AN INVESTIGATION OF THE BEHAVIOUR OF THE EPITHELIUM OF RADIAL FOREARM FLAPS TRANSPLANTED INTO THE ORAL CAVITY

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...TO MY FAMILY

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DECLARATION

Patients in this study were all diagnosed to have oral tumours requiring excision and reconstruction of the resulting defect by a free radial forearm flap. Biopsies from the normal forearm skin and normal oral mucosa were taken intra-operatively by the plastic surgery team at Canniesburn Hospital and passed to the investigator who attended the theatre.

The investigator was registered with the General Medical Council during the period of the study and for thirty months attended the weekly combined clinic and was responsible for producing detailed description of the appearanace of the flap, including photography, and when appropriate taking a biopsy. The investigator processed all the biopsy specimens and produced and analysed the semithin and thin sections.

While receiving radiotherapy, patients were seen by the investigator at weekly intervals at the Head and Neck Clinic in the Western Infirmary, Glasgow, where swabs were taken from the surface of the intraoral flap of seven of them. These swabs and others from healthy volunteers were all collected and processed for scanning electron microscopy by the investigator who also undertook the analysis of the material.

CONTENTS

SUMMARY

This project is a collaborative study between the Anatomy Department, Canniesburn Hospital and the Western Infirmary, Glasgow, and consists of two parts.

I. A scanning electron microscopical study documenting the variation in the surface pattern of buccal epithelial cells obtained by repeated swabbing of the buccal mucosa at a defined site in normal control subjects, and investigating the possibility of using oral swabs to monitor the dose of radiotherapy without the need for biopsies. From this study it is concluded that:

1) There is considerable variation in the surface morphology of normal buccal epithelial cells as seen by scanning electron microscopy of oral swabs.

2) These variations seem to reflect the level at which the cells lie within the epithelium.

3) Adjacent cells can have different surface patterns.

4) The superficial and deep surfaces of one cell may show different patterns.

5) These findings in normal buccal epithelium suggest caution in attributing particular patterns to the effect of specific doses of radiation.

6) Swabs from the surface of intraoral radial forearm flaps are not helpful in monitoring the behaviour of the flap.

II. A clinical, histological and ultrastructural study of the behaviour of free radial forearm flaps transplanted into the oral cavity for treatment of oral tumours. In

particular the study aimed to document the structural basis of a change in the gross appearance of some of the flaps from skin to that of oral mucosa. Data was collected from 53 patients from whom a total of 77 biopsies were taken at different periods following their treatment by either surgery alone or surgery and radiotherapy. The clinical observations were correlated with the histological and ultrastructural findings and it is concluded that:

1) Radiotherapy is not essential for the skin to change to an appearance similar to mucosa.

2) The time of onset of the change is variable.

3) The change can start anywhere in the flap and may involve the whole flap or parts of it.

4) The change to an appearance resembling oral mucosa is reversible.

5) The epidermis of the changed flaps showed signs of hyperactivity i.e. parakeratosis, acanthosis and increased glycogen content. The dermis showed various grades of inflammatory reaction.

6) Transmission electron microscopy showed the tonofilaments in keratinocytes of the changed flaps to be dispersed in the cytoplasm, as seen in cells of normal lining mucosa, with persistence of fat droplets in the cells of the stratum corneum.

7) Radiotherapy at the dose given to these patients produces permanent damage to the hair follicles.

8) Candidal infection is probably an important factor in producing the change.

INTRODUCTION

During follow-up of patients at Canniesburn Hospital in whom the radial forearm flap had been used to reconstruct the oral defect created by excision of a tumour, it was noted that the behaviour of the flaps, as assessed by clinical inspection, seemed to depend on whether or not radiotherapy was used. In non-irradiated patients, the flap usually continued to behave as if it was in its original site. On the other hand, when radiotherapy was used, the clinical impression was that the skin sometimes changed in texture, colour and consistency, to the extent that it was sometimes difficult to distinguish it from adjacent mucosa.

The observation that the epidermis of the flap may, following radiotherapy, adopt the characteristics of oral epithelium conflicted with a widely held view that the regional characteristics of epidermis are determined by its contact with its native dermis; while its appearance may change under the influence of extrinsic factors, such changes are reversible and are not regarded as representing a genotypic change.

Although increasing number of patients were being treated for intraoral neoplasms by excision of the tumour and reconstruction by a flap often followed by radiotherapy, the behaviour of the skin component of the intraoral flaps had received little attention; only a few reports described the flaps in their new site in the oral cavity (Ariyan, 1979; Lawson, 1985; Wei et al., 1989; Ogunbekun, 1990).

The study by Ogunbekun (1990) was a comprehensive immunocytochemical study that compared the pattern of keratinisation in irradiated and non-irradiated intraoral forearm flaps. She found that there was a change in the keratin pattern of the epidermis of the forearm flaps towards a more proliferative and less keratinised form compared with normal controls. There was no significant difference between irradiated and non-irradiated flaps.

The other reports considered patients treated by surgery alone and described either the gross appearance of the flap only or a limited histological study with no controls. None of them provided an explanation for the changes in the gross and histological appearances.

Further study of the behaviour of intraoral flaps must correlate the clinical observations with the histological and ultrastructural appearance. In addition, the apparent association of changes in gross appearance and radiotherapy required confirmation.

A major limitation to the study of the early and late effects of radiotherapy on the intraoral flaps was the need for repeated biopsies, an approach that causes inconvenience to the patient and was difficult to justify ethically. In addition, because of reduced healing ability, biopsies could not be taken while the patient was receiving the course of treatment.

Part of this project, therefore, aimed to explore the possibility of studying the effects of radiotherapy by a

less invasive technique. The development of such a method would considerably benefit current radiotherapy practice. In irradiating the mouth it is important that there is sufficient exposure to eradicate neoplastic cells but not so much as to risk causing carcinogenesis de novo; a means of determining when the optimum dosage had been given would make an important improvement in treatment.

Robertson et al. (1987) suggested that study of exfoliated cells by the scanning electron microscope might form the basis of such a technique. If such a technique were developed it would allow closer follow-up of the patient and eliminate the need for biopsies. In their study Robertson and his colleagues reported that in patients treated with radiotherapy, cells collected on oral swabs had a highly variable surface morphology compared with that in untreated subjects. Further work was required to determine whether the changes described in the surface morphology of epithelial mucosal cells are specific radiation effects. Positive confirmation would allow a simple method of assessing radiation dosage to be developed which would be most useful in clinical practice and would also provide additional means of investigating the nature of the changes in gross appearance of the flaps of patients at Canniesburn Hospital.

Aims of the project

This project includes two studies and aims to answer the following questions:

1) Has scanning electron microscopy of oral swabs a

role to play in assessing the behaviour of intraoral radial forearm flaps or in monitoring radiotherapy?

2) Had the epidermis of free radial forearm flaps of the Canniesburn patients survived in the oral cavity, or had it been replaced by regenerating oral epithelium?

3) Can the apparent change to mucosa be confirmed histologically?

4) Is the observed alteration to a mucosal appearance a genuine or a reactive change?

5) What is the aetiology of any morphological change in the flap?

