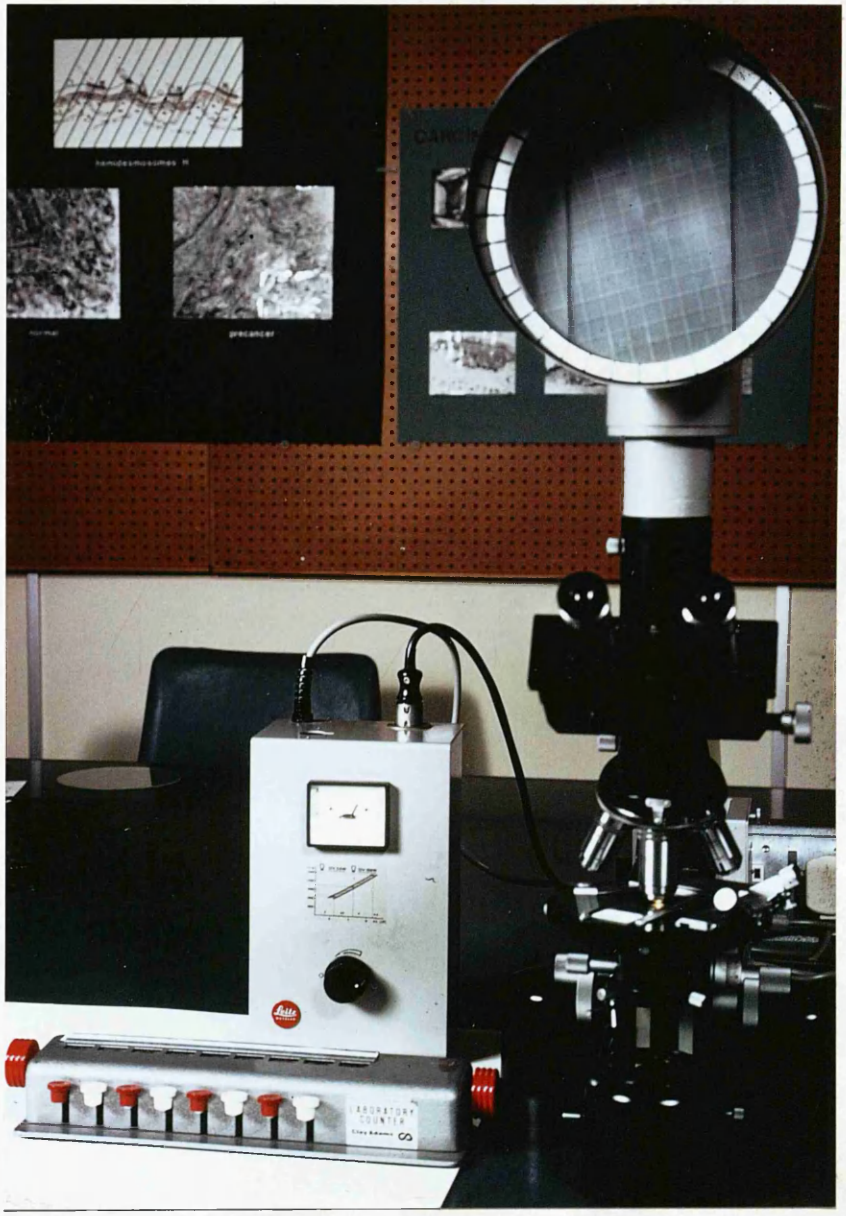


Figure 2.9 Leitz Ortholux microscope and projection screen upon which are marked two vertical lines to delineate the column used for stereological analysis. A counting grid is also superimposed on the screen.

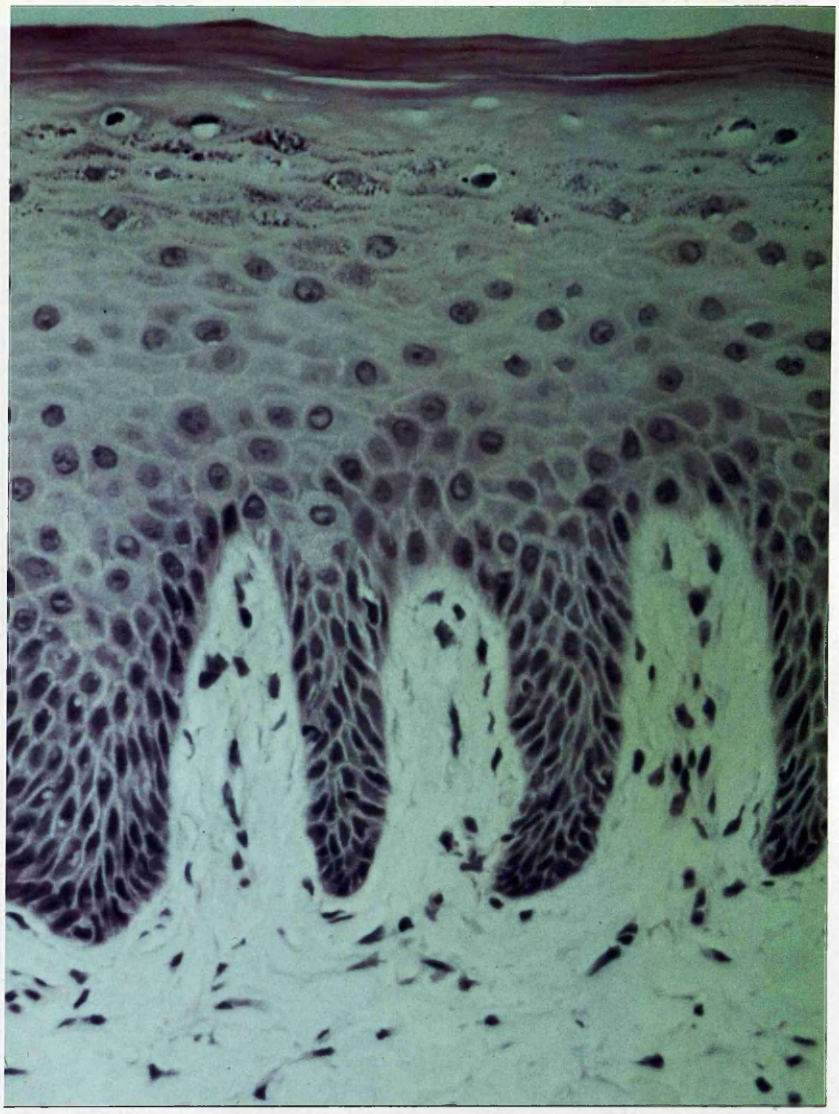


Figure 2.10 Column of epithelium between lines on the screen x 350.

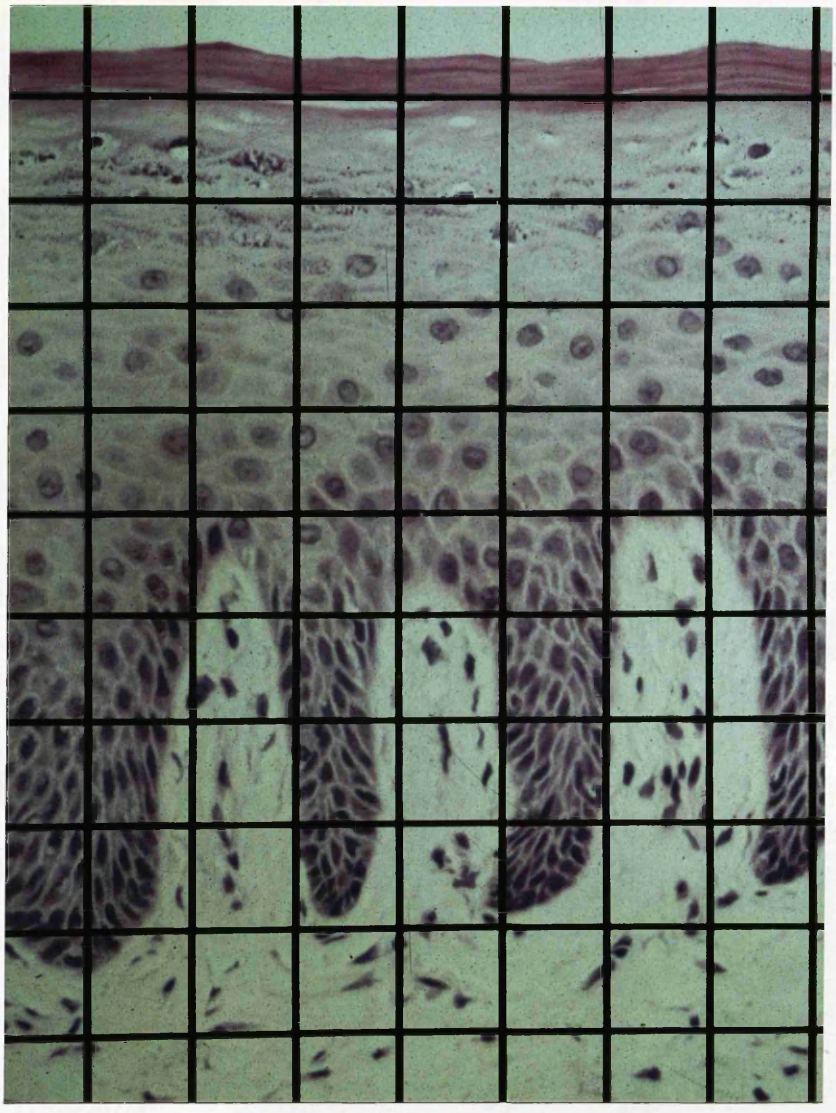


Figure 2.11 Square grid superimposed on column of epithelium x 350.

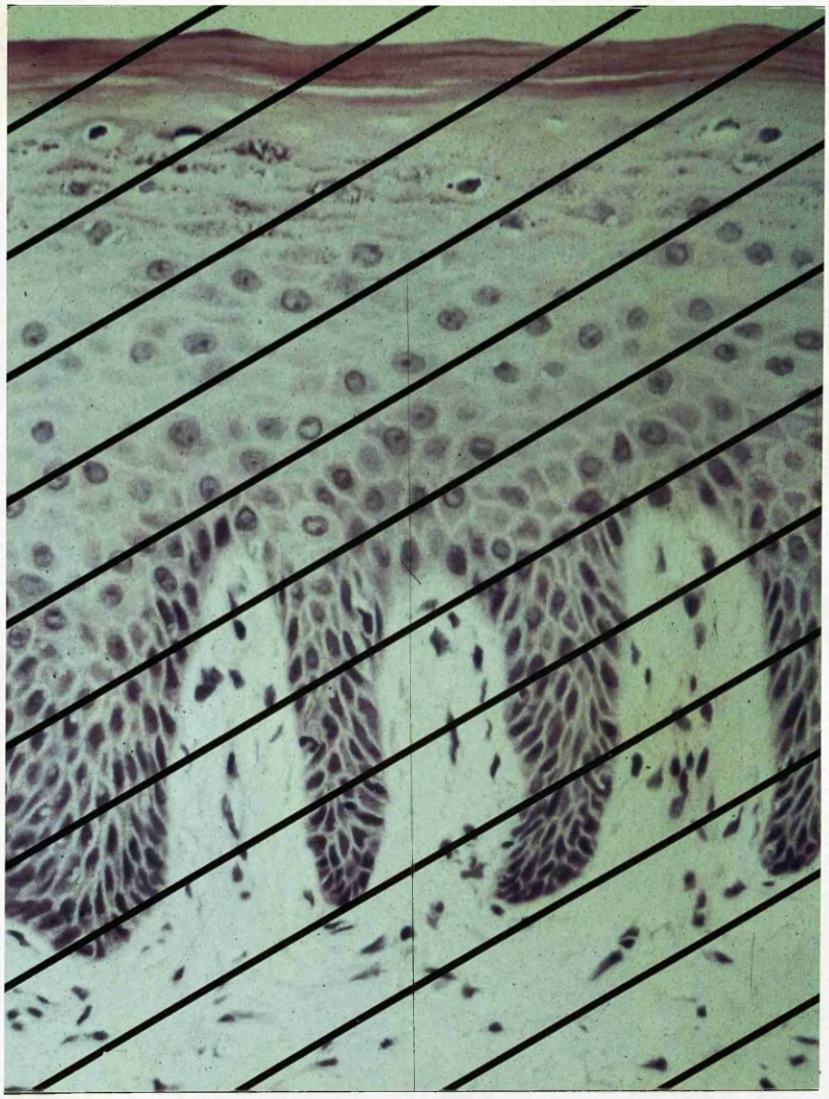


Figure 2.12 Line grid superimposed on column of epithelium x 350.

	Case Number	Mean Point Count (p_e)	Epithelial Thickness (μm)
Maxilla	1	64.0	242.6
	2	71.2	270.0
	3	52.2	197.9
	4	59.5	225.6
	5	76.2	288.9
	6	69.2	262.4
	Mean		247.9
Mandible	1	57.7	218.8
	2	65.7	249.1
	3	58.7	222.5
	4	32.7	124.0
	5	46.3	175.6
	7	69.0	261.6
	8	43.7	165.7
	9	49.5	187.7
	Mean		200.6

Table 2.2 Mean point count and epithelial thickness in specimens from maxilla and mandible in the preliminary post-mortem study.

Epithelial Thickness (μm) Maxilla	Rank	Epithelial Thickness (μm) Mandible	Rank
242.6	9	218.8	6
270.0	13	249.1	10
197.9	5	222.5	7
225.6	8	124.0	1
288.9	14	175.6	3
262.4	12	261.6	11
		165.7	2
		187.7	4

$R_1 = 61$ $R_2 = 44$

$$U = n_1 n_2 + \frac{n_1 (n_1 + 1)}{2} - R_1$$

$$= 6 \times 8 + \frac{6 (6 + 1)}{2} - 61$$

$$= 8$$

$$P = 0.02$$

Table 2.3 The Mann-Whitney U test applied to epithelial thickness in specimens from maxilla and mandible in the preliminary post-mortem study (Siegel, 1956).

	Case Number	Intercept Point Count on Basement Membrane I_{bm}	Intercept Point Count on Surface I_s	$\frac{I_{bm}}{I_s}$
Maxilla	1	101	36	2.81
	2	117	35	3.34
	3	65	38	1.71
	4	115	37	3.11
	5	149	35	4.25
	6	83	36	2.31
	Mean			2.92
Mandible	1	103	37	2.78
	2	104	36	2.89
	3	204	35	5.82
	4	70	37	1.89
	5	95	36	2.64
	7	80	36	2.22
	8	68	36	1.89
	9	113	36	3.14
	Mean			2.87

Table 2.4 Intercept point counts on the basement membrane, the surface and the epithelial morphology in specimens from maxilla and mandible in the preliminary post-mortem study.

	Case Number	Mean Point Count (p_k)	Stratum Corneum Thickness (μm)
Maxilla	1	6.7	3.9
	2	13.2	7.8
	3	6.2	3.6
	4	26.2	15.4
	5	42.2	24.8
	6	22.5	13.2
	Mean		11.45
Mandible	1	14.0	8.2
	2	14.0	8.2
	3	12.2	7.2
	4	23.5	13.8
	5	12.3	7.2
	7	21.2	12.5
	8	15.5	9.1
	9	17.2	10.1
	Mean		9.54

Table 2.5 Mean point count and stratum corneum thickness in specimens from maxilla and mandible in the preliminary post-mortem study.

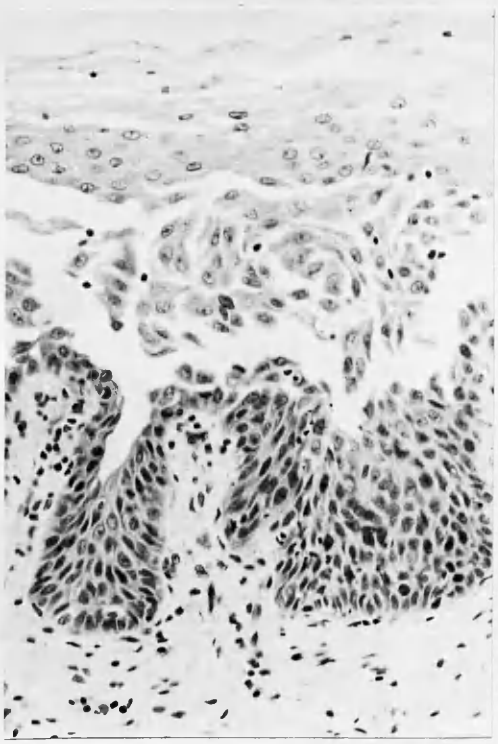
Case Number	Maxilla	Mandible
1	Incomplete Orthokeratosis	Complete Parakeratosis
2	Incomplete Parakeratosis	Complete Parakeratosis
3	Incomplete Parakeratosis	Incomplete Parakeratosis
4	Incomplete Orthokeratosis	Complete Parakeratosis
5	Incomplete Orthokeratosis	Incomplete Parakeratosis
6	Complete Parakeratosis	-
7	-	Complete Parakeratosis
8	-	Complete Parakeratosis
9	-	Incomplete Parakeratosis

Table 2.6 Degree of keratinisation in specimens from maxilla and mandible in the preliminary post-mortem study.

	Maxilla	Mandible
Incomplete Orthokeratosis	3	-
Complete Parakeratosis	1	5
Incomplete Parakeratosis	2	3

Table 2.7 Number of specimens of each degree of keratinisation in the preliminary post-mortem study.

A



B

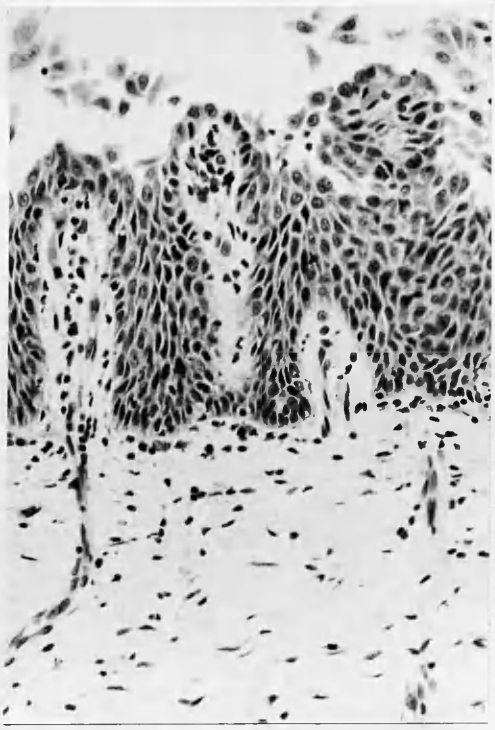


Figure 2.13 Stages in post-mortem autolysis x 200.

A Superficial layers of epithelium have separated from the deeper layers.

B Superficial layers of epithelium are completely lost.

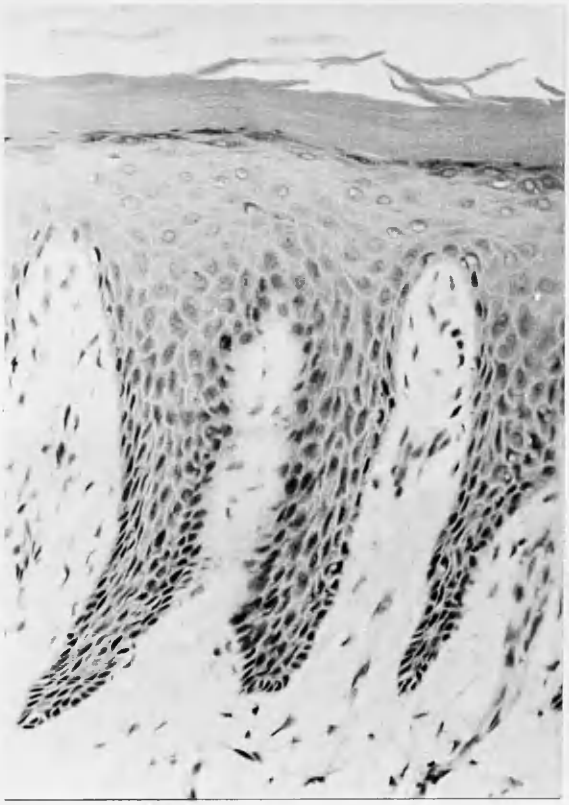


Figure 2.14 Epithelium from crest of ridge in the maxillary first molar area x 200.

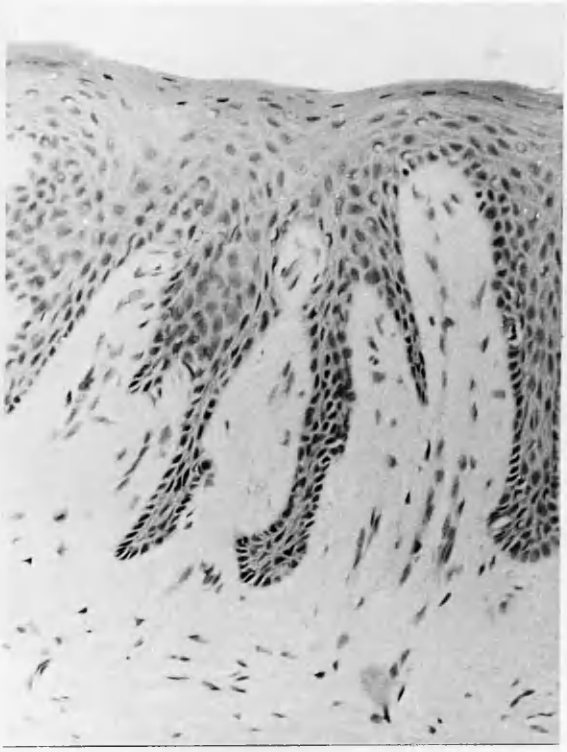


Figure 2.15 Epithelium from crest of ridge in the mandibular incisor area x 200.

Case Number	Age (Years)	Sex
1	56	Male
2	60	Male
3	51	Male
4	79	Female
5	57	Female
6	78	Female
7	70	Male
8	66	Female

Mean age of male cases : 59.25 years.

Mean age of female cases : 70.0 years.

Table 3.1 Age and sex of the cases in post-mortem study of intact palates.

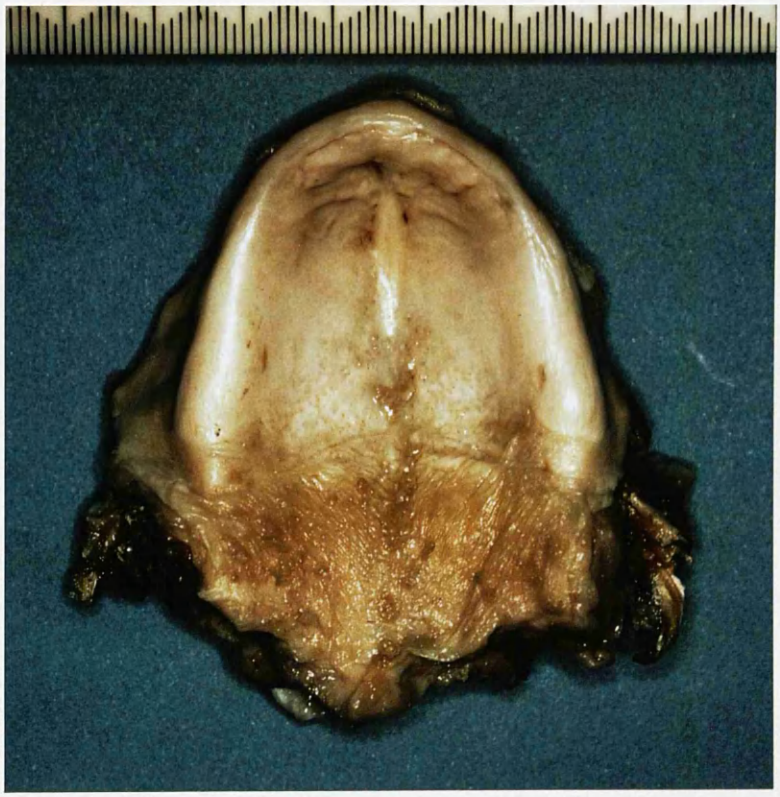


Figure 3.1 Intact palate obtained post-mortem.

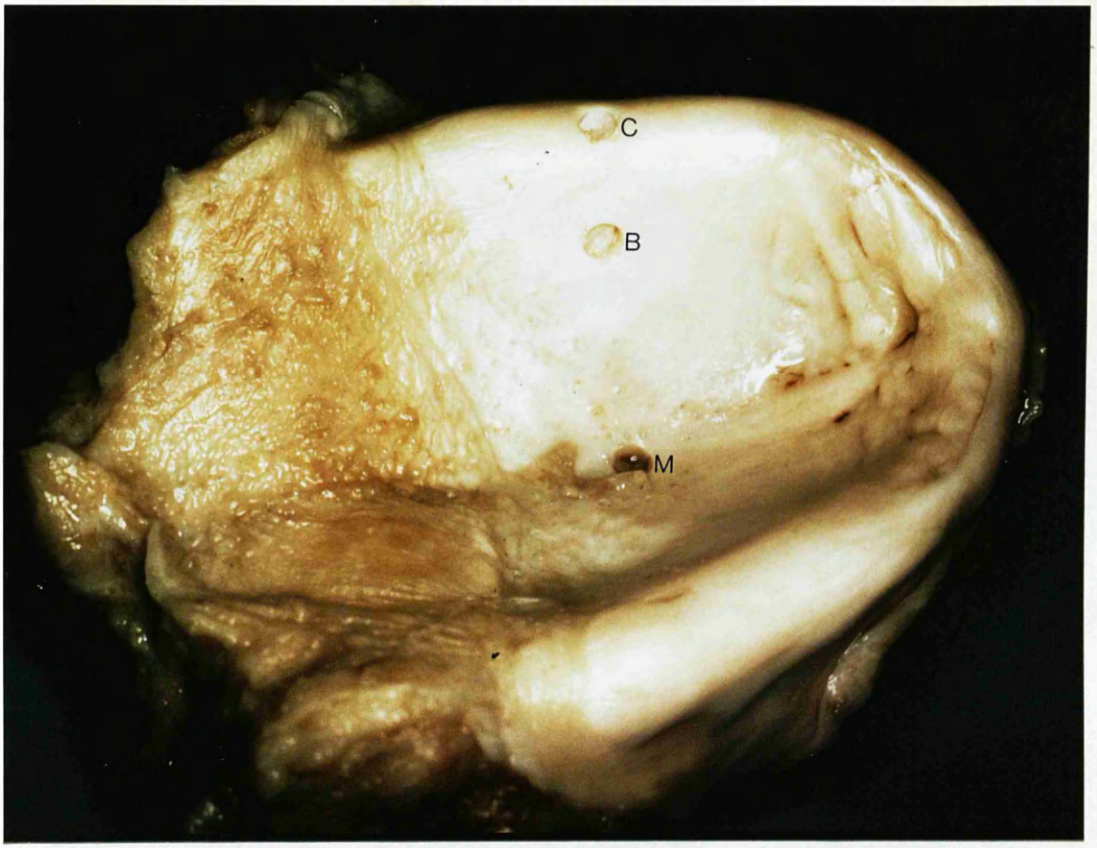


Figure 3.2 Intact palate showing sites of three of the specimens obtained using the trephine-type biopsy punch prior to decalcification.

C = Crest of ridge.

B = Biopsy site.

M = Midline.

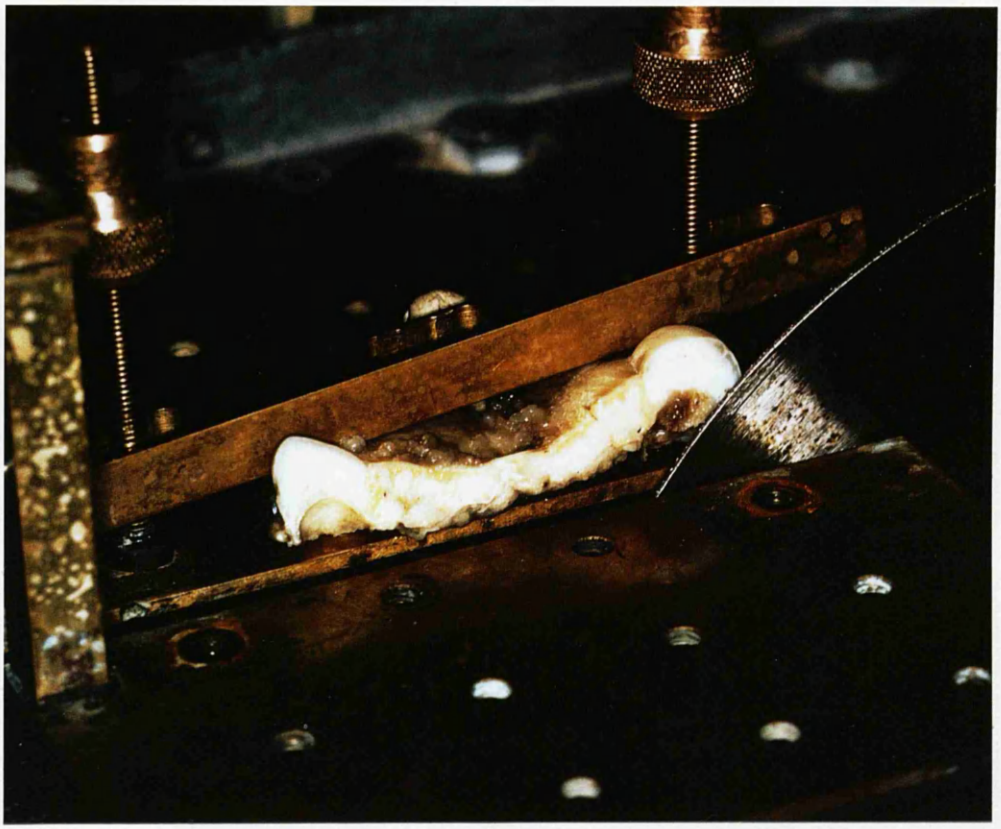


Figure 3.3 Circular saw used to cut the first palate into blocks.

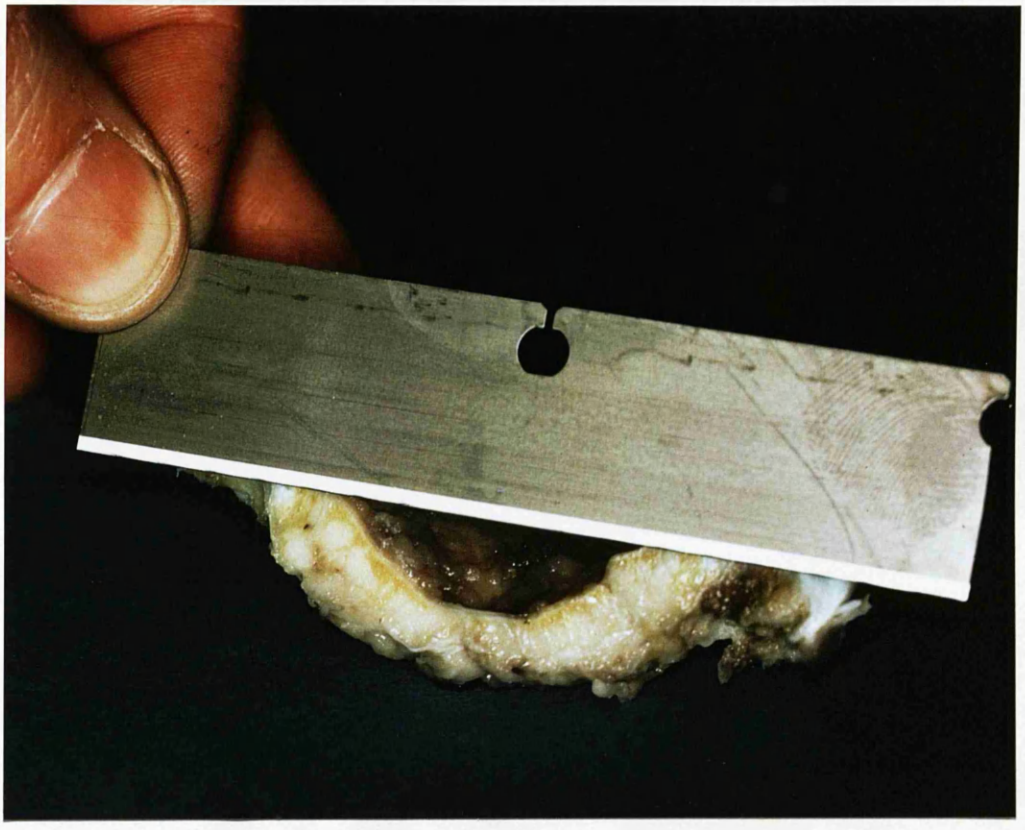


Figure 3.4 Dermatome knife used to cut decalcified palates into blocks.

Figure 3.5 Processed block from palate embedded in paraffin wax.

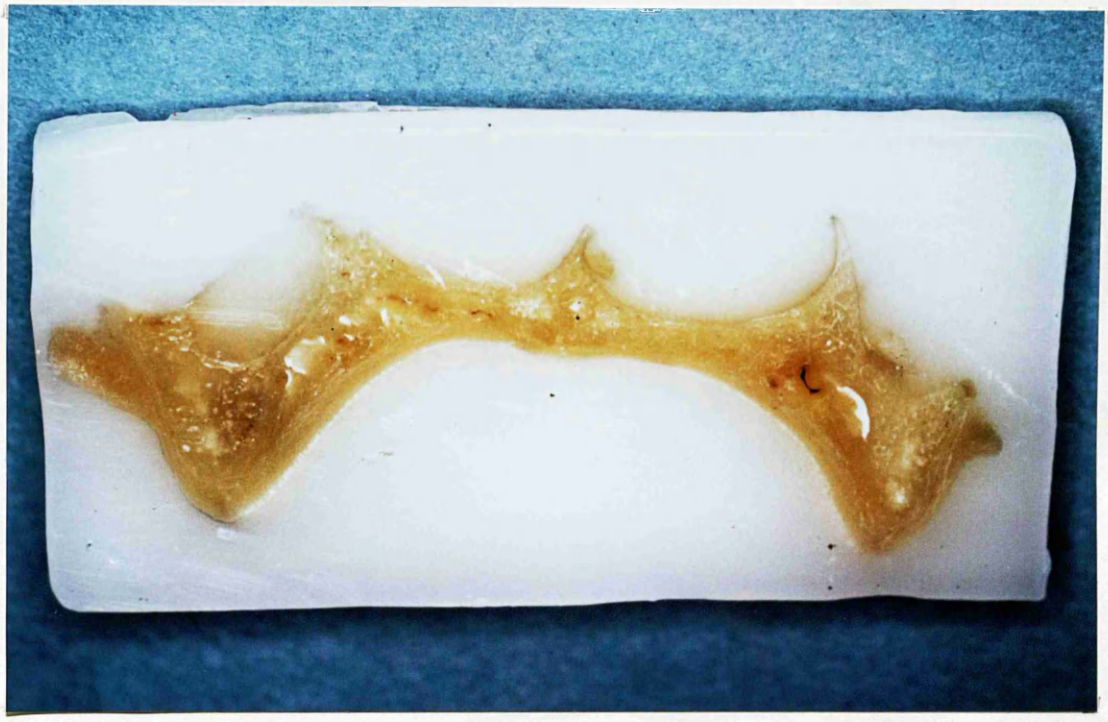


Figure 3.6 Processed block from palate embedded in paraffin wax and trimmed for section cutting.