TREATMENT OF INTRAORAL TUMOURS AT CANNIESBURN HOSPITAL

The plastic surgery unit at Canniesburn Hospital is the regional centre to which most patients with the oral tumours in the West of Scotland are referred for management. In this unit a combined plastic surgery/radiotherapy clinic is well established and its staff includes plastic surgeons, a radiotherapist-oncologist, a dentist, a dietician, a speech therapist and a nurse counsellor; a team approach to therapy is emphasised.

On the first visit to the clinic, the patient with suspected intraoral tumour is examined clinically and preliminary staging is carried out using the TNM classification (UICC, 1987) (Appendix I). All the patients undergo examination under general anaesthesia (EUA) to assess the extent of the tumour and to take a biopsy. The biopsy is sent to the Glasgow Dental Hospital for examination by an oral pathologist. The patient is usually discharged the next day. One week later, on the second visit to the clinic, the patient is informed of the result of the biopsy and the plan for treatment is discussed. The treatment that is usually adopted at Canniesburn Hospital is as follows:

Stage I	:	surgery alone
Stages II, III, IV	:	surgery followed by radical radiotherapy.

The surgical procedure involves wide excision of the tumour in stage I together with radical or functional neck

dissection in stages II, III and IV. The defect resulting from excision is reconstructed by means of a flap. In the past a pedicled flap was used and the procedure was a multistage one, but advances in microsurgical techniques have made it possible to reconstruct the oral cavity in the majority of the cases in one stage using a free flap. Among the different flaps available for use, the free radial forearm flap has gained wide popularity for intraoral reconstruction.

The excision specimens are sent to the oral pathologist. The pathology report may change the preliminary clinical staging since the histopathology may show the tumour to extend to adjacent tissues which look grossly normal. In addition, lymph nodes, although impalpable clinically, may be infiltrated with tumour cells, while some clinically palpable lymph nodes may show reactive changes only. Pathologists use the pTNM classification and their finding may alter subsequent treatment.

Six weeks after surgery the patient receives a radical course of radiotherapy in the form of 60 Gy in 30 fractions over a period of 6 weeks (5 days a week) using a Philips SL75, 5MV linear accelerator (Fig. 1). This treatment is carried out at the Beatson Oncology Centre in the Western Infirmary or Belvedere Hospital, Glasgow.

The technique used in radiotherapy was described by Robertson et al. (1985, 1986). A wedged pair set-up is used

to treat the oral cavity; this technique reduces the dose to the contralateral parotid salivary gland to 20 Gy and minimises the incidence of xerostomia i.e. dry mouth (Ching et al., 1981; Mira et al., 1981; Robertson et al., 1985, 1986). The anterior triangles of the neck are treated by single anterior field. The cervical part of the spinal cord is protected by means of a lead shield applied to the midline of the lower field (Fig. 2).

Following discharge, the patient attends the combined clinic at Canniesburn Hospital. Unless otherwise indicated, the routine follow-up is once every 2 months for 6 months, once every 3 months for another 6 months, every 4 months for 1 year, then 6 monthly till the end of the fifth year after surgery and annually thereafter.

THE FREE RADIAL FOREARM FLAP (THE CHINESE FLAP)

The free radial forearm flap was developed by a team of Chinese military surgeons in 1978. Since that time the flap has gained wide popularity and been used extensively in reconstruction of many deformities in different parts of the body; e.g. release of post-burn contractures (Mühlbauer et al., 1982; Song et al., 1982), nasal reconstruction (Shaw, 1981) and hand surgery (Biemer & Stock, 1983; Soutar & Tanner, 1984).

Because of the quality of the forearm skin and the consistent anatomy of its blood supply, Mühlbauer et al. (1982) suggested that the radial forearm flap might be better than other free flaps in reconstructing soft-tissue defects of the head and neck, including the oral cavity. A few months later Soutar and his colleagues (1983) opened the way for the use of the flap in oral reconstruction when they reported highly successful treatment of 10 cases of oral cancer by excision and repair using a free radial forearm flap. Since then the flap has been widely used in the oral cavity and pharynx (Harii et al., 1985; Boorman & Green, 1986; Soutar & McGregor, 1986; Muldowney et al., 1987; Acatürk et al., 1989; Kawashima et al., 1989; Percival & Earley, 1989; Hatoko et al., 1990). This success has made the flap the first choice in oral reconstruction in many centres.

The vascular basis of the free radial forearm flap:

The free radial forearm flap is a fasciocutaneous flap

based on the radial artery. The vascular anatomy of the forearm is partly responsible for its successful use in reconstructive surgery.

Arterial blood supply of the forearm

The blood supply of the forearm comes from the brachial artery through its two terminal branches, the radial and ulnar arteries.

Radial artery

The radial artery is usually smaller than the ulnar artery but appears as the direct continuation of the brachial artery. At its origin it lies on the tendon of biceps and soon passes onto the supinator muscle, then, covered by the brachio-radialis it descends successively on the insertion of the pronator teres, the lateral part of the flexor digitorum superficialis, flexor pollicis longus and pronator quadratus (Cormack & Lamberty, 1986). It leaves the anterior aspect of the forearm by turning laterally around the wrist, deep to the extensor tendons of the thumb, traversing the 'anatomical snuff box' to reach the dorsum of the hand. It enters the palm by passing between the heads of the first dorsal interosseous muscle and forms the deep palmar arch.

Just below its origin the radial artery gives off the radial recurrent artery, which ascends and anastomoses with the radial collateral branch of the profunda brachii artery.

Throughout its course the radial artery gives many branches to adjacent muscles, and just before it reaches

the wrist it gives off two branches: the palmar carpal artery which shares in the formation of the palmar carpal arch and the superficial palmar artery which ends either by supplying the thenar muscles or by anastomosing with the terminal part of the ulnar artery forming the superficial palmar arch.

The radial artery gives off a series of small perforators that supply the skin of the lateral half of the anterior surface of the forearm, and most of the lateral border the forearm. The proximal perforators are larger but less frequent than the distal ones. These perforators anastomose freely with each other and with perforators from other arteries.

In its course in the forearm the radial artery is accompanied by two venae comitantes, which together are invested in a condensation of deep fascia that separates the flexor from the extensor compartment, the lateral intermuscular septum (Soutar, 1986). The superficial branch of the radial nerve lies on its lateral side.

Ulnar artery

The ulnar artery is the larger of the two terminal branches of the brachial artery. It leaves the cubital fossa by passing deep to the ulnar head of the pronator teres muscle and then passes obliquely on the flexor digitorum profundus covered by the flexor digitorum superficialis to reach the medial side of the forearm. It then descends vertically and crosses the flexor

retinaculum, passing to the hand where it ends by forming the superficial palmar arch. It is accompanied by two venae comitantes; the ulnar nerve lies medial to its lower half.

The ulnar artery gives the anterior and posterior ulnar recurrent vessels, these in turn give off small branches to the skin. The former supplies the skin in front of the elbow and the latter the skin behind it (Cormack & Lamberty, 1986).

In the lower part of the cubital fossa the ulnar artery gives off the common interosseous trunk which ascends to reach the upper border of the interosseous membrane where it divides into anterior and posterior branches. The anterior interosseous artery runs deeply on the anterior surface of the interosseous membrane and supplies adjacent muscles. It also supplies a small area of skin on the lateral border of the forearm by means of three perforating branches. The artery ends by piercing the interosseous membrane above the pronator quadratus to reach the posterior compartment where it joins the posterior interosseous artery.