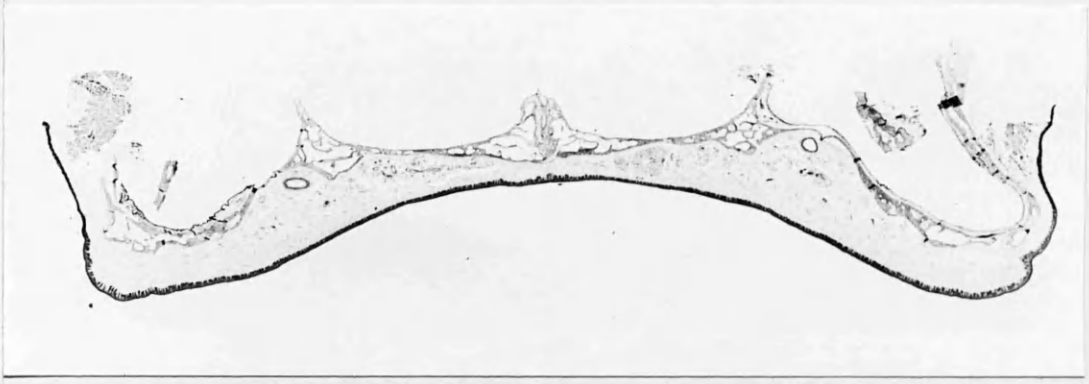


Figure 3.7 Cross-section of palate in first molar area x 2.6.

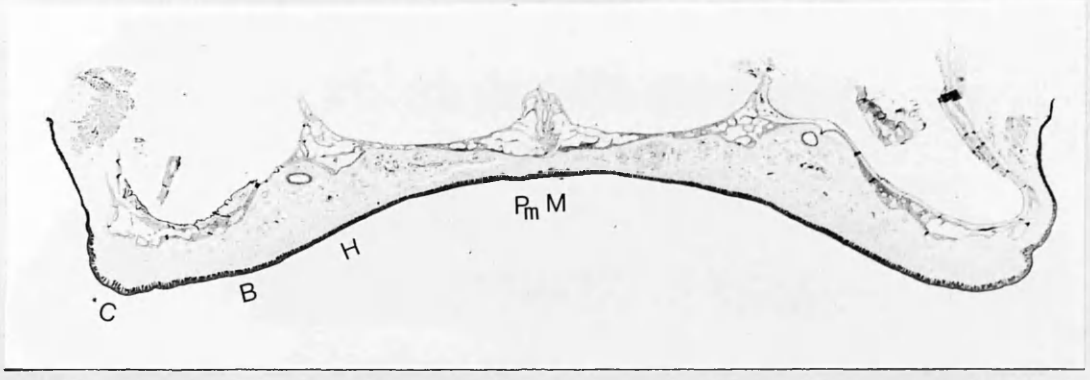


Figure 3.8 Cross-section of palate with sites for detailed analysis marked x 2.6.

C = Crest

B = Biopsy

H = Halfway

P_m = Paramedian

M = Midline

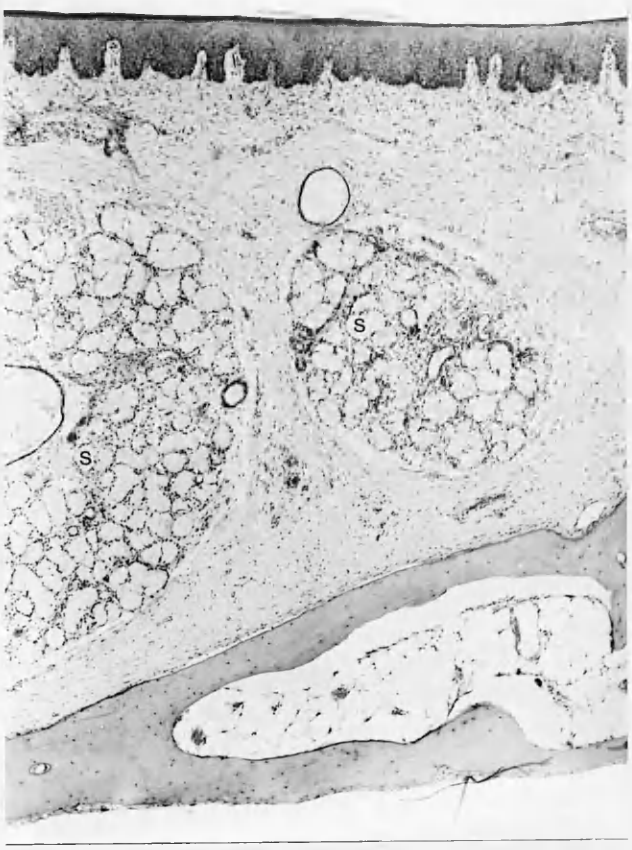


Figure 3.9 Palatal salivary gland tissue(s) x 30.

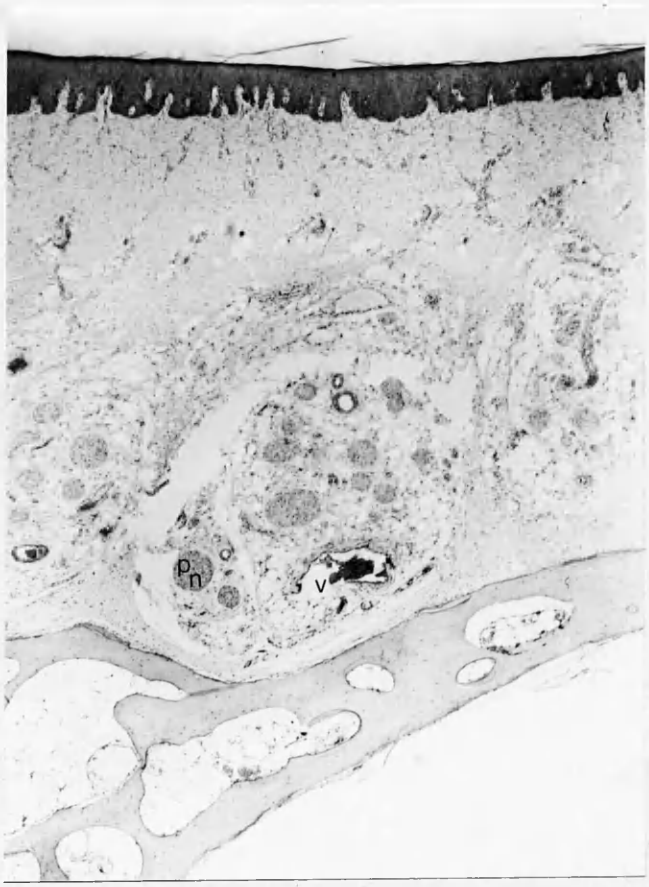


Figure 3.10 Palatal blood vessel(v) and nerves (p_n) x 30.

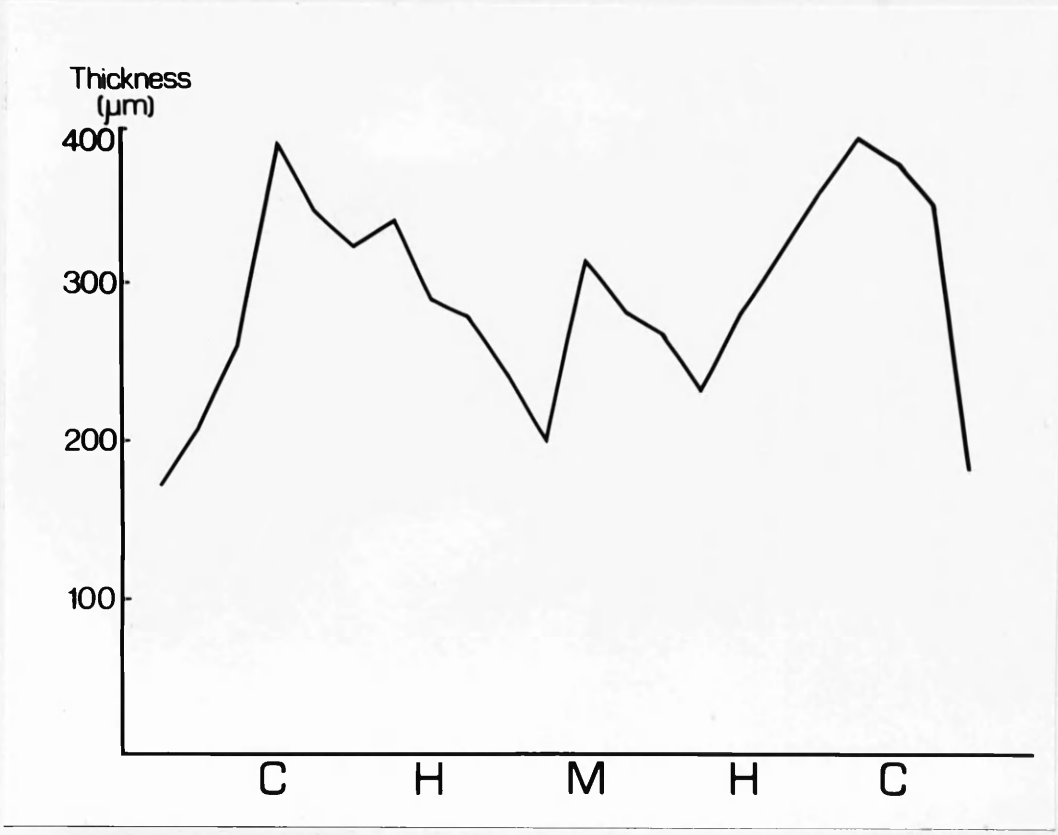


Figure 3.11 Graph of epithelial thickness across one palate.

C = Crest.

H = Halfway.

M = Midline.

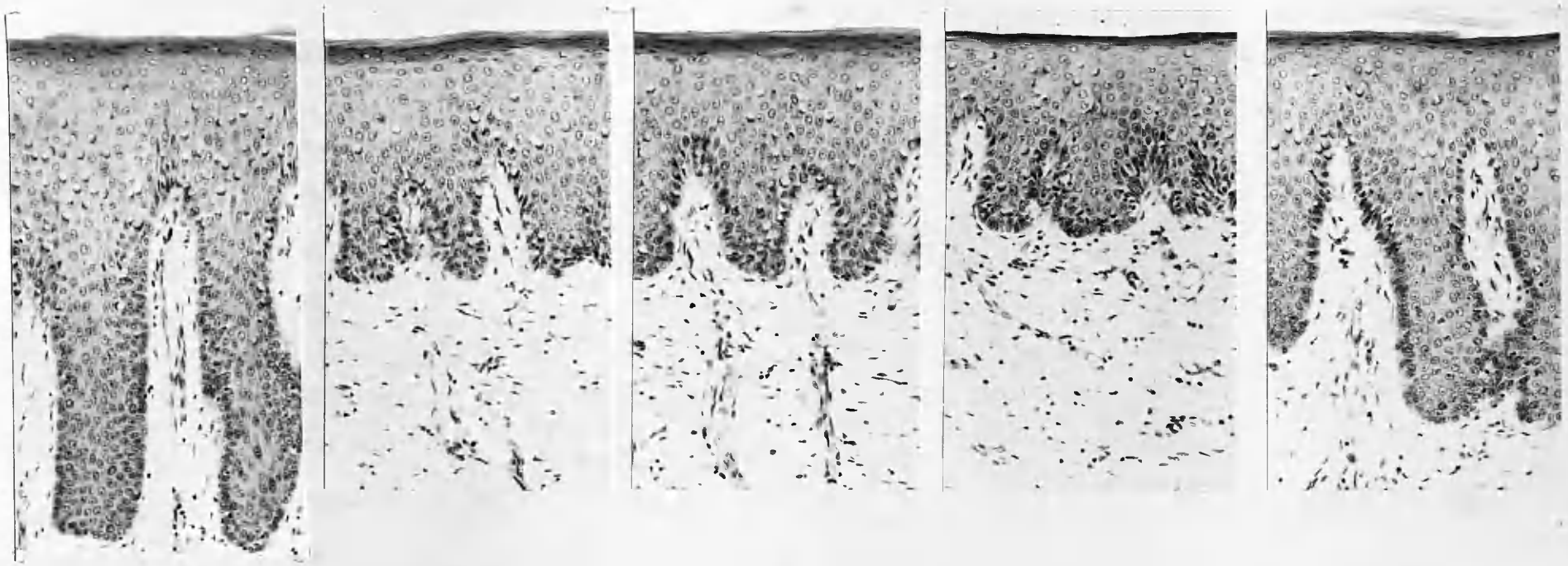


Figure 3.12 The histological appearance of the epithelium at each of the sites across one palate at the same magnification x 100.

Case Number	Crest	Biopsy	Halfway	Paramedian	Midline	Paramedian	Halfway	Biopsy	Crest
1	284.3	248.1	149.0	154.9	182.3	145.1	138.2	222.5	347.1
2	384.3	260.8	252.9	152.9	223.5	160.8	232.3	242.2	298.1
3	227.4	258.8	274.5	217.6	290.2	221.5	252.9	241.2	252.9
4	182.3	225.5	231.3	184.3	166.6	168.6	264.7	239.2	233.3
5	123.5	186.2	183.3	176.5	164.7	123.5	150.0	182.3	201.9
6	237.2	248.1	219.6	170.6	201.9	180.4	245.1	277.4	241.2
7	305.9	254.9	277.4	156.8	247.0	221.6	221.6	265.7	298.1
8	243.1	143.1	72.5	103.9	107.8	76.5	90.2	149.0	164.7

Table 3.2 Site variation in epithelial thickness (μm).

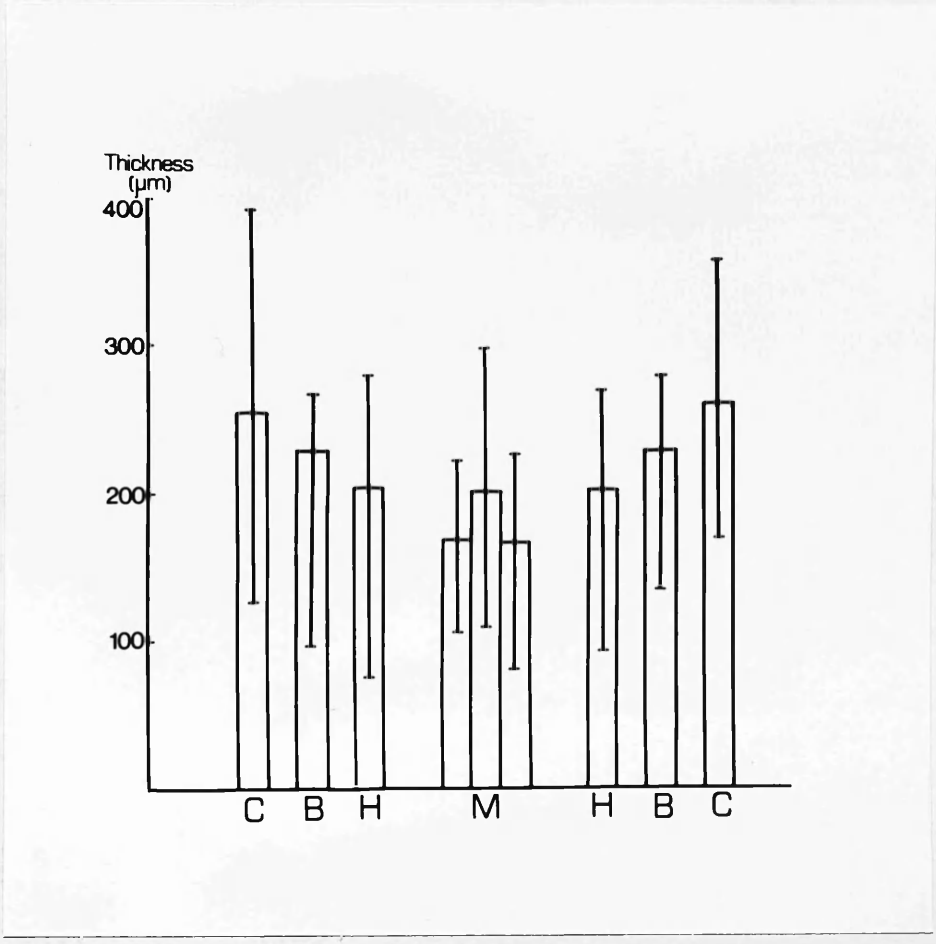


Figure 3.13 Histogram of mean values and range of values of epithelial thickness at each of the sites from the eight cases.

Epithelial Thickness. Crests (µm)	Epithelial Thickness. Paramedians (µm)	Difference (di)	Rank of Difference	Rank with less Sign
284.3	154.9	129.4	11	
384.3	152.9	231.4	16	
227.4	217.6	9.8	2	
182.3	184.3	-2.0	1	1
123.5	176.5	-53.0	4	4
237.2	170.6	66.6	7	
305.9	156.8	149.1	14	
243.1	103.9	139.2	13	
347.1	145.1	202.0	15	
298.1	160.8	137.3	12	
252.9	221.5	31.4	3	
233.3	168.6	64.7	6	
201.9	123.5	78.4	9	
241.2	180.4	60.8	5	
298.1	221.6	76.5	8	
164.7	76.5	88.2	10	

$T = 5$

$n = 16$

$P < 0.01$

Table 3.3 The Wilcoxon matched-pairs signed-ranks test applied to the differences between the epithelial thickness of the crests and the paramedian sites (Siegel, 1956).

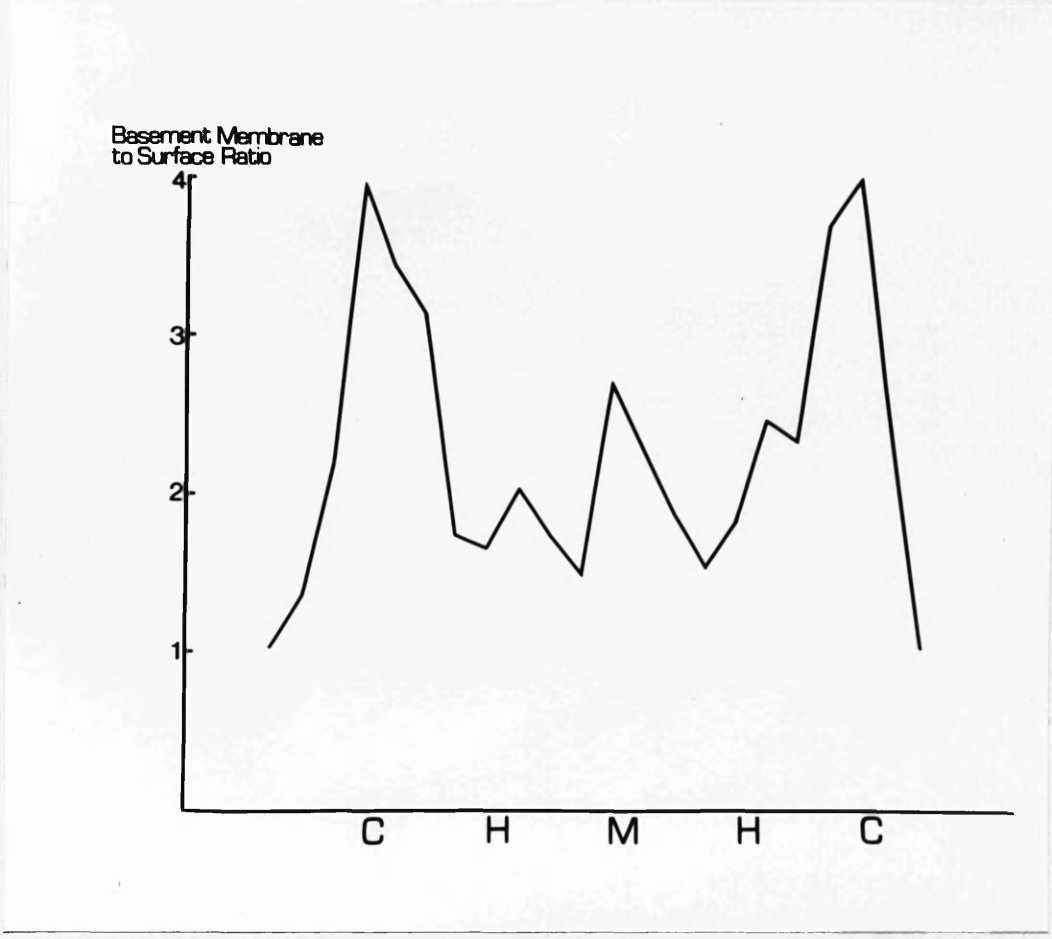


Figure 3.14 Graph of epithelial morphology across one palate.

Case Number	Crest	Biopsy	Halfway	Paramedian	Midline	Paramedian	Halfway	Biopsy	Crest
1	5.69	2.03	1.66	2.03	2.53	1.82	1.33	2.70	4.47
2	4.09	3.35	2.74	1.91	2.07	2.30	2.60	2.14	3.02
3	2.94	2.09	1.96	2.46	2.94	1.96	2.30	2.54	2.70
4	3.26	2.89	2.78	2.50	2.24	2.33	2.41	2.35	4.31
5	2.33	2.33	2.35	2.20	3.00	1.94	1.67	2.50	4.04
6	5.29	4.43	3.29	2.25	3.07	2.33	2.59	3.52	3.95
7	3.97	3.16	1.66	1.48	2.70	2.31	1.81	2.33	4.07
8	4.77	1.98	1.12	1.61	1.90	1.55	1.27	1.94	3.44

Table 3.4 Site variation in epithelial morphology.

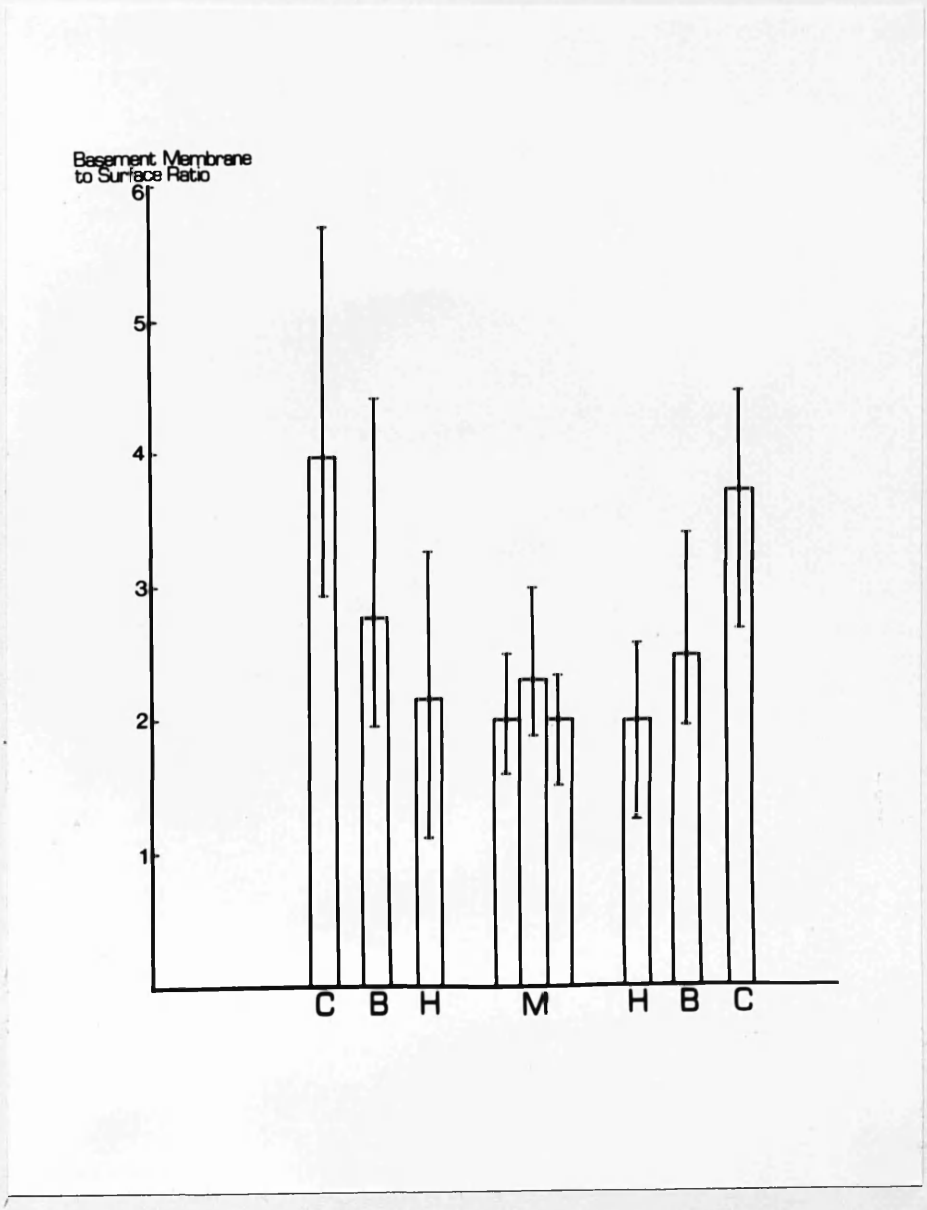


Figure 3.15 Histogram of mean values and range of values of epithelial morphology at each of the sites from the eight cases.

EPITHELIAL MORPHOLOGY	EPITHELIAL THICKNESS (μm)	RANKS		di	di^2
A	B	A	B		
1.39	131.3	2	2	0	0
3.38	207.8	12	9	3	9
5.29	237.2	17	11	6	36
4.87	250.9	16	15	1	1
4.00	245.0	15	13.5	1.5	2.25
3.29	219.5	11	10	1	1
2.50	176.4	6	4	2	4
2.25	170.5	3	3	0	0
3.07	201.9	9	8	1	1
2.33	180.3	4	5	-1	1
2.40	196.0	5	6	-1	1
2.59	245.0	7	13.5	-6.5	42.25
3.12	256.8	10	16	-6	36
3.94	297.9	13	17	-4	16
3.95	241.1	14	12	2	4
2.62	198.0	8	7	1	1
1.13	74.5	1	1	0	0
					Σdi^2 155.5

$$r_s = 1 - \frac{6 \Sigma di^2}{n^3 - n}$$

$$r_s = 1 - \frac{6(155.5)}{17^3 - 17}$$

$$= 0.81$$

$$P < 0.01$$

Table 3.5 The Spearman rank correlation coefficient test applied to the relationship between epithelial morphology and epithelial thickness in case number 6 (Siegel, 1956).

Case Number	P
1	< 0.01
2	< 0.01
3	> 0.05
4	< 0.01
5	< 0.01
6	< 0.01
7	< 0.01
8	< 0.01

Table 3.6 The significance levels from the Spearman rank correlation coefficient tests between the epithelial thickness and the epithelial morphology of the eight cases.

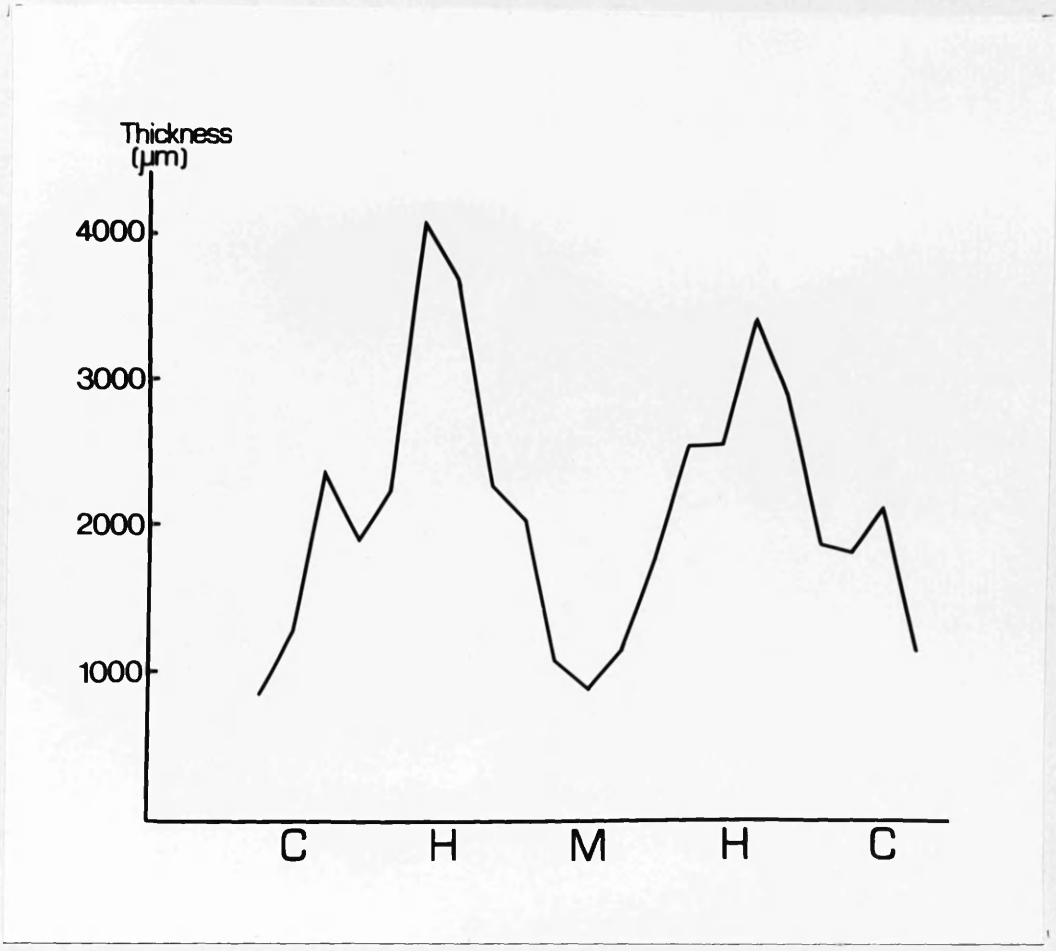


Figure 3.16 Graph of connective tissue thickness across one palate.

Case Number	Crest	Biopsy	Halfway	Paramedian	Midline	Paramedian	Halfway	Biopsy	Crest
1	1883.6	1491.7	4525.7	1504.3	1074.5	1251.5	5461.2	2389.2	1997.3
2	2174.3	3059.2	2073.2	556.2	745.8	505.6	2705.3	3539.6	1997.3
3	3628.1	3312.1	3501.7	1744.5	1466.4	1377.9	3615.5	2591.5	1959.4
4	2781.1	3691.3	4272.8	1529.6	1049.2	1428.5	3792.5	3666.0	1997.3
5	2490.4	4462.5	4222.3	1125.1	1023.9	809.1	2446.1	2389.2	1908.8
6	3375.3	4095.9	4399.3	1946.8	1883.6	2401.9	4355.0	4057.9	3198.3
7	1314.7	2098.5	3874.7	1074.5	897.5	1163.0	3040.4	2136.4	2136.4
8	2541.0	3092.2	3779.9	2262.9	1428.5	1226.2	3666.1	3324.8	2111.2

Table 3.7 Site variation in connective tissue thickness (μm).

Case Number	Crest	Biopsy	Halfway	Paramedian	Midline	Paramedian	Halfway	Biopsy	Crest
1	-	-	2073.2	-	-	-	1226.2	151.7	-
2	-	-	-	-	-	-	-	-	-
3	-	-	120.1	455.1	-	-	-	-	-
4	-	-	366.6	-	-	-	644.7	-	-
5	-	-	619.4	-	-	-	828.0	-	-
6	-	-	-	-	-	-	410.8	-	-
7	-	-	-	164.3	-	-	-	-	-
8	-	-	1302.1	971.7	-	-	316.0	202.2	-

Table 3.8 Site variation in salivary gland tissue (μm).

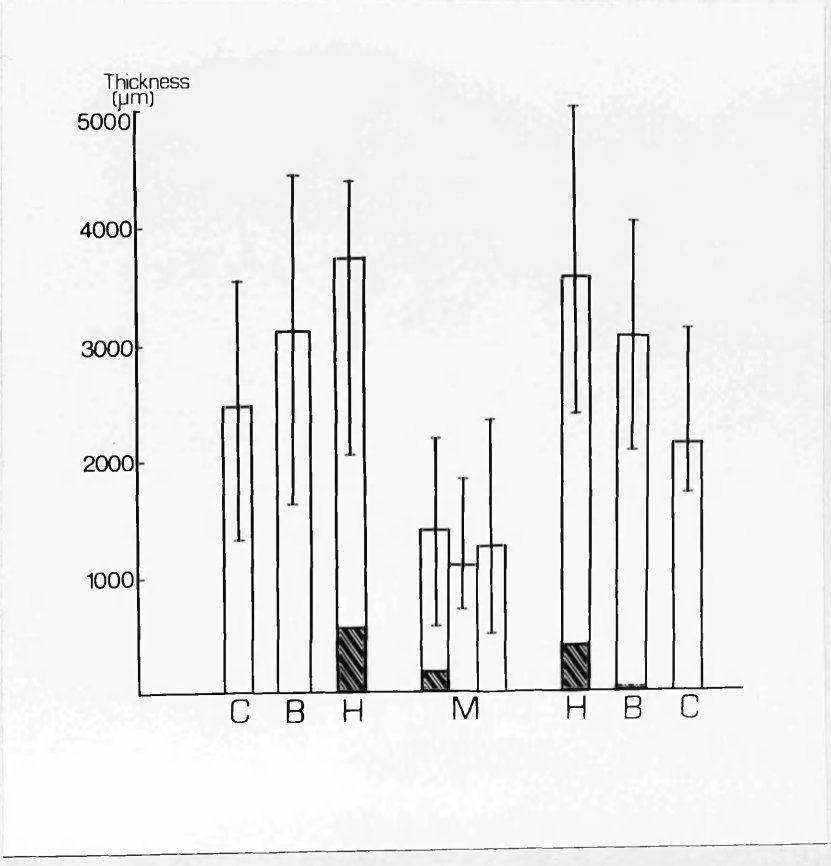


Figure 3.17 Histogram of mean values and range of values of connective tissue thickness and mean values of salivary gland tissue thickness (shown in black) at each of the sites from the eight cases.

Case Number	Age (Years)	Sex	Epithelial Thickness (μm)	Epithelial Morphology
1	56	Male	201.9	2.53
2	60	Male	235.6	2.59
3	51	Male	239.7	2.34
4	79	Female	204.1	2.60
5	57	Female	156.7	2.25
6	78	Female	199.6	2.95
7	70	Male	225.8	2.24
8	66	Female	123.9	2.10

Table 3.9 Total mean values of epithelial thickness and epithelial morphology of the eight cases.

Case Number	Buccal	Crest	Biopsy	Midline
1	20.8	20.7	25.1	14.7
2	9.0	15.1	12.6	19.9
3	17.3	17.4	14.9	15.2
4	13.8	9.9	20.2	12.7
5	11.6	18.2	26.7	16.7
6	6.4	5.3	6.6	4.5
7	11.3	8.5	18.7	10.4
8	13.8	19.0	18.2	16.2
Mean	13.0	14.3	17.9	13.8

Table 3.10 Site variation and mean values of stratum corneum thickness (μm).