The posterior interosseous artery appears on the back of the forearm between the supinator and the abductor pollicis longus and descends between the superficial and deep layers of the extensor muscles. It supplies the periosteum of the radius and adjacent muscles then gives cutaneous branches to the skin over the middle of the back of the forearm.

The median artery may originate from either the ulnar,

common interosseous or the anterior interosseous artery. It is a small branch that runs with the median nerve, but is occasionally larger, and may run down to the wrist and give one or two perforators to the skin of the distal part of the forearm.

Along its course the ulnar artery gives a few fasciocutaneous perforators that reach the skin along the fascial septum between the flexor carpi ulnaris and flexor digitorum superficialis (Cormack & Lamberty, 1986).

The ulnar artery also gives off muscular branches, palmar and carpal branches and a deep palmar branch. The muscular branches may give rise to perforators to the skin.

Venous drainage of the forearm

The veins draining the forearm are divided into superficial and deep veins that communicate at many points in their course. The deep veins are venae comitantes accompanying the arteries and receive tributaries corresponding to the arterial branches. They unite to form the brachial veins. Superficial veins include the cephalic, basilic and median vein of the forearm and their tributaries. The venous drainage of the skin and superficial fascia is mainly by way of the superficial veins. Communicating veins are present between the superficial and deep veins.

Cephalic vein

The cephalic vein is commonly formed over the 'anatomical snuff box' and runs on the radial side of the

flexor aspect of the forearm: it receives tributaries from both sides of the forearm. It ascends in front of the elbow superficial to the groove between the brachioradialis and biceps brachii, continuing its course in the arm.

Basilic vein

The basilic vein begins at the medial end of the dorsal venous arch and ascends posteromedially in the forearm. It is joined with the cephalic vein in the forearm by the median cubital vein.

Median vein of the forearm

The median vein of the forearm drains the superficial palmar venous plexus, it joins either the basilic or the median cubital vein.

Blood supply of skin and superficial fascia (Fig. 3):

The blood supply of the skin and superficial fascia of the forearm comes from the perforator arteries that originate from the radial and ulnar arteries and their main branches. These perforators are variable in size and anastomose freely with each other. The rich anastomoses are principally found in two plexuses, one between the dermis and the superficial fascia (prefascial) and the other at the junction between the reticular and papillary layers of the dermis. Because of the extensive anastomoses between the perforating arteries in territories supplied by the radial and ulnar arteries, it is possible to elevate large fasciocutaneous flaps based on the radial artery alone (Cormack & Lamberty, 1986). The largest recorded radial forearm flap was reported by Mühlbauer et al. (1982); it

measured 25 cm x 12 cm and was used to release a post-burn contracture in the neck.

Each cutaneous artery is accompanied by one or two veins at least until the opening in the fascia through which the artery passes to the prefascial plexus and beyond (Timmons, 1986).

The advantages of the radial forearm flap:

The radial forearm flap has a number of advantages over other techniques (Mühlbauer et al., 1982; Soutar & McGregor, 1986).

(1) Constant neurovascular anatomy that allows the use of a vascularised segment of the radius and some of the cutaneous nerves of the forearm should these be required for reconstruction.

(2) Long blood vessels of relatively large calibre suitable for anastomosis under "loupe" magnification and allowing the use of a long vascular pedicle.

(3) Adequate drainage by one venous anastomosis.

(4) Thin pliable skin that conforms to the contours of the oral cavity and tolerates post-operative radiotherapy without breaking down.

(5) Thin layer of fat that does not usually require further revising surgery.

(6) Short post-operative hospitalisation with early mobilisation and return to oral feeding.

Preoperative assessment of the patients:

An Allen test is performed to determine whether the

hand will survive without the radial artery (Allen, 1929). The test is done by applying sufficient pressure above the wrist to occlude the radial and ulnar arteries while the patient opens and closes the fist. The hand will become pale in 10 - 20 seconds and the patient is asked to open the hand to a relaxed position avoiding hyperextension. The ulnar artery is then released; capillary filling will appear within a few seconds. A delay of more than 15 seconds means that the circulation of the hand would be compromised by the absence of the radial artery and the surgeon should look for an alternative flap. It is important to carry out this test properly to avoid false positives (Ejrup et al., 1966; Greenhow, 1977). Soutar (1986) even advised repeating the Allen test at various times during the pre-operative assessment.

The radial artery is palpated and its course in the forearm carefully marked. The subcutaneous veins are also identified and marked (Song et al., 1982). Any recent cannulation of the superficial veins of the upper limb may be a relative contraindication for using the forearm flap from that limb (Hallock, 1986). This does not apply if the flap is planned to be drained by the vena comitantes of the radial artery (Soutar, 1991).

Design of the flap and the vascular anastomosis:

The size and shape of the radial forearm flap can be planned to cover the defect and to include in addition to the vessels, a cutaneous nerve and a piece of bone from the radius. The flap is usually taken from the non-dominant

upper limb.

The use of the forearm flap in oral reconstruction requires two teams of surgeons working at the same time: one team to excise the tumour and prepare the recipient site and the other team to elevate the flap and close the donor defect. The surgical technique was described by Soutar et al. (1983).

The design of the flap is drawn on the forearm with sterile methylene blue dye (Fig. 4). The upper limb is then elevated to assist venous drainage and an arm tourniquet is inflated to create a bloodless field.

The margins of the flap are incised down to the deep fascia, isolating and preserving a proximal subcutaneous vein if required. The deep fascia is incised and the flap is raised sub-fascially (Fig. 5). If the donor defect is to be closed by an ulnar-based transposition flap, the surgeon has to rely totally on the venae comitantes of the radial artery for drainage of the radial forearm flap. The intermuscular septa which pass deeply from the deep fascia must be divided. This allows the radial artery and its venae comitantes to be elevated and freed. If part of the radius is to be included, the lateral intermuscular septum must be preserved intact. The superficial branch of the radial nerve is preserved because it supplies sensation to the hand and fingers (Mühlbauer et al. 1982).

In the neck, the most commonly used arteries for the anastomosis are the facial and superior thyroid arteries

while the veins are tributaries of the internal jugular vein, the external jugular vein and tributaries of the anterior jugular veins (Soutar & McGregor, 1986). The anastomoses are either end-to-end or end-to-side for both artery and veins.

Although not necessary, many surgeons reconstruct the radial artery in the forearm using a reverse venous graft (Mühlbauer et al., 1982; Soutar et al., 1983). In a recent report, Barsdley et al. (1990) reconstructed the radial artery in 12 cases out of 100 and found half the reconstructed vessels occluded at the time of follow-up.

The donor defect:

The donor defect may be closed in different ways:

1) directly if the defect is small or elliptical (Mühlbauer et al., 1982);

2) using a split thickness skin graft from another donor site (Soutar et al., 1983; Moore et al., 1985; Harii et al., 1985);

3) by taking a split thickness graft from the flap itself to cover the donor defect and transferring the remaining de-epithelialised flap into the oral cavity (Kawashima et al., 1989);

4) using tissue expansion allows direct closure of the wound (Hallock, 1988; Masser, 1990);

5) by a transposition flap based on the ulnar artery (Elliot et al., 1988); this is the technique currently used at Canniesburn Hospital (Fig. 4 - 6).