	Buccal	Crest	Biopsy	Midline
Orthokeratosis	-	4	6	3
Orthokeratosis/ Parakeratosis	4	2	-	3
Parakeratosis	4	2	2	2

Table 3.11 Number of cases of each type of keratinisation at each of the sites.

	Buccal	Crest	Biopsy	Midline
Incomplete/ Complete Orthokeratosis	-	2	2	1
Incomplete Orthokeratosis	-	2	4	2
Complete Orthokeratosis/ Parakeratosis	-	-	-	1
Incomplete Orthokeratosis/ Parakeratosis	4	2	-	2
Incomplete Parakeratosis	4	2	2	2

Table 3.12 Number of cases of each degree of keratinisation at each of the sites.

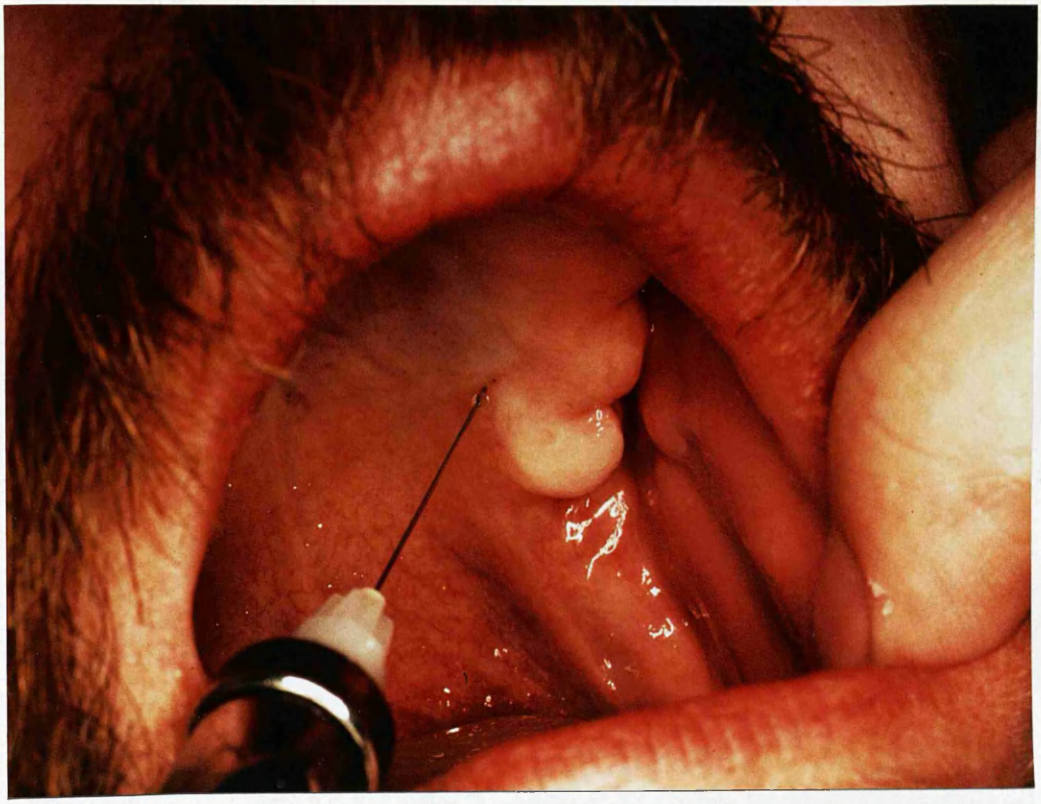


Figure 4.1 Local anaesthetic injection being administered remote from the biopsy site.

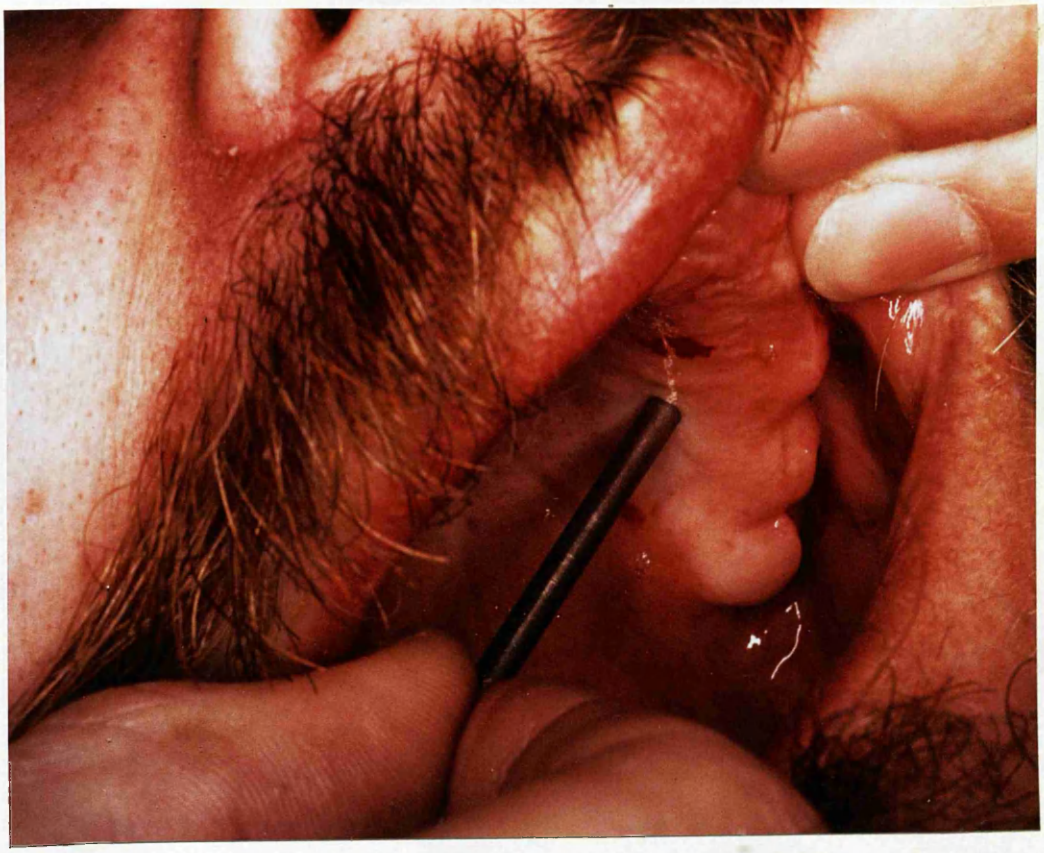


Figure 4.2 Trephine-type biopsy punch being used to obtain specimen.

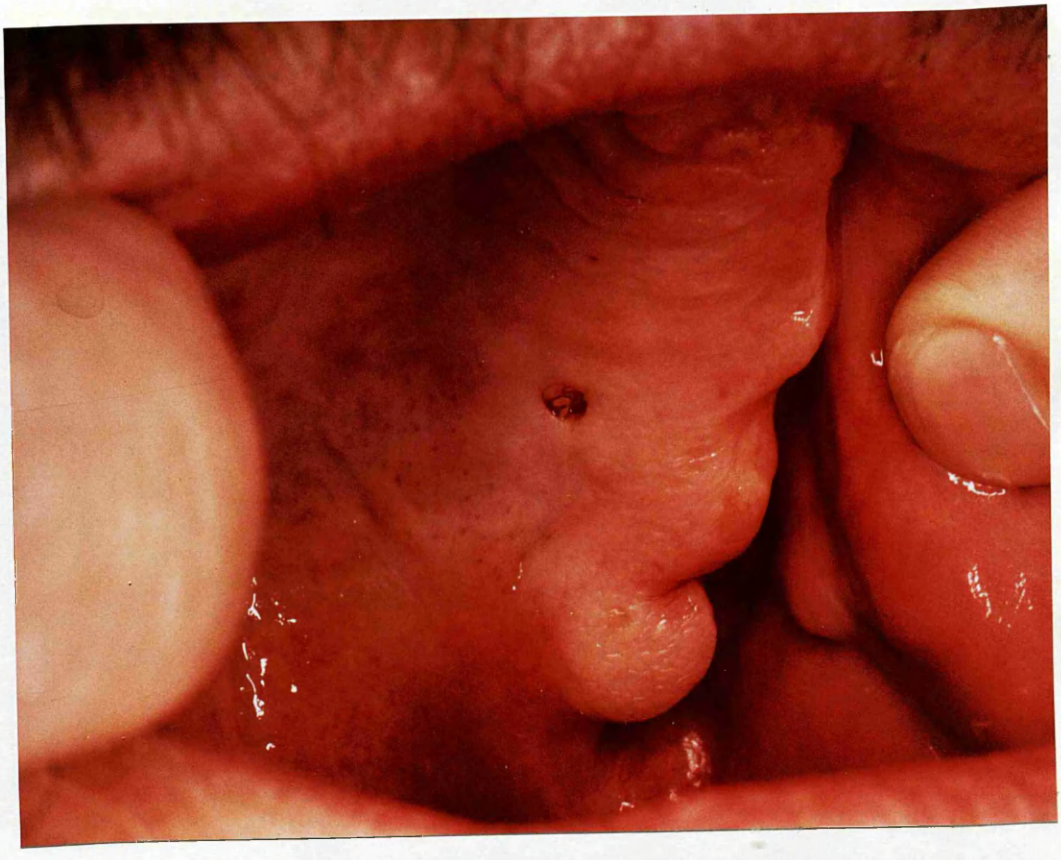


Figure 4.3 Initial appearance of the wound.

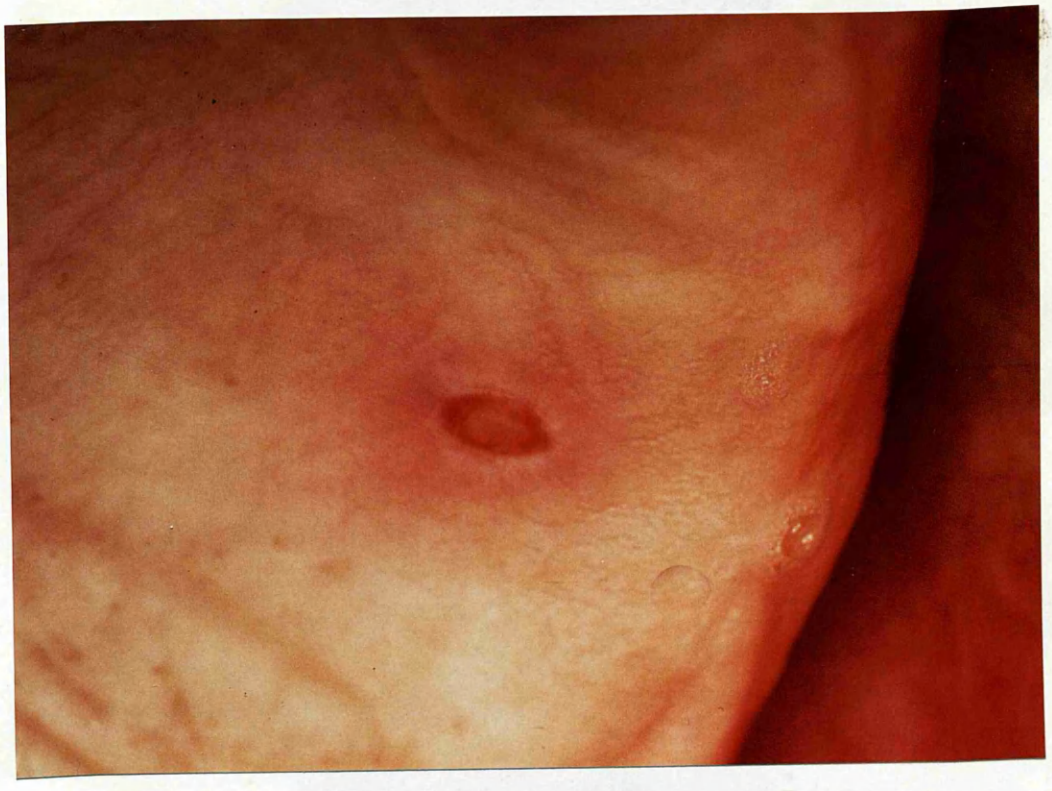
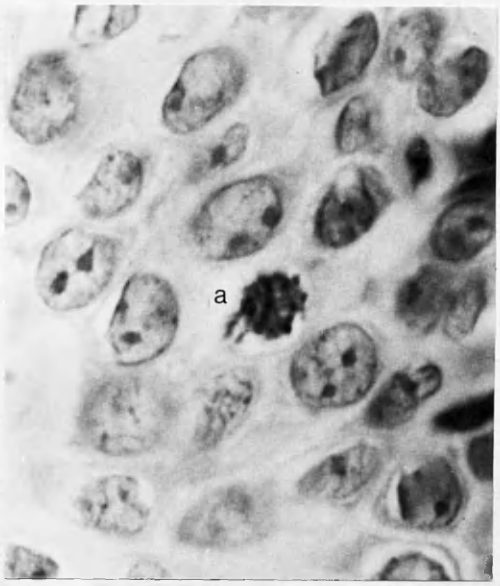


Figure 4.4 Healing wound after five days in a different patient.

A.



B.

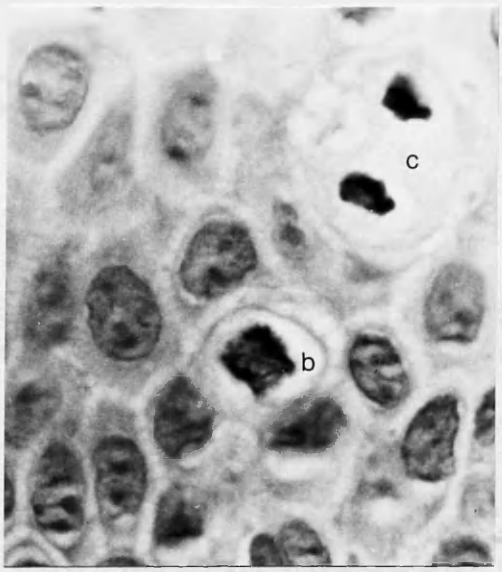


Figure 4.5 Stages of mitosis x 1150.

a - Prophase

b - Anaphase

c - Telophase

Case Number	Age	Smoking	Epithelial Thickness(μm)	Epithelial Morphology	Stratum Corneum Thickness(μm)	Degree of Keratinisation	Mitoses per mm Surface Length	Cells per mm Surface Length
1	44	Y	161.7	2.36	9.1	I./C. O.	0.48	1133.5
2	34	Y	268.6	2.51	11.4	C. O.	0.54	1793.7
3	20	Y	222.4	1.82	13.0	C. O.	0.62	1310.3
4	60	Y	232.5	3.06	19.9	C. O.	0.62	1703.1
5	67	N	307.7	3.38	20.0	I./C. O.	0.55	1880.9
6	40	Y	232.5	2.86	15.3	I./C. O.	0.35	1742.0
7	17	Y	295.1	3.67	17.0	I. O.	0.54	1647.5
8	31	Y	310.3	2.78	16.0	I. O.	0.96	1980.9
9	74	N	161.7	2.39	17.5	I./C. O.	1.04	1091.9
10	51	Y	283.1	3.28	21.4	I. O.	0.55	1430.8
11	53	Y	247.1	1.97	15.2	I./C. O.	1.06	903.5
12	47	N	165.5	2.55	23.2	C. O.	0.44	1522.5
13	47	-	252.8	2.62	14.4	C. O.	0.70	1772.6
14	69	N	396.2	1.94	30.8	C O.	0.98	1614.2
15	63	N	299.5	2.39	21.5	C. O.	0.42	1555.8
16	55	N	299.5	3.28	19.8	C. O.	0.76	1989.2
17	61	Y	294.5	2.55	35.3	I. O.	0.81	1564.2
18	54	Y	311.5	2.97	47.0	I./C. O.	0.71	2089.3
19	63	Y	317.9	3.02	20.7	I./C. O.	0.55	1886.5
20	42	Y	284.4	2.58	20.1	I. O.	1.58	1483.6
21	43	Y	302.7	2.19	19.9	C. O.	0.94	1561.4
Mean	49.3		268.9	2.68	20.4		0.72	1602.7

Table 4.1 Male non-denture wearers.

Case Number	Age	Smoking	Epithelial Thickness(μm)	Epithelial Morphology	Stratum Corneum Thickness(μm)	Degree of Keratinisation	Mitoses per mm Surface Length	Cells per mm Surface Length
22	48	Y	176.3	1.48	14.6	C. O.	0.81	1077.4
23	28	Y	300.2	2.42	21.2	C. O.	0.48	1338.3
24	72	N	308.4	3.00	20.9	I./C. O.	1.19	1275.8
25	63	N	283.8	2.36	20.1	I. O.	0.83	1099.6
Mean	52.75		267.2	2.31	19.2		0.83	1197.8

Table 4.2 Female non-denture wearers.