THE SKIN

The skin (Fig. 7) is the largest organ of the body; it covers its surface and is continuous with several mucous membranes. It is composed of two layers: a cellular epidermis which rests on a basement membrane that separates it from the underlying mainly fibrous layer, the dermis or corium. Beneath the dermis is a layer of loose connective tissue known as the hypodermis. The fibrous elements of the dermis merge with those of the hypodermis and there is no clear-cut boundary between these two layers.

The colour of the skin depends on the presence of melanin pigment particularly in the deeper layers of the epidermis, the thickness of the horny layer, the carotein content and the vascularity of the dermis.

The epidermis: (Fig. 8 & 55)

The epidermis is a renewing tissue formed of keratinised stratified squamous epithelium; it forms an uninterrupted cellular investment covering the outer surface of the body, but is locally specialised to form the various skin appendages: hairs, nails and glands. Its cells produce the fibrous protein keratin, which is essential to the protective function of the skin, and melanin, the pigment that protects against ultraviolet irradiation. The epidermis gives rise to two main types of gland: sweat and sebaceous.

The thickness of most of the epidermis ranges between 0.07 - 1.12 mm, but in the thick epidermis of the palms and soles may reach 1.4 mm. The thinnest epidermis is found on

the eyelids, unexposed flexor surfaces of the trunk and limbs, and on the scrotum. While thick epidermis is associated with thick dermis on the palms and soles, thick dermis accompanies thin epidermis in the skin of the upper back.

The structural organisation of the epidermis is most obvious in thick skin; in sections perpendicular to the surface, four principal layers can be distinguished: stratum basale, stratum spinosum, stratum granulosum and stratum corneum.

Stratum basale

This layer consists of one layer of cells lying on the basement membrane to which the cells are attached by hemidesmosomes. The cells are cuboidal-columnar with relatively large nuclei and basophilic cytoplasm. Cell division occurs mostly in this layer, which is therefore sometimes referred to as the stratum germinativum.

In the epidermis of many mammals and in thin epidermis in man, the process of cell division in the basal layer, and subsequent upwards migration of daughter cells, is highly ordered. The surface squames are hexagonal or polygonal in outline, and each overlies a vertical column of 3 - 4 cells, surrounded by a peripheral ring of 6 - 7 cells. The group of basal cells is called an epidermal proliferative unit (EPU). In the central cluster there is a stem cell which divides to produce another stem cell and a daughter cell, which in turn produces several post-mitotic

cells. These mature in the peripheral ring and then move upwards into the column of cells which therefore represents the progeny of a single basally situated epidermal proliferative unit.

Most mitoses occur in cells in the basal layer, and the spindle axis is most frequently parallel to the basement membrane, so that both new daughter cells remain temporarily in the basal layer (Potten, 1981). It is estimated that at any given time one or two mitotic figures are found per 1000 basal cells (Stenn, 1988).

Stratum spinosum

The keratinocytes in this layer show a flattened polyhedral form with their long axis parallel to the surface and the nucleus somewhat elongated in this direction (Fawcett, 1986). Processes of adjacent cells connect at desmosomes and appear with the light microscope as spines giving this layer its name. This arrangement provides much tensile strength and coherence to the epidermis (Williams et al., 1989). The thickness of this layer is variable over different parts of the body.

Stratum granulosum

The name of this layer comes from the intensely basophilic keratohyalin granules which appear in the cytoplasm of the keratinocytes, which are more squamous than those in the stratum spinosum. In thick epidermis the stratum granulosum ranges between one and five cells in thickness but it may be absent or interrupted in thin epidermis. The nuclei of the cells become pyknotic and

other cellular organelles degenerate (Williams et al., 1989).

Stratum corneum

The most superficial part of the epidermis consists of several layers of flat, closely packed, cornified, non-viable cells. In haematoxylin and eosin stained sections it appears as a characteristic eosinophilic layer. Ultrastructurally, desmosomes are still seen but their halves are widely separated (Fawcett, 1986). This layer is only a few cells deep in thin epidermis but it may be more than 50 cells deep in thick epidermis (Williams et al., 1989).

Cells of the epidermis:

Keratinocytes

Keratinocytes are the most abundant cells of the epidermis. They form the fibrous protein keratin which contributes to the physical toughness and is partly responsible for its impervious properties. Their life of about 15 - 30 days passes through three distinctive phases: (1) growth and multiplication, (2) maturation and upwards movement and, (3) desquamation (Stenn, 1988).

Most of the cell division occurs within the basal layer but not all the cells have the same proliferative ability (Stenn, 1988).

The basal keratinocytes are cuboidal-columnar cells which are firmly attached to the basal lamina by hemidesmosomes and to surrounding cells by desmosomes.

Their nuclei vary from euchromatic with prominent nucleoli (in stem or young cells) to heterochromatic in older keratinocytes (Williams et al., 1989). Within the cytoplasm there are bundles of intermediate filaments known as tonofilaments which end in the desmosomes (Fig. 55). The usual cellular organelles are present in the basal keratinocytes but the Golgi apparatus is poorly developed and centrioles are occasionally seen (Breathnach, 1971).

As keratinocytes move upwards in the epidermis, they flatten, develop more intercellular connections and their content of tonofilaments increases.

Two types of keratohyalin granules accumulate in the cytoplasm of the upper spinous and granular layers: both types are non-membrane bound and irregular when demonstrated by electron microscopy. The smaller is the dense homogeneous deposit (DHD) also known as the single granule (SG), which is rich in sulphhydryl groups, and the larger is the composite granule (CG), which contains histidine-rich protein, RNA, polysaccharide and lipids (Bernstien, 1983).

In keratinocytes of the upper spinous layers the Golgi apparatus produces membrane-bound lamellated granules, known as membrane-coating granules or Odland bodies, which vary in diameter between 100 - 500 nm. These granules acquire a peripheral position in the cytoplasm of the cells of the stratum granulosum. They become attached to the inner surface of the plasma membrane and discharge their contents into the intercellular space, where they are

believed to form the primary intercellular barrier to water. These granules were also suggested to secrete lipids that participate in the formation of the bilayers of the stratum corneum (Elias, 1981).

Melanocytes

Melanocytes develop from cells which, in man, leave the neural crest by the tenth week of embryonic development and start to enter the epidermis about the eleventh week (Breathnach, 1971).

The number of melanocytes varies between races and even between different regions of the body of the same individual. However, it is the melanin in the keratinocytes which is the main determinant of differences in skin and hair colour between individuals (Jimbow et al., 1976). Rosdahl and Rorsman (1983) quantified the number of melanocytes in different parts of the body; in the forearm there were 1130 ± 160 cells/mm^2.

Melanocytes rest on the basement membrane slightly below the level of keratinocytes (Fig. 9) and are widely separated from each other.

Ultrastructurally, melanocytes have a small rounded nucleus and numerous dendritic processes that spread between the keratinocytes. Melanin is contained in melanosomes which are small, electron-dense, oval or rounded cytoplasmic granules. Cytofilaments may be present in the cytoplasm but no tonofilaments (Breathnach, 1971).

From one melanocyte, melanosomes are transferred

through its cytoplasmic processes to a group of basal keratinocytes. A melanocyte and its associated keratinocytes are known as an epidermal-melanin unit (Fig. 9). Within the keratinocytes, the granules may remain separate or aggregate into larger membrane-limited structures, compound melanosomes. It has been estimated that a single epidermal melanocyte is capable of transferring melanosomes to a pool of keratinocytes varying from 4 - 12 in number (Breathnach, 1971).

The keratinocytes are thought to play an active role in the transfer of melanin by engulfing portions of the melanocyte processes, and thereby to be capable of controlling not only the rate of transfer but, through feedback, the actual rate of melanogenesis within the melanocyte (Gordon et al., 1989). Melanosomes accumulate above the nucleus of the keratinocyte forming the so-called "supra-nuclear cap" protecting it from incoming radiation. The melanosomes in the more superficial layers of the skin are more uniformly distributed within the cells.

Melanocytes can also be found in the dermis, especially in the sacral region where they are responsible for the bluish coloration noted at birth known as the "Mongolian spot".

Langerhans cells

Langerhans cells are antigen-presenting cells and important in immune responses involving the skin (Silberberg, 1971; Stingl et al., 1978).

These dendritic cells, like melanocytes, appear as

clear cells by light microscopy but are detectable by stains for ATPase (Stenn, 1988). They are scattered in all epidermal layers with variable density in different parts of the body, being more numerous in thick epidermis.

The Langerhans cell has an irregularly indented nucleus and, ultrastructurally, the cytoplasm contains characteristic racket-shaped structures known as Birbeck granules. The cytoplasm contains no tonofilaments and the cell has no desmosomal attachments to its neighbours.

In addition to histocompatibility antigen (MHC), it is now believed that Langerhans cells carry receptors for immunoglobulin (Fc) and complement (C3). They also bear surface antigens common to most B- and some T-lymphocytes, monocytes and macrophages. They are believed to be important in helper T-cell activation (Stenn, 1988).

Merkel cells

These cells are more numerous in skin areas performing a sensory function; they are especially numerous in the finger tips.

The origin of Merkel cells is debatable, it has been suggested that they originated from the neural crest (Winkelmann & Breathnach 1971) or from neighbouring keratinocytes (Moll et al., 1984).

Microscopically, Merkel cells are found in the basal epidermal layer; the nucleus is irregular, and the cytoplasm is clear. The electron microscope has shown the cytoplasm to contain cytokeratin filaments and

neurosecretory granules similar to chromaffin granules; because of the latter, they are classified as APUD cells (Stenn, 1988). Merkel cells are attached to neighbouring cells by desmosomes. They are innervated by long myelinated nerve fibres and are believed to have a sensory function.

Dermal-epidermal junction:

The dermal-epidermal junction is a complex layered structure which appears as an undulating line in vertical histological sections. The epidermis projects down forming the rete ridges that surround finger-like projections of the dermis called dermal papillae.

Electron microscopy has shown the dermal-epidermal junction to be divided into three components: epidermal, dermal and a basal lamina separating the dermis from the epidermis.

The epidermal component consists of the plasma membranes of the basal keratinocytes and the hemidesmosomes that attach them to the basal lamina. Along the cytoplasmic surface of the hemidesmosomes there is an electron-opaque thickening of the plasma membrane called the attachment plaque, towards which the tonofilaments converge (Stenn, 1988).

The dermal contribution is in the form of connective tissue fibres including anchoring fibrils, microfibrils condensed to form bundles and collagen fibres.

The basal lamina is formed of two parts, the lamina lucida and lamina densa.

The lamina lucida is an electron-lucent amorphous

layer that lies adjacent to the hemidesmosomes. It contains anchoring filaments that course from the inner leaflet of the plasma membrane to the lamina densa. The lamina lucida contains a unique 900 Kd protein known as laminin and bullous pemphigoid antigen. It does not contain collagen and is digested by proteolytic enzymes.

The lamina densa is an electron-dense layer that contains Type IV collagen and heparan sulphate.

In addition to supporting the epidermis and providing for its adhesion to the underlying dermal connective tissue, the dermal-epidermal junction provides a permeability barrier between the vascular dermis and the avascular epidermis and is believed to play an important role in the interaction between them (Woodley, 1987).

The dermis:

The dermis is made predominantly of connective tissue elements, i.e collagen and elastic fibres embedded in a ground substance and has a scant cellular population. In addition, it contains blood vessels, lymphatics, nerves and the epithelial adnexa.

The dermis is divided into two parts: a superficial part, known as the papillary dermis made of loose connective tissue, and a deep part made of dense irregular connective tissue, known as the reticular dermis.

The thickness of the dermis varies from about 0.5 mm in the eyelids to greater than 5 mm over the back.

The collagen fibres are produced by fibroblasts and

laid down as bundles of variable size in a random pattern. The predominant orientation gives rise to Langer's tension lines. The collagen in the papillary dermis is predominantly Type III and to a lesser extent Type I, while in the reticular dermis it is mainly Type I and to a lesser extent Type III.

The elastic fibres extend from the papillary dermis towards the epidermis, probably helping to bind them together. In the reticular dermis the elastic fibres surround the collagen bundles giving the dermis its mechanical properties.

The ground substance in which the fibres are embedded is an amorphous matrix, made up by proteoglycans, metabolic products of dermal cells, water and ions.

The cellular population of the dermis consists mainly of fibroblasts, macrophages, mast cells and occasional lymphocytes.

Blood vessels of the dermis:

The dermis is supplied by perforating vessels from the main arteries or their branches; these perforators form two plexuses: one at the level of the dermal-hypodermal interface and the other, the papillary plexus, at the junction of the reticular and the papillary dermis. Vessels arising from these plexuses anastomose freely.

The avascular epidermis depends totally on the dermis for its nourishment; capillary loops originating from the papillary plexus enter the dermal papillae to serve the nutritional needs of the epidermis.

Pasyk and her co-workers (1989) have shown that the density of capillaries is greater in the papillary than in the reticular dermis. They also found that it varies in different body regions; the head and neck have the highest density of capillaries in both the reticular and papillary dermis while the lower extremities have the lowest density.

Arteriovenous shunts have been proven in the dermis at two levels, the deep and the superficial dermis.

Dermal lymphatics are more numerous in the upper reticular dermis and follow a similar pattern to the blood vessels. The lymphatic capillaries have a distended lumen, thin walls, and prominent valves (Stenn, 1988).

Dermal blood vessels are associated with myelinated nerve fibres which also form plexuses with the vascular plexuses.

Age and regional variations of the skin:

The basic structure of the skin is similar all over the body but it shows genetically determined regional variations which reflect functional demands.

As mentioned earlier, the two components of the skin, the epidermis and dermis, vary in thickness over most of the body. In general, but excluding hands and feet, the skin is thicker on the dorsal and extensor surface than the ventral or the flexor surface and is thicker in men than women (Stenn, 1988).

Skin colour varies between races but also within individuals; the skin is darker in exposed areas such as

face and hands as well as at the scrotum and nipple. The skin also shows gradual changes in appearance, texture and mechanical properties with age.

Normal ageing is accompanied by epidermal and dermal atrophy; the thinned epidermis is accompanied by flattening of the dermal papillary ridges (Montagna & Carlisle, 1979) which results in decreasing the resistance to shearing forces. The dermal changes include thickening of the elastic fibres and a decrease in the amount of collagen in exposed areas (Lavker, 1979). Skin elasticity decreases with age (Lèvêque et al., 1980).