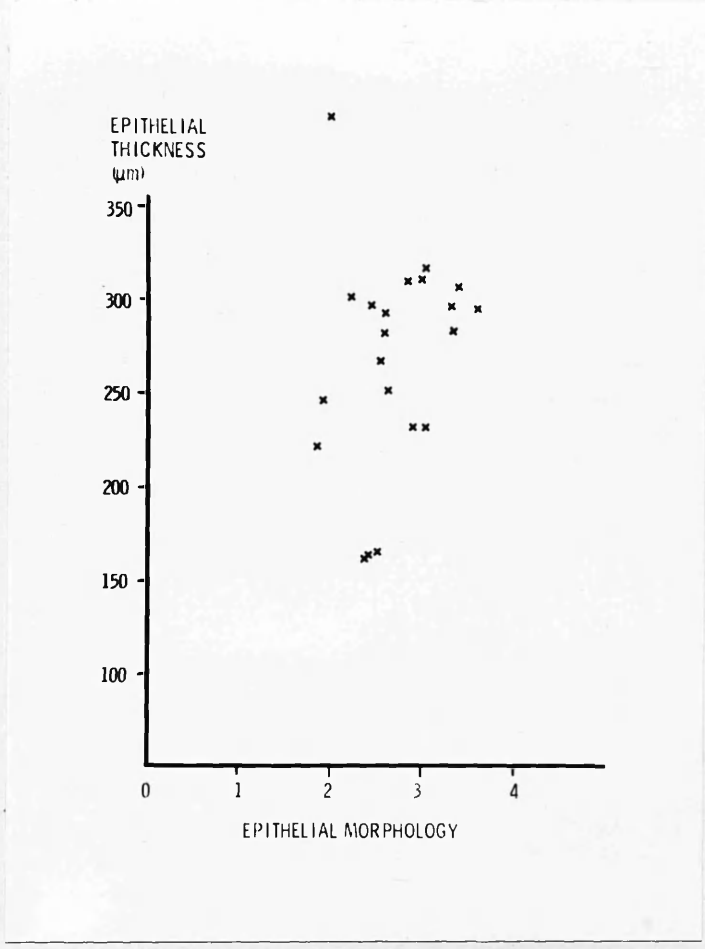


Figure 4.6 Scatter diagram plotted between the epithelial thickness and the epithelial morphology.

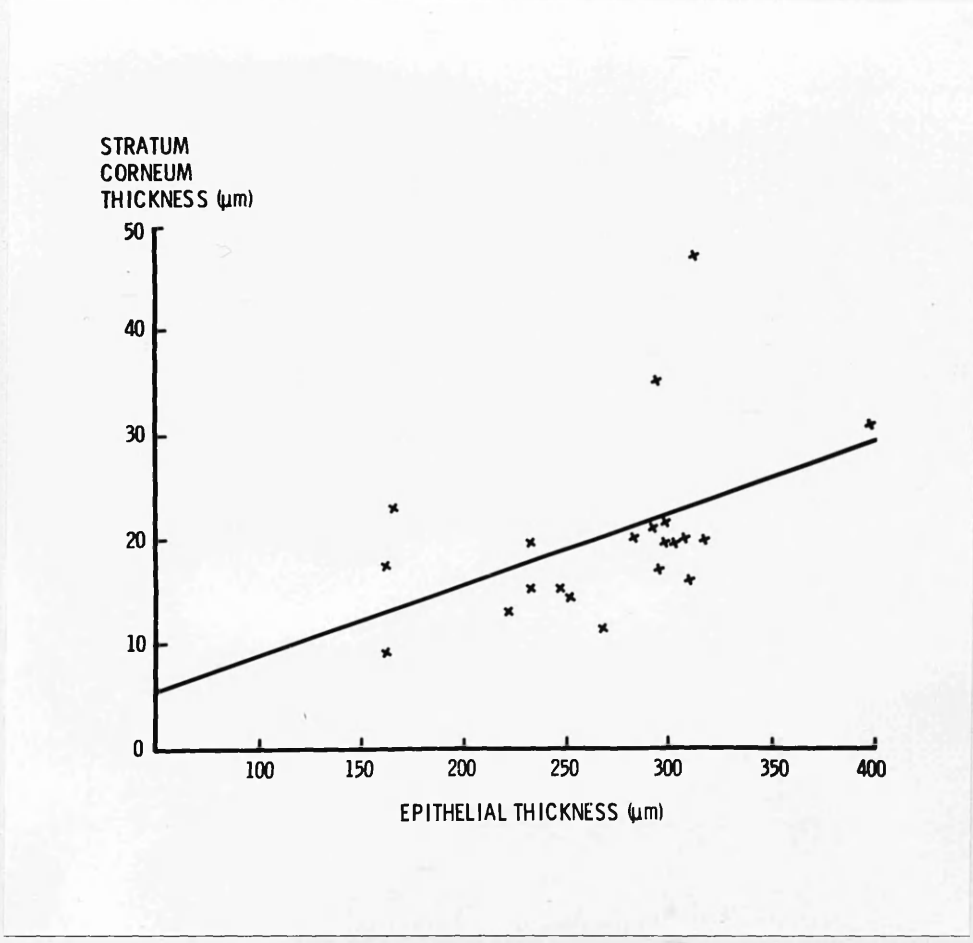


Figure 4.7 Scatter diagram plotted between the epithelial thickness and the stratum corneum thickness.

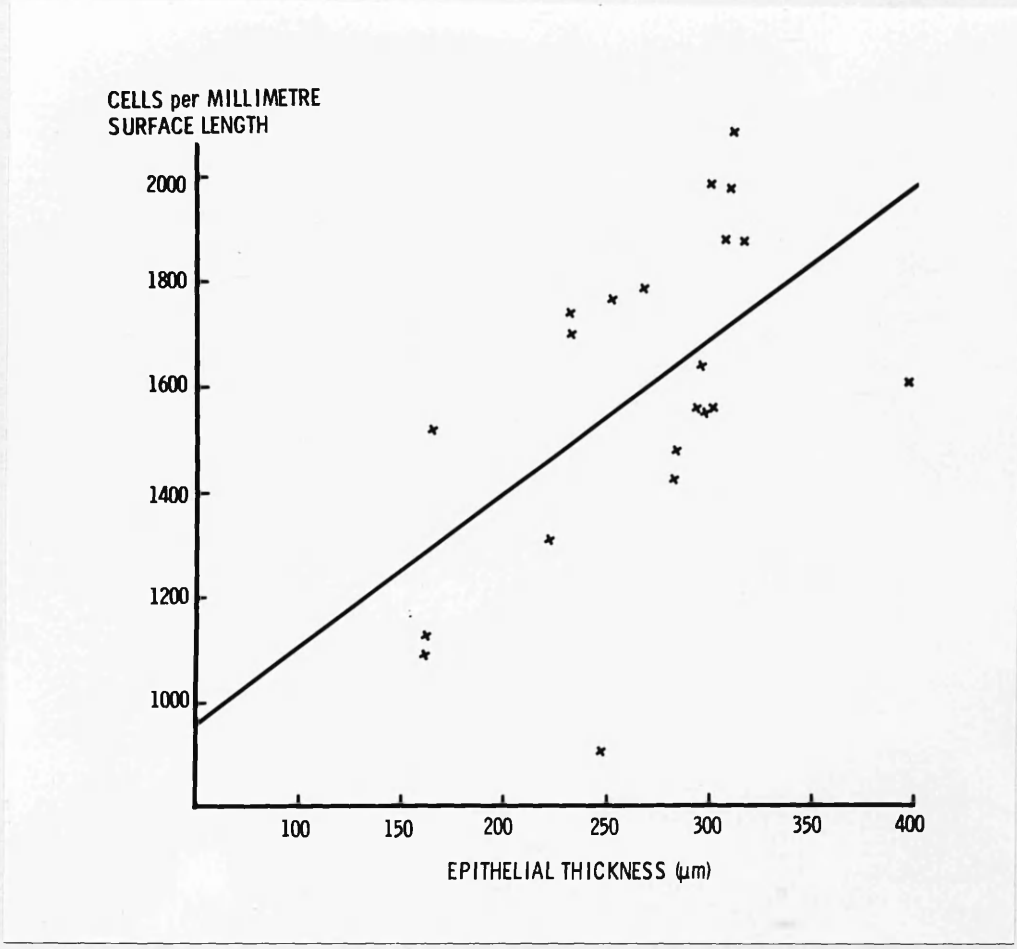


Figure 4.8 Scatter diagram plotted between the epithelial thickness and the number of cells per millimetre surface length (cell density).

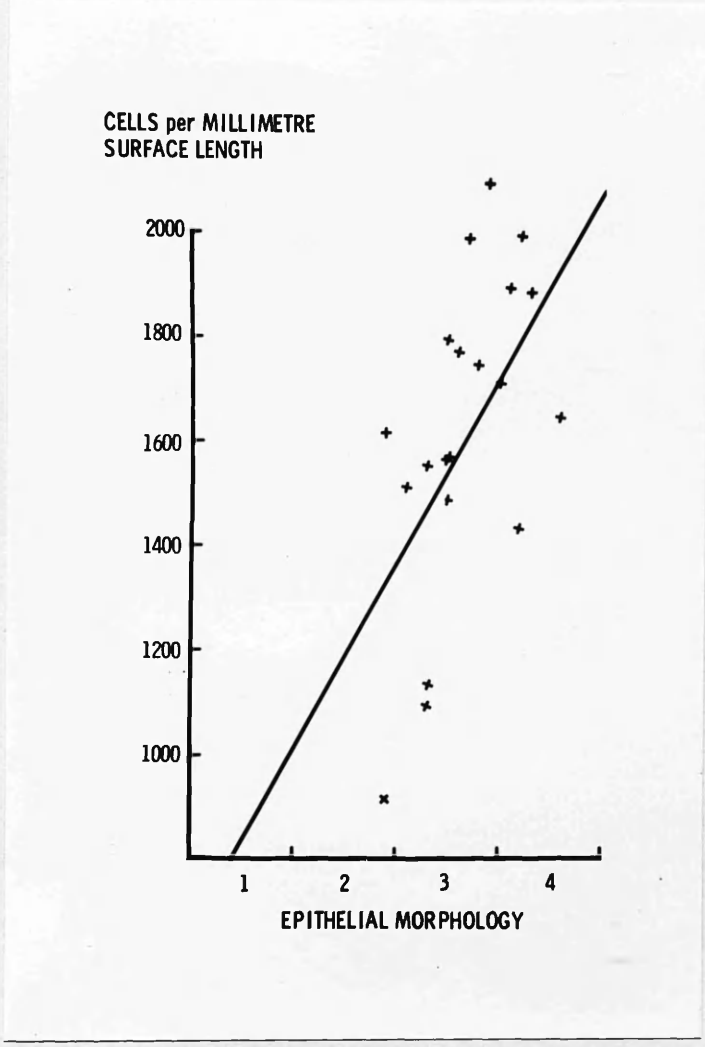


Figure 4.9 Scatter diagram plotted between the epithelial morphology and the cell density.

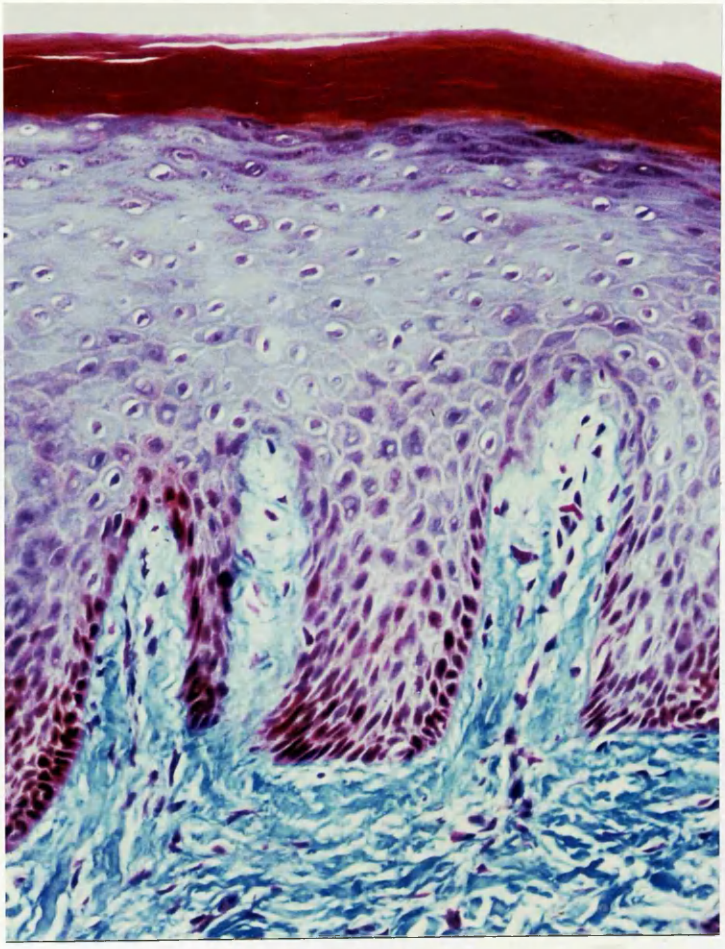


Figure 4.10 Complete orthokeratosis.

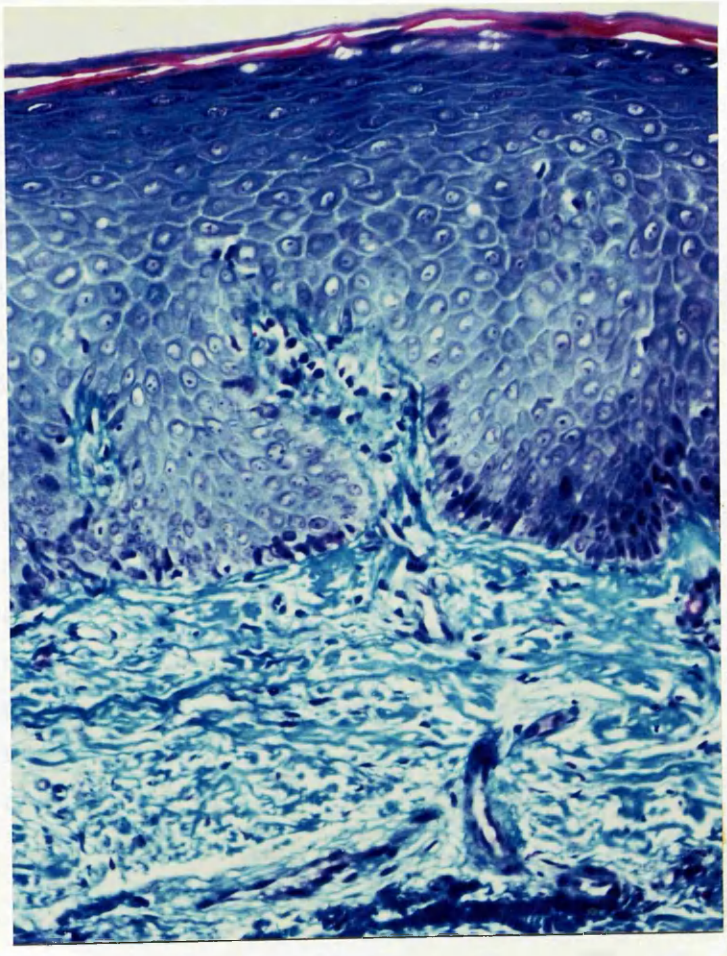


Figure 4.11 Incomplete orthokeratosis.

Degree of Keratinisation	Age	Ranks			
A	B	A	B	di	di^2
I./C.O.	44	9	8	1	1
I./C.O.	53	9	12	-3	9
C.O.	34	17	4	13	169
C.O.	20	17	2	15	225
C.O.	47	17	9.5	7.5	56.25
C.O.	47	17	9.5	7.5	56.25
C.O.	60	17	15	2	4
I./C.O.	67	9	19	-10	100
I./C.O.	40	9	5	4	16
C.O.	69	17	20	-3	9
C.O.	63	17	17.5	-0.5	0.25
I. O.	17	3	1	2	4
I. O.	31	3	3	0	0
C.O.	55	17	14	3	9
I./C.O.	74	9	21	-12	144
I. O.	51	3	11	-8	64
I. O.	61	3	16	-13	169
I./C.O.	54	9	13	-4	16
I./C.O.	63	9	17.5	-7.5	56.25
I. O.	42	3	6	-3	9
C.O.	43	17	7	10	100
				$\leq di^2$ =	1217

$$r_s = 1 - \frac{6 \leq di^2}{n^3 - n} = 1 - \frac{6 \times 1217}{21^3 - 21} = 0.21$$

$P > 0.05$

Table 4.3 The Spearman rank correlation coefficient test between the degree of keratinisation and age.

Incomplete orthokeratinisation = 1
(in this example rank is 3 because of ties)

Incomplete/complete orthokeratinisation = 2
(in this example rank is 9 because of ties)

Complete orthokeratinisation = 3
(in this example rank is 17 because of ties)

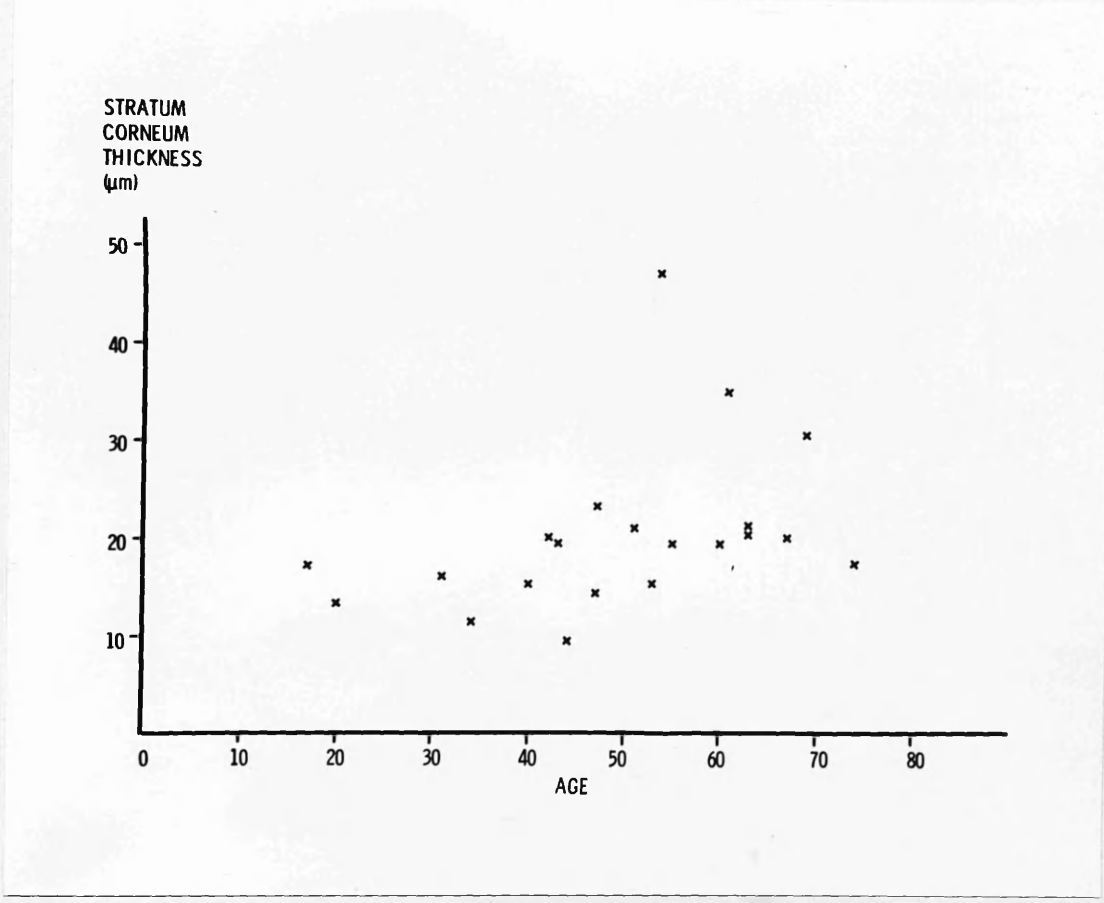


Figure 4.12 Scatter diagram plotted between age and the stratum corneum thickness. No linear relationship is present.