Epithelial adnexa:

Sweat glands

In human skin there are two general types of sweat gland, eccrine and apocrine. The former is found in all skin except that of the glans penis, the lip margin and the nail beds. On the other hand, apocrine sweat glands are concentrated in the axillae, the areola of the nipples, the umbilicus and the anogenital area. The ceruminous glands of the external auditory meatus and Moll's glands of the eyelids are modified apocrine glands. Apoeccrine glands which show a mixture of eccrine and apocrine features were described recently in the axillary skin (Sato et al., 1987).

Because sweat glands in the forearm skin are of the eccrine type they will be considered in some detail.

Eccrine sweat glands (Fig. 7)

The eccrine sweat gland is a simple coiled tubular

gland that develops as a downgrowth from primitive epidermis. The secretory part of the gland is situated in the deep part of the dermis or occasionally in the hypodermis. The duct opens directly on to the skin surface; its intraepidermal part is known as the acrosyringium.

The secretory part is formed of three types of cell: clear, dark and myoepithelial cells.

The clear cells rest on the basement membrane and extend to the lumen. In some areas these cells are separated from the basement membrane by the myoepithelial cells. They secrete serous fluid that contains variable amounts of glycogen. Ultrastructurally, the clear cells contain many mitochondria, variable amounts of glycogen and small amounts of rough endoplasmic reticulum.

The dark cells lie adjacent to the lumen of the gland. The cytoplasm of these cells contains an abundance of mucous granules and rough endoplasmic reticulum. It is believed that dark cells secrete the mucoid substance in the eccrine sweat but this has not been proven biochemically (Collins, 1989).

The myoepithelial cells are situated between the clear cells and the basement membrane. They are widely believed to expel the newly formed sweat to the surface but some workers consider their function to be one of support for the secretory and duct systems (Sato et al., 1979; Sato, 1987). These cells are better developed in the ducts than in secretory regions but they are absent from the

transitional zone between the secretory part and the duct (Collins, 1989).

The intradermal duct segment is lined by bi-layered epithelium, the luminal cells of which bear numerous microvilli on their surface.

The intraepidermal part of the duct (acrosyringium) runs a spiral course and is lined by two or more layers of cells which are keratinised and dead in the stratum corneum.

The epithelial lining of the ducts is important in the formation of new epidermis during wound healing (Mehregan, 1986).

Hair follicles (Fig. 7)

The hair follicle passes through three phases in its growth cycle: a) anagen or growing phase, b) catagen or involuting phase and c) telogen, the resting phase.

The histological appearance of the hair follicle varies in these three phases; the following account describes the hair follicle in the anagen phase.

In longitudinal sections, the deepest part of the hair is seen as a bulb-like expansion which surrounds a dermal connective tissue papilla. The outermost part of the hair follicle is a downgrowth of the epidermis known as the external root sheath which consists of several layers of cells except over the bulb where it is only one layer thick.

The cells of the matrix of the bulb are actively dividing and give rise to the three-layered internal root

sheath which forms the keratin of the hair. The three layers of the internal root sheath are known as the cuticular, Huxley's and Henle's layers. The hair shaft also consists of three layers: the medulla, cortex and cuticle.

A smooth muscle known as the arrector pili muscle is attached to the connective tissue sheath that surrounds the hair follicle. Elastic fibres run parallel to the muscle fibres. On contraction, the muscle erects the hair and elevates the skin in the immediate vicinity of the follicle.

Sebaceous glands (Fig. 7)

The sebaceous glands are derived from the developing hair follicle and become fully developed by the 17th week of intrauterine life (Thody & Shuster, 1989). Several glands are usually associated with one hair follicle.

Sebaceous glands are alveolar holocrine glands that discharge their secretion (sebum) through the sebaceous duct into the upper part of the associated hair follicle. This secretion is under hormonal control and contains necrotic cells and their fatty products. It helps to make the skin waterproof and prevents excess water loss from the epidermal cells.

New cells are produced by mitotic activity of the cells at the periphery of the gland. The basal cells are attached to adjacent cells by desmosomes and contain free ribosomes, rough and smooth endoplasmic reticulum, glycogen granules and a few lipid droplets.

Keratinisation and desquamation:

Cells of the stratum corneum are fully differentiated anucleated keratinocytes filled with filaments of the fibrous protein, keratin. Before reaching the stratum corneum the keratinocyte undergoes a series of biochemical and morphological changes known as keratinisation.

Keratinisation has been described in two phases: i) a synthetic and ii) a transformation or degradation phase (Matoltsy, 1976; Lavker & Leyden, 1979).

i) Synthetic phase: In this phase the differentiation products, which include tonofilaments, keratohyalin granules and membrane-coating granules, are accumulated in the keratinocyte in increasing number and size as they move up in the epidermis from the basal to the granular layer. The first two products remain in the cell to be used in the formation of the horny cell while the membrane-coating granules are discharged from the cell into the intercellular space where they form part of the intercellular matrix.

ii) Transformation phase: This phase occurs at the level of the stratum granulosum and follows the synthetic phase. Lysosomes rupture and their enzymes are released into the cytoplasm and activated leading to degradation of the remaining cellular components. Tonofilaments and keratohyalin granules are resistant to the action of lysosomal enzymes. The components become consolidated by

loss of fluids resulting in the formation of the flattened horny cells.

The fate of the degraded organelles has not been studied in detail. Lavker and Matoltsy (1976) suggested that they pass through the thickened membrane of the transformed cells to the intercellular space where they are reabsorbed by the epidermis.

Abnormal keratinisation may result in a variety of clinical disorders; parakeratosis which is characterised by a keratin layer which retains pyknotic nuclei is seen in a number of inflammatory skin diseases and is believed to result from recent disturbance in the metabolism of keratinocytes (McGregor & McGregor, 1986).

Hyperkeratosis, another disorder, is characterised by an increase in the thickness or density of the stratum corneum and is seen in chronic diseases like lichen planus.

On the other hand dyskeratosis features premature or abnormal keratinisation of individual epidermal cells as seen in benign (e.g. benign familial pemphigus) and malignant conditions (e.g. poorly differentiated squamous cell carcinoma).

The rate of the cell loss from the surface of the epidermis varies in different regions of the body and depends, in part, on environmental factors like friction, temperature and relative humidity. Marks et al., (1982) invented a special device (Cohesograph) to measure the rate of desquamation in different parts of the body and found it higher in the forearm skin (1309 ± 328 cells/cm^2/hour) than

in the skin of other areas examined (upper arm, abdomen, thigh, and back). In another report the rate of "horn" production of the forearm skin was found to be less than that of the palm and scalp; 0.1 $g/m^2/day$ in the forearm compared to 3.5 $g/m^2/day$ and 2.1 $g/m^2/day$ in the palms and scalp respectively (Ebling & Rook, 1972).

The mechanism of desquamation is not clearly understood, but the cells are believed to be shed intact (Kessel & Kardon, 1979). Much of the present knowledge on desquamation came from studying icthyosis, a dermatological disorder characterised by accumulation of visible scales on the skin surface. This condition was found to be due to disturbed lipid metabolism (Elias, 1983; Williams & Elias, 1983).

Epidermal-Dermal Interaction:

As in many cell populations, in epidermis there is continuous cell turnover and a balance between cell formation and loss. In epidermis mitosis occurs in the basal layer, cells move to progressively more superficial positions while undergoing structural and functional changes and at the end of their life-time are shed from the superficial surface. Despite these continuous processes, the normal regional structural patterns are maintained throughout life.

The factors which control the development of skin in embryonic and fetal life and ensure its subsequent maintenance are still the subject of many investigations and may be relevant in explaining the changes in intraoral radial forearm flaps under investigation in this project.

Experience with full thickness skin grafts in man has shown that these grafts retain the characteristics of the donor site and never acquire the features of the skin of the recipient site. These observations are consistent with experimental results which indicate that the regional characteristics of the epidermis are determined by the dermis.

A large body of information has been gathered which shows the dermis to have a most important role in the growth and maintenance of epidermis. The central concepts are described in this section.

Role of dermis in epidermal proliferation

It has been shown that both embryonic and adult dermis has the ability to promote epidermal cell proliferation and that the coordinated proliferation of basal keratinocytes is dependant on dermal factors. These factors are non-specific and can be supplied by non-dermal substrates.

Wessels (1963) found that the epidermis of different body regions of the 11-day chick embryo ceased to proliferate when isolated from the dermis but resumed mitotic activity when recombined with it own dermis. In these experiments non-dermal tissues such as cartilage and muscle were not effective.

Similar findings were reported in adult human skin by Briggaman and Wheeler (1968). These workers and others found that freeze-thawed dermis and collagen can replace viable dermis under optimal culture conditions but that heat killed dermis, agar or Millipore filters were ineffective (Dodson, 1967; Briggaman & Wheeler, 1971).

The nature of dermal stimuli in embryonic and adult dermis has also been investigated. Redler and Lustig (1968) suggested that these two tissues differ in the nature of dermal stimuli produced because of the inability of adult dermis to maintain the proliferation of embryonic epidermis. It has also been shown that the proliferation rate of normal human epidermis is increased when combined with peritumoral and cicatricial dermis. This indicates that the dermis is endowed with embryonic features that become evident under pathological conditions.

Role of dermis in epidermal cell stratification and keratinisation

Epidermal cells are transformed into keratinised non-viable cells before being desquamated from the skin surface. Several experiments, many on chicks, have suggested that keratinisation is an intrinsic property of the epidermal cells. For example, Moscona (1959) found that isolated chick extraembryonic ectoderm *in vitro* became transformed into keratinised epithelium following separation from the dermis. This indicated that keratinisation could occur in isolation from the dermis. These findings were later confirmed by Mcloughlin (1961) who found that two-layered epidermis, isolated *in vitro* from 5-day chick embryo limb bud, became transformed into a disorganised mass, the outermost cells of which flattened and formed keratin. Eventually all cells differentiated, became keratinised and died. In these experiments normal stratification of the epidermis was lost.

The role of the dermis in epidermal stratification has been demonstrated by combining the single-layered chick chorionic ectoderm with dorsal or tarsometatarsal dermis. The chorionic epithelium was transformed into stratified keratinising epidermis which was typical of that normally associated with the dermis (Fisher & Sawyer, 1979; Sawyer, 1979).

The ability of the dermis to produce region specific epidermis has been also demonstrated in the adult organism.

Billingham and Silvers (1968) combined dermis from the trunk, ear or foot pad of guinea pigs with heterotopic epidermis. The resulting epidermis was similar to the one that normally covered the dermis.

Other work, e.g., Karasek & Charlton (1971), has shown that, in culture, epidermal cells will show stratification when grown on non-dermal substrates such as collagen gels.

Morphogenesis of epidermal adnexa

Many experiments involving different types of recombinations have been carried out in order to investigate the role of epidermal-dermal interactions in the morphogenesis of epidermal adnexa. They include, among others, combinations of epidermis and dermis from different sites (heterotopic), different inbred strains (heterogenetic) and combinations where the epidermis or dermis had been treated by a hormone or a vitamin.

a) Heterotopic recombinations:

In these recombinations the epidermis from one part of the body was combined with dermis from another part that had a different pattern of epidermal adnexa.

When combining embryonic epidermis from a glabrous region, such as mouse foot, with dermis from a region which would form appendages it was noted that appendages developed (Kollar, 1970; Dhouailly, 1973); similarly when epidermis from an appendage-forming area was combined with dermis from a glabrous area, no appendages formed. These studies showed that formation of adnexa was induced by the dermis.

It was also found that when the recombinants were taken from chick embryos at the same stage of development, the resulting appendages were similar to those found in the original site of the dermis. On the other hand, when the dermis was younger than the epidermis the type of appendages was similar to that at the original site of the epidermis (Rawles, 1963; Sawyer, 1983). These experiments demonstrated that the age of the recombinants is important in determining the role played by each of the dermis and epidermis in the formation of appendages.

b) Heterogenetic recombinations:

Heterogenetic recombinations in mouse embryos have been used to study malformations of hair and coat colour. Such recombinations have shown that the genes responsible for the coat colour act through the dermis (Poole, 1974 & 1975).

c) Effects of retinoids and hydrocortisone:

Injecting retinoids into the amniotic cavity of 10 - 12 days chick embryo resulted in the so-called chemical ptilopody i.e., the formation of feathers on the foot scales (Dhouailly et al., 1980). In heterotypic recombinations of treated epidermis with non-treated dermis feathers developed at the distal tip of the scales while in the reverse combinations of treated dermis with untreated epidermis scales developed normally. These results indicate that retinoids act on the epidermis (Cadi et al., 1983).

On the other hand, injecting hydrocortisone at 6 days

of incubation resulted in complete absence of feathers (Zust, 1971). As with retinoids, heterotypic recombinations have shown that hydrocortisone affects primarily the epidermis; when treated embryonic dermis was combined with normal embryonic epidermis, feathers developed normally while in reverse combinations of normal dermis with treated epidermis no feathers developed (Stuart et al., 1972).

Role of the basement membrane

The basement membrane is believed to be involved in the epidermal-dermal interactions but its exact contribution is incompletely understood.

Terzaghi and Klein-Szanto (1980) found that tracheal epithelial cells grew normally when associated with non-tracheal stroma, e.g., from the bladder and small intestine, while fibroblasts that were not associated with basement membrane failed to support the normal growth of these cells. From these results, they suggested that the basement membrane has an important role in the development and subsequent maintenance of the epithelium; this view is supported by other workers (Hay, 1978; Osman & Ruch, 1981). However, Mackenzie and Hill (1984) succeeded in cultivating the epithelium on an inverted connective tissue which lacked the basement membrane.

Gipson et al., (1983) have shown that the basement membrane can induce the epidermal cells to produce hemidesmosomes. When they cultured keratinocytes in contact with basement membrane devoid of anchoring fibrils the keratinocytes did not produce hemidesmosomes. In contrast

hemidesmosomes were rapidly formed when the basement membrane contained anchoring fibrils.

It has also been found that the ultrastructure of the dermal-epidermal junction in chick embryos differs in feather-forming, scale-forming and glabrous skin (Démarchez et al., 1981. The differences were related to morphogenesis of cutaneous appendages.

Further work is required to elucidate any role of the basement membrane in epidermal-dermal interaction.

Mechanism of the dermal influence on epidermal growth and proliferation

The mechanism by which the dermis influences epidermal growth and proliferation has also been the subject of extensive research. Two mechanisms have been postulated to explain this influence:

1) the dermis provides a substrate for the attachment and orientation of basal keratinocytes. This effect is non-specific and can be replaced by other non-dermal substrates.