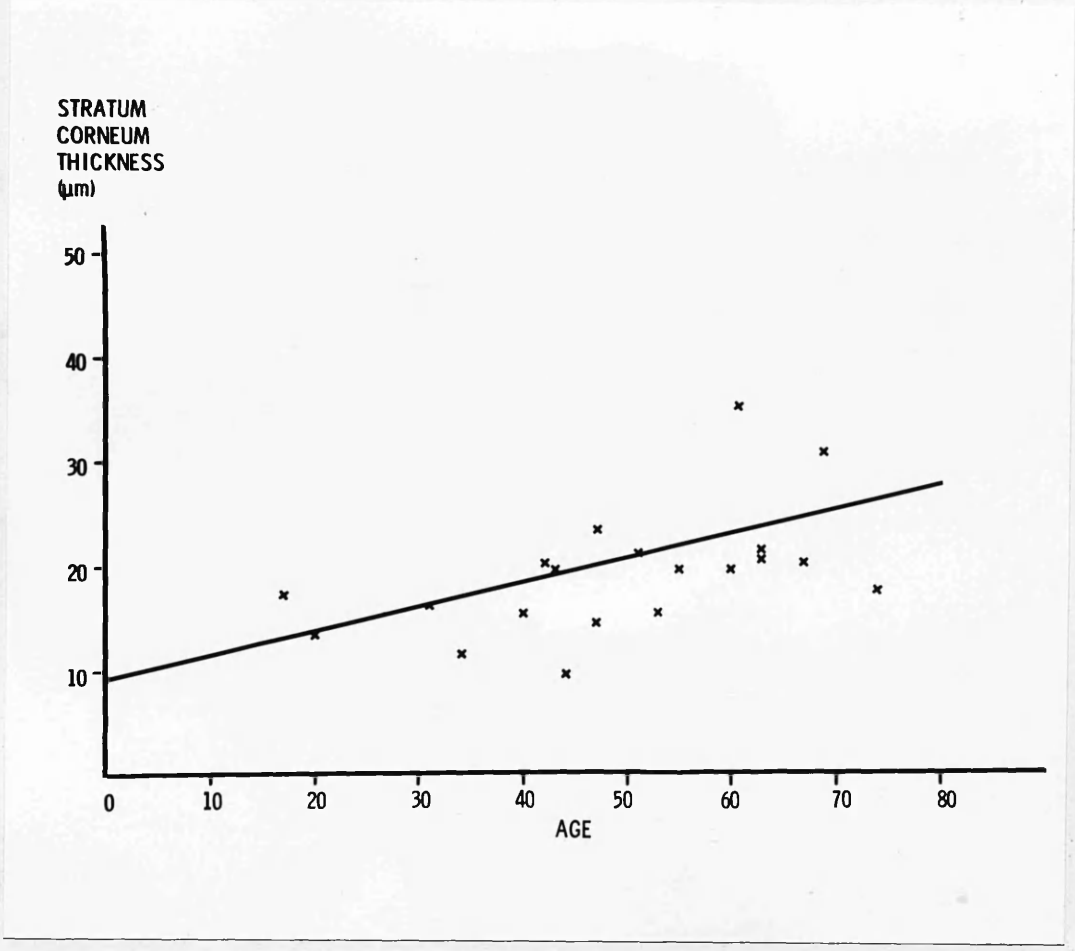


Figure 4.13 Scatter diagram plotted between age and the stratum corneum thickness omitting the "high point". Linear relationship is present.

	I.O.	I/C.O.	C.O.	TOTAL
Smokers	5	5	4	14
Non-Smokers	0	2	4	6
TOTAL	5	7	8	20

Table 4.4 Degree of keratinisation and smoking habits.

Epithelial Thickness (μm) Smokers	Rank	Epithelial Thickness (μm) Non-Smokers	Rank
161.79	1.5	165.58	3
247.11	7	307.78	16
268.60	8	396.26	20
222.46	4	299.57	13.5
232.58	5.5	299.57	13.5
232.58	5.5	161.79	1.5
295.14	12		
310.31	17		
283.14	9		
294.51	11		
311.58	18		
317.90	19		
284.40	10		
302.73	15		

$R_1 = 142.5$ $\qquad$ $R_2 = 67.5$

$$U = n_1 n_2 + \frac{n_1 (n_1 + 1)}{2} - R_1$$

$$= 14 \times 6 + \frac{14(14 + 1)}{2} - 142.5$$

$$= 46.5$$

$$P > 0.05$$

Table 4.5 The Mann-Whitney U Test applied to epithelial thickness in smokers and non-smokers.

Case Number	Age	Smoking	Epithelial Thickness(μm)	Epithelial Morphology	Stratum Corneum Thickness(μm)	Degree of Keratinisation	Mitoses per mm Surface Length	Cells per mm Surface Length	Length of Denture Experience (Months)	Hours of Denture Wearing per Day
1	45	Y	195.2	2.03	18.1	I. O.	0.62	1321.7	24	24
2	36	Y	252.8	2.64	6.5	I. P.	0.42	1341.1	28	24
3	21	Y	221.2	1.61	9.3	I./C. O.	0.56	1815.9	18	24
4	61	Y	221.2	2.13	16.1	I./C. O.	0.57	1096.2	14	24
5	68	N	299.5	2.36	22.7	I./C. O.	0.49	1404.9	15	24
6	41	Y	175.7	2.57	19.9	I. O.	0.39	982.9	14	24
7	18	Y	303.3	3.75	0.0	N.K.	0.42	1779.8	6	24
8	32	Y	231.9	2.22	9.3	I. O.	-	1461.2	12	24
9	74	N	210.4	1.67	14.4	I. O.	1.35	1044.0	7	20
10	51	Y	233.8	2.05	14.1	I./C. O.	0.73	1063.5	5	20
26	56	Y	178.8	2.18	23.9	I. O.	1.00	1002.9	24	14
27	69	Y	186.4	1.75	13.1	I. O.	0.46	1010.7	300	15
28	51	N	277.4	2.03	24.4	I. O.	-	1302.2	132	24
29	62	N	166.8	1.78	10.3	I./C. O.	0.42	1171.7	14	24
30	73	N	296.4	2.08	10.1	I./C. P.	1.57	1332.8	36	24
31	53	N	302.1	1.84	19.8	I. O.	0.77	1030.1	84	15
32	69	Y	314.1	2.72	19.9	I. O.	1.58	1338.3	324	14
33	62	N	249.6	1.39	10.7	I. O.	0.75	1121.8	96	16
34	57	N	292.6	1.91	14.8	I. O.	0.83	1160.6	240	16
35	75	N	231.9	1.94	7.9	I. P.	0.65	968.9	240	16
36	57	Y	219.9	2.25	12.8	I. O.	0.31	1199.5	24	24
37	76	N	194.0	1.57	9.8	I. P.	0.26	652.5	96	24
38	51	N	212.3	1.91	9.5	I. P.	0.27	902.4	96	24
39	59	Y	241.4	2.35	13.9	I./C. O.	0.34	910.7	300	16
40	38	N	252.8	2.69	14.9	C. O.	1.15	1402.2	84	16
41	42	N	243.3	3.11	17.2	I. O.	0.50	1529.9	216	21
42	30	N	286.3	3.44	8.8	I. P.	-	1746.5	120	24
Mean	52.9		240.4	2.22	13.83		0.77	1225.7	95.1	20.7

Table 5.1 Male denture wearers.

Case Number	Age	Smoking	Epithelial Thickness(μm)	Epithelial Morphology	Stratum Corneum Thickness(μm)	Degree of Keratinisation		Mitoses per mm Surface Length	Cells per mm Surface Length	Length of Denture Experience (Months)	Hours of Denture Wearing per Day
22	50	Y	155.5	1.69	16.4	I.	O.	0.69	791.4	27	24
23	29	Y	292.6	3.00	12.7	I.	O.	0.65	1396.6	21	24
24	73	N	237.0	2.37	12.7	I.	O.	0.37	885.6	6	20
25	63	N	223.7	1.66	6.6	I.	O.	0.63	1049.6	7	24
Mean	53.75		227.2	2.18	12.1			0.59	1030.8	15.25	23

Table 5.2 Female denture wearers.

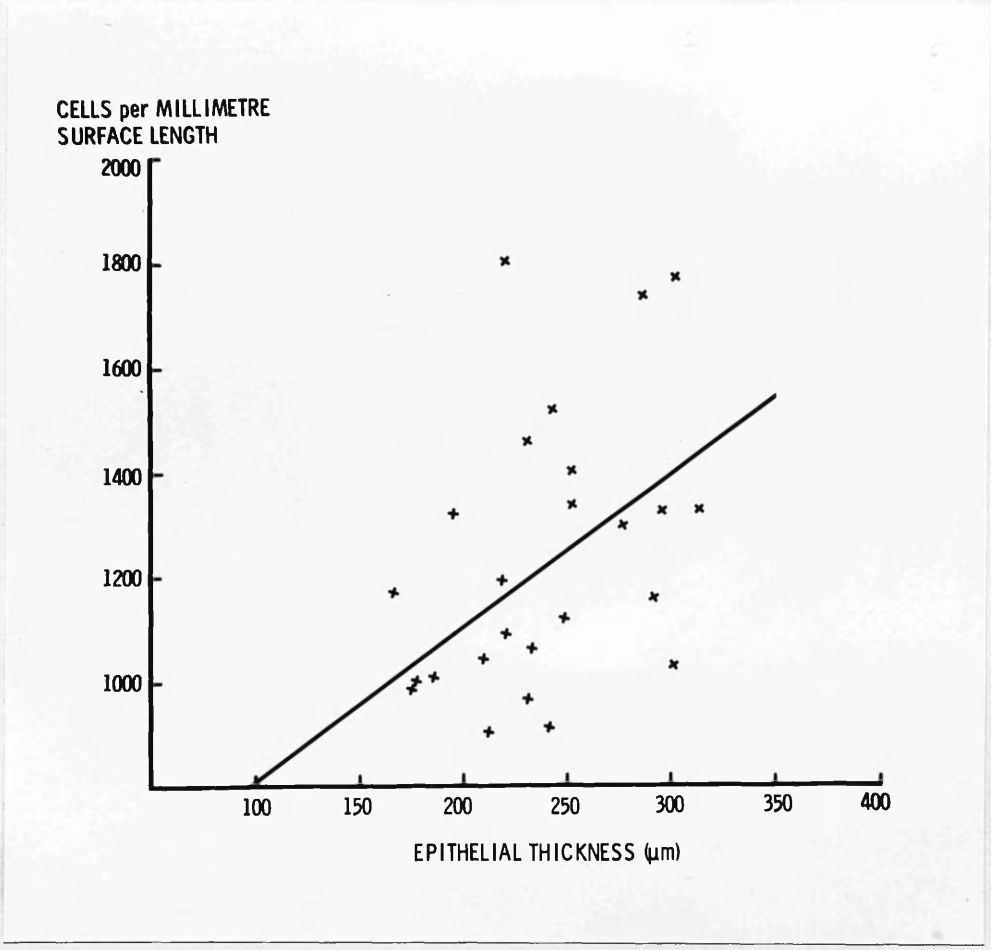


Figure 5.1 Scatter diagram plotted between the epithelial thickness and the cell density.

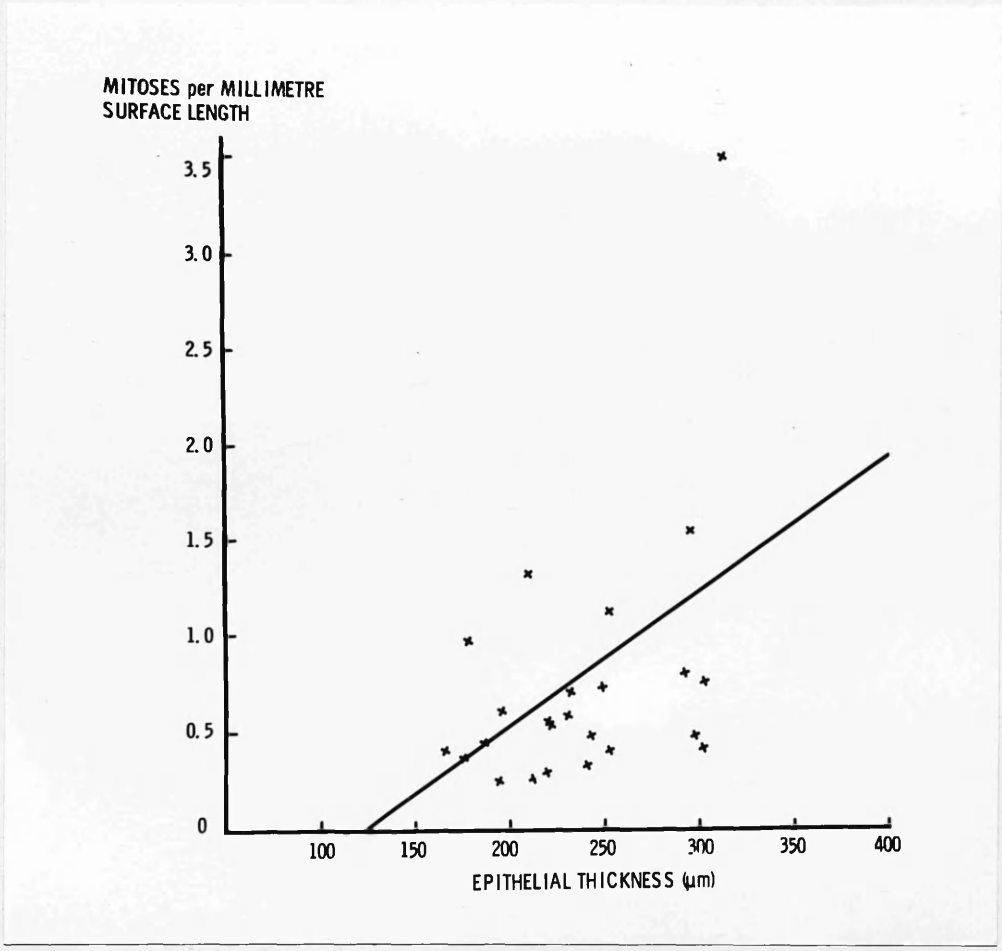


Figure 5.2 Scatter diagram plotted between the epithelial thickness and the number of mitoses per millimetre surface length.

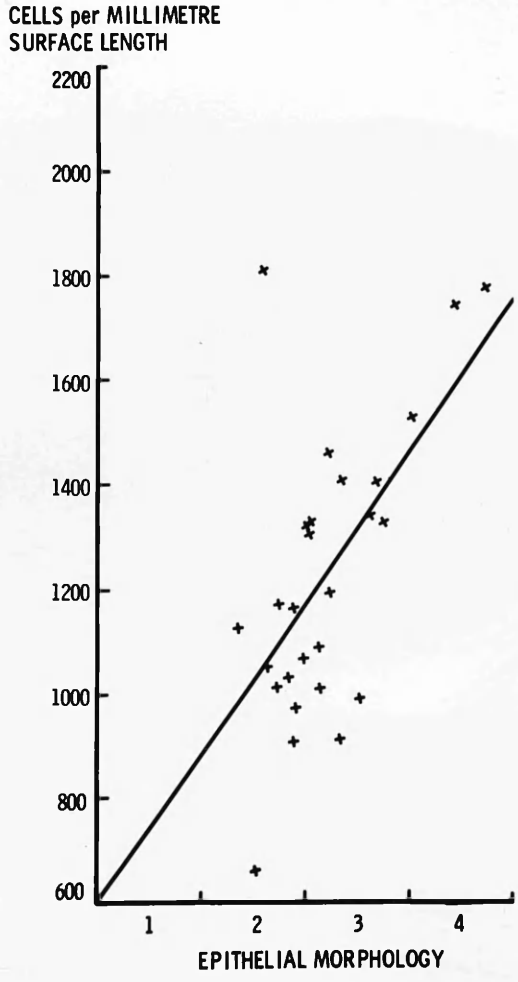


Figure 5.3 Scatter diagram plotted between the epithelial morphology and the number of cells per millimetre surface length.

Degree of Keratinisation	Ordinal Scale
Non-keratinisation	1
Incomplete parakeratinisation	2
Incomplete/complete parakeratinisation	3
Incomplete orthokeratinisation	4
Incomplete/complete orthokeratinisation	5
Complete orthokeratinisation	6

Table 5.3 Ordinal scale of the degrees of keratinisation.

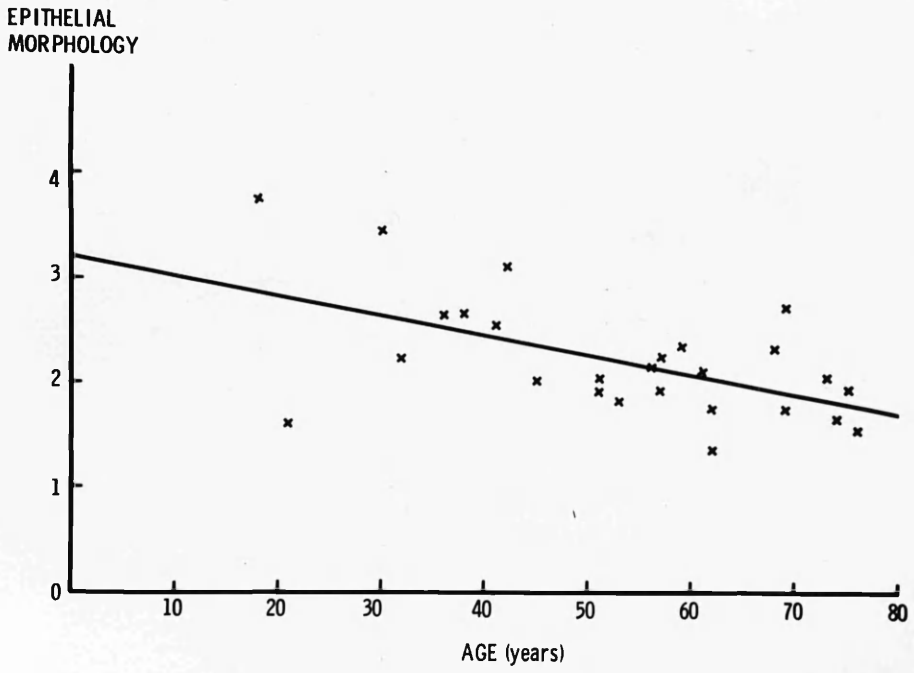


Figure 5.4 Scatter diagram plotted between age and epithelial morphology.

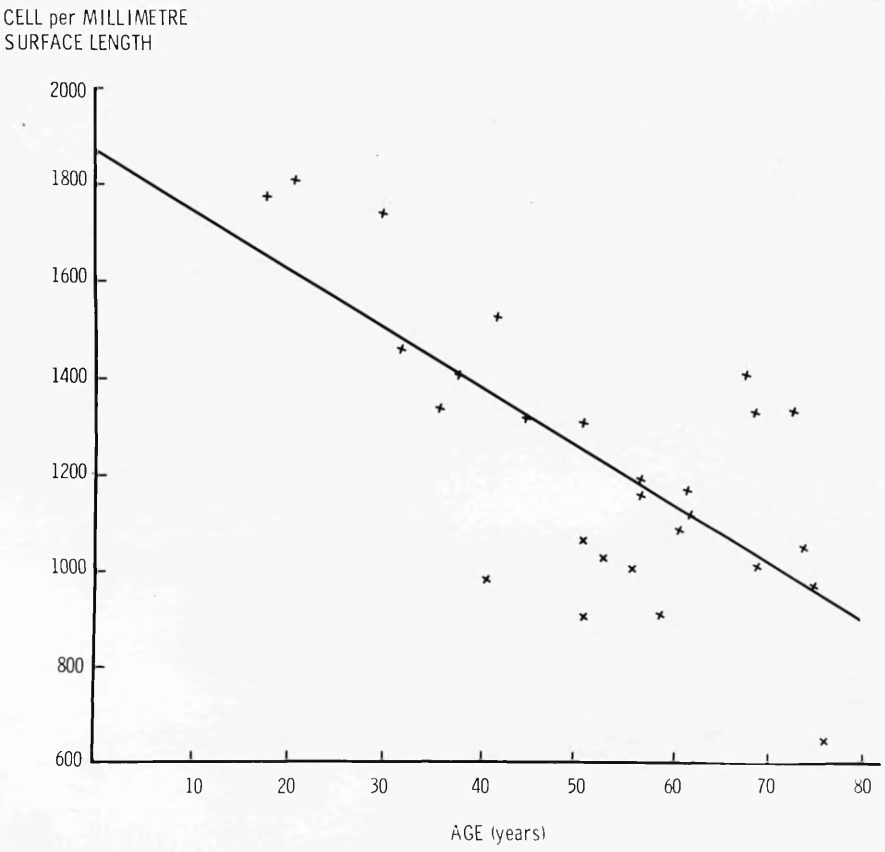


Figure 5.5 Scatter diagram plotted between age and cell density.

	N.K.	I.P.	I./C.P.	I.O.	I./C.O.	C.O.	TOTAL
SMOKERS	1	1	0	7	4	0	13
NON SMOKERS	0	4	1	6	2	1	14
TOTAL	1	5	1	13	6	1	27

Table 5.4 Degree of Keratinisation and smoking habits.

	N.K.	I.P.	I./C.P.	I.O.	I./C.O.	C.O.	TOTAL
24 hrs	1	4	1	5	4	0	15
not 24 hrs	0	1	0	8	2	1	12
TOTAL	1	5	1	13	6	1	27

Table 5.5 Degree of keratinisation and the number of hours of denture-wearing per day.

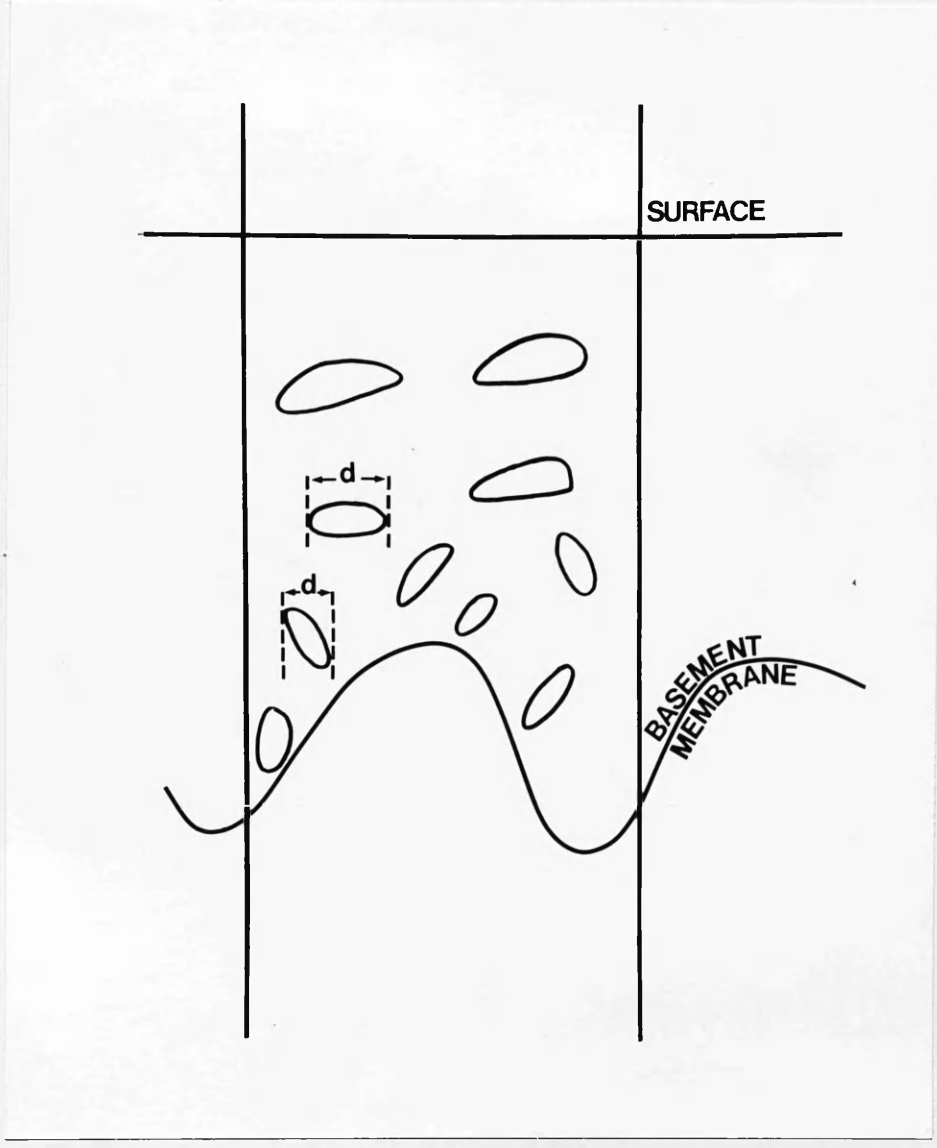


Figure 6.1 Diagramatic representation of a column of epithelium showing the nuclear projection (d).

	Non-denture group			Denture group		
	Mean	Standard Deviation	Number	Mean	Standard Deviation	Number
Age	49.3	15.3	21	52.9	16.4	27
Epithelial Thickness (µm)	268.9	58.2	21	240.4	43.8	27
Epithelial Morphology	2.68	0.50	21	2.22	0.56	27
Stratum Corneum Thickness (µm)	20.4	8.5	21	13.8	5.7	27
Mitoses per mm surface length	0.72	0.29	21	0.77	0.69	24
Mitoses per 1000 viable cells	0.48	0.27	21	0.65	0.52	24
Cells per mm surface length	1602.7	307.8	21	1225.7	282.6	27

Table 6.1 Mean values, standard deviations and the number of observations in the entire group of males.

	Non-denture group	Denture group
Complete Orthokeratosis	9	1
Incomplete/Complete Orthokeratosis	7	6
Incomplete Orthokeratosis	5	13
Incomplete/Complete Parakeratosis	0	1
Incomplete Parakeratosis	0	5
Non-keratosis	0	1

Table 6.2 Number of cases of each degree of keratinisation in the entire group of males.

	Non-denture group	Denture group
Orthokeratosis	21	20
Parakeratosis	0	6
Non-keratosis	0	1

Table 6.3 Number of cases of each type of keratinisation in the entire group of males.

Case Number	Epithelial Thickness (µm)		Epithelial Morphology		Stratum Corneum Thickness (µm)		Mitoses per mm Surface Length		Mitoses per 1000 Cells		Cells per mm Surface Length	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	161.7	195.2	2.36	2.03	9.1	18.1	0.48	0.62	0.42	0.47	1133.5	1321.7
2	268.6	252.8	2.51	2.64	11.4	6.5	0.54	0.42	0.30	0.31	1793.7	1341.1
3	222.4	221.2	1.82	1.61	13.0	9.3	0.62	0.56	0.47	0.31	1310.3	1815.9
4	232.5	221.2	3.06	2.13	19.9	16.1	0.62	0.57	0.36	0.52	1703.1	1096.2
5	307.7	299.5	3.38	2.36	20.0	22.7	0.55	0.49	0.29	0.35	1880.9	1404.9
6	232.5	175.7	2.86	2.57	15.3	19.9	0.35	0.39	0.20	0.40	1742.0	982.9
7	295.1	303.3	3.67	3.75	17.0	0.0	0.54	0.42	0.33	0.24	1647.5	1779.8
8	310.3	231.9	2.78	2.22	16.0	9.3	0.96	-	0.49	-	1980.9	1461.2
9	161.7	210.4	2.39	1.67	17.5	14.4	1.04	1.35	0.95	1.29	1091.9	1044.0
10	283.1	233.8	3.28	2.05	21.4	14.1	0.55	0.73	0.38	0.69	1430.8	1063.5
22	176.3	155.5	1.48	1.69	14.6	16.4	0.81	0.69	0.75	0.87	1077.4	791.4
23	300.2	292.6	2.42	3.00	21.2	12.7	0.48	0.65	0.36	0.47	1338.3	1396.6
24	308.4	237.0	3.00	2.37	20.9	12.7	1.19	0.37	0.93	0.42	1275.8	885.6
25	283.8	223.7	2.36	1.66	20.1	6.6	0.83	0.63	0.75	0.60	1099.6	1049.6

Table 6.4 Direct follow-up group.

Epithelial Thickness (um)

Case Number	Before	After	Difference
1	161.7	195.2	-33.5
2	268.6	252.8	15.8
3	222.4	221.2	1.2
4	232.5	221.2	11.3
5	307.7	299.5	8.2
6	232.5	175.7	56.8
7	295.1	303.3	-8.2
8	310.3	231.9	78.4
9	161.7	210.4	-48.7
10	283.1	233.8	49.3
22	176.3	155.5	20.8
23	300.2	292.6	7.6
24	308.4	237.0	71.4
25	283.8	223.7	60.1

Mean of difference = 20.75

Standard deviation of difference = 38.21

$$t = \frac{\sqrt{n} \times \text{mean of difference}}{\text{standard deviation of difference}}$$

$$= \frac{\sqrt{14} \times 20.75}{38.21}$$

$$P > 0.05$$

Table 6.5 Paired t test applied to difference in epithelial thickness in the direct follow-up group.

	Mean Difference	Standard Deviation of Mean Difference	t	Significance (P)
Epithelial Thickness(μm)	20.75	38.21	2.03	> 0.05
Epithelial Morphology	0.40	0.52	2.88	< 0.02
Stratum Corneum Thickness(μm)	4.19	7.01	2.24	< 0.05
Mitoses per mm Surface Length	0.06	0.28	0.77	> 0.05
Mitoses per 1000 cells	- 0.04	- 0.23	-0.63	> 0.05
Cells per mm Surface Length	219.4	357.1	2.30	< 0.05

Table 6.6 Change in the values of the parameters in the direct follow-up group and the results of the paired t tests.

Case Number	Before	After	Change	Sign
1	I./C.O.	I.O.	decrease	-
2	C.O.	I.P.	decrease	-
3	C.O.	I./C.O.	decrease	-
4	C.O.	I./C.O.	decrease	-
5	I./C.O.	I./C.O.	no change	0
6	I./C.O.	I.O.	decrease	-
7	I.O.	N.K.	decrease	-
8	I.O.	I.O.	no change	0
9	I./C.O.	I.O.	decrease	-
10	I.O.	I./C.O.	increase	+
22	C.O.	I.O.	decrease	-
23	I.O.	I.O.	no change	0
24	I./C.O.	I.O.	decrease	-
25	I.O.	I.O.	no change	0

n = the number of pairs where difference shows a sign.

x = the number of fewer signs.

In this case, n = 10

x = 1

P = 0.02

Table 6.7 Change in degree of keratinisation in direct follow-up group and sign test applied to the change (Siegel, 1956).

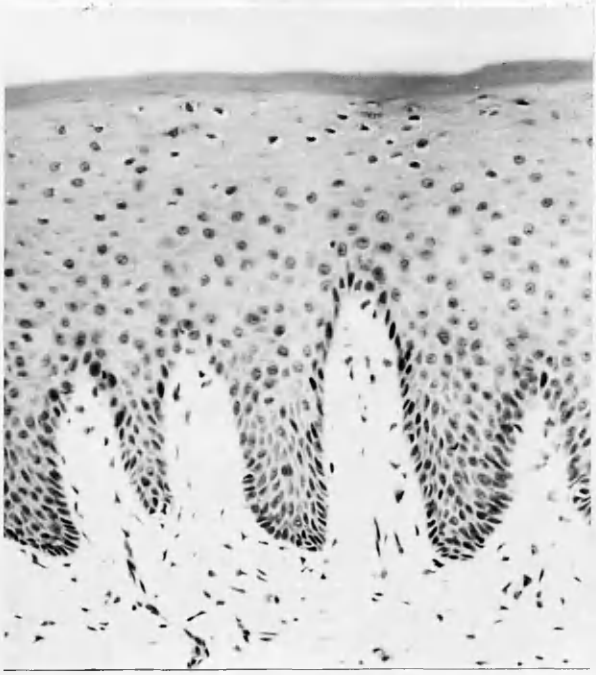


Figure 7.1 The typical appearance of normal mucosa x 200.

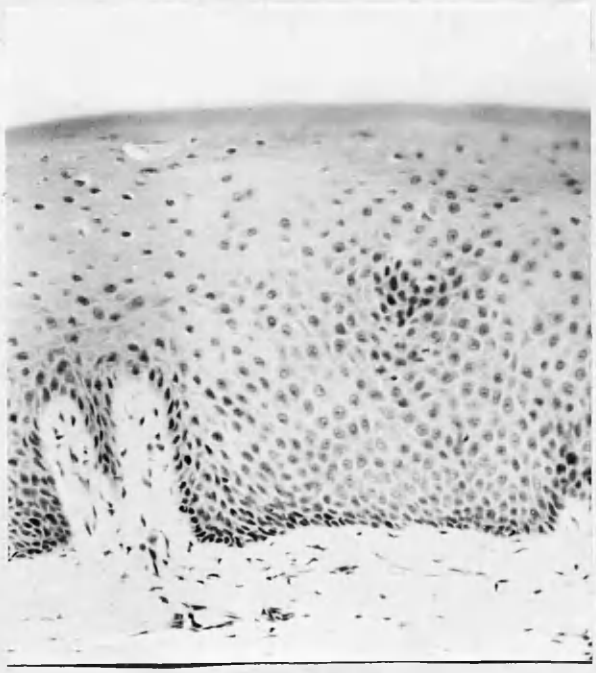


Figure 7.2 The typical appearance of clinically normal mucosa under a denture x 200.