2) the dermis produces diffusible factors that enhance epidermal growth and /or differentiation.

It is likely that both mechanisms are involved in the development and maintenance of epidermis.

It seems likely that fibroblasts are important in dermo-epidermal interactions since they maintain and organise the connective tissue and secrete diffusible

factors that stimulate epidermal growth (Mackenzie & Fusenig, 1983; Coulomb et al., 1989).

It is also believed that the extracellular matrix may play an important role in transmission of messages from the dermis to the epidermis. This matrix includes interstitial collagen (types I and III), fibronectin, sulphated and non-sulphated glycosaminoglycans (GAG's) and the bullous pemphigoid antigen; some of these are important constituents of the basement membrane (Sengel, 1986).

Other factors regulating epidermal growth and proliferation

The proliferation and differentiation of epidermal cells are integrated balanced processes. Several local and systemic factors have been postulated as regulators for these processes in vivo and in vitro. They include cyclic nucleotides (Voorhees & Duell, 1971; Marcello & Duell, 1979), growth factors (Greaves, 1980; Nanney et al., 1984; Nanney, 1990), retinoids (Bailly et al., 1990; Siegenthaler et al., 1990; Zouboulis et al., 1991), prostaglandins (Kragballe et al., 1985), chalones, calcium and calmodulin (Tucker et al., 1984; Fairley et al., 1985) and histamine (Flaxman & Harper, 1975; Iizuka et al., 1979). The exact mechanism by which these factors affect the proliferation and differentiation of keratinocytes is not yet clearly understood.

The behaviour of skin autografts:

The trauma which results from transferring the skin from one site in the body to another initiates a series of changes in the transplanted skin which may persist for a variable period after the graft is healed in its new position. The survival of free grafts depends on the re-establishment of an adequate circulation. In this respect, the behaviour of free grafts may not be identical to that of free flaps, such as the radial forearm flaps in the present study, because free flaps are provided from the start with a functional circulation, while free grafts are relatively ischaemic for a few days.

Working on rabbit skin grafts, Medawar (1944) has shown that skin autografts pass through three phases:

1) a period of primary union and vascularisation: this phase is completed by the fourth day and is characterised by invasion of the dermis of the graft by blood and lymphatic vessels accompanied by a mild traumatic inflammatory reaction and an increase in epithelial thickness. The blood vessels are dilated and filled with stagnated blood. The inflammatory cells are monocytes that migrated from the dermal blood vessels.

2) a period of generalised hyperplasia: in this phase dermal and epidermal elements show increased activity. The epidermis becomes thickened up to seven times its original thickness. Lymphatic capillaries are abundant and, in early stages, they may be found to contain mononuclear phagocytes and degenerating red and white blood cells.

3) a period of partial retrograde differentiation: in this phase the changes regress; the epithelium thins out, the great majority of blood capillaries disappear and the graft becomes similar to the surrouding skin.

Scothorne and Scothorne (1953), working on human full-thickness skin grafts in the lower limbs of patients undrgoing treatment for burns, have shown that the surgical trauma and ischaemia may cause localised sloughing of the epithelium in the immediate post-operative period. By the fifth day, the surviving epithelium was found to be increased in thickness. This increase in epithelial thickness was due to cellular hyperplasia and hypertrophy. The cells of the stratum corneum were parakeratotic and the stratum granulosum was absent but reappeared by the seventh day indicating the restoration of normal keratin synthesis. In biopsies taken 7 - 20 days after grafting the epithelium was hyperkeratotic but otherwise showed a gradual return to normal structure. The keratinocytes of the grafted skin accumulated glycogen for variable periods. This accumulation was considered a sign of epidermal irritation and may be associated with abnormal keratinisation (Montagna & Parakkal, 1974).

The origin of the new blood vessels has long been debated. Haller and Billingham (1967) have shown that the intrinsic blood vessels of the graft are reutilised and are necessary for its survival.

Using the stereomicroscope, Converse (1977) described

the surface of human forearm grafts to be pale in the first postoperative day. In the second day vessels gradually became dilated and a sluggish blood flow soon occurred which increased after the fifth day. The number of blood vessels increased up to the seventh or eighth days and the dilated vessels disappeared so that by the tenth day the vascular pattern was almost the same as in adjacent skin.

These experiments all suggest that the early changes in the appearance of the epidermis are initiated by trauma and ischaemia; once the graft is healed in place and established its definitive circulation the epidermis returns to its appearance before grafting.

Recent advances in keratinocyte culture have made it possible to use the generated epidermis as an autograft in treatment of burns. Following grafting, these sheets developed a fully stratified epidermis by the 6th day with a well-developed stratum granulosum, the cells of which contained abundant keratohyalin granules and lamellar bodies. The lamellar bodies also found in the intercellular spaces in the stratum granulosum. In the first two weeks, mitotic figures were frequent in the stratum basale. The dermal-epidermal junction formed by the fourth week. The rete ridges reappeared 5 - 18 months after grafting. Some samples showed focal parakeratosis, dyskeratosis or hyperkeratosis in biopsies taken up to 1 year after grafting. The connective tissue of the deep fascia beneath the cultured grafts also showed changes; in the first

three months, collagen fibres became progressively more densely arranged and oriented parallel to the epidermis. The fibres and vasculature of the connective tissue resembled true dermis within 4 - 5 years (Compton et al., 1989).

Experience with flaps in oral reconstruction:

A few reports describe the gross appearance of different flaps used in oral reconstruction in their new site.

O'Brien (1970) reported one case of lip reconstruction using a pedicled sternomastoid flap, in which cyanosis and oedema were noted in the cutaneous part of the flap which then became vermilion in colour. The significance of these observations is unclear.

Ariyan (1979) used pedicled sternomastoid flaps in reconstructing oral defects and noted that sloughed flap epithelium was replaced by non-keratinised epithelium which was non-pigmented in black patients.

Pectoralis major myocutaneous flaps were used by Lawson (1985) who stated that "the natural history of the cutaneous portion of the flap is such that the epithelium changes from keratinising to nonkeratinising epithelium resulting in a pseudomucous membrane consistency with time".

Similar observations on the skin part of the pectoralis major myocutaneous flap were reported by Wei and his colleagues (1989). They examined biopsies taken from 14 patients at different post-operative periods by light microscopy and scanning electron microscopy and found that the epidermis was desquamated with loss of its appendages. The flaps used to reconstruct the floor of the mouth showed maximal desquamation while those in the lateral part of the

mouth showed the least desquamation.

Kawashima et al. (1989) used de-epithelialised forearm flaps in intraoral and oropharyngeal reconstruction. They found that these flaps show a mucosa-like appearance in the early postoperative period which on histological examination revealed a thinned stratum granulosum and parakeratosis one month after surgery, but returned to the normal skin appearance at 6 months after operation. They attributed the reversiblity of the change to regeneration from retained hair follicles.

In a brief communication, Zolite and O'Neill (1989) reported a change in the appearance of an intraoral radial forearm flap 6 months after surgery and described it as leukoplakia. This ill-defined term is used by clinicians to describe white patches seen in the mucosa of the mouth and vulva. It is attributed to hydration of a thickened horny layer (McGregor & McGregor, 1986; Lever & Schaumburg-Lever, 1983).

The response of the skin to radiotherapy:

The gross and microscopical effects of radiotherapy on the skin depend on several variables: radiation dose, time over which the dose is given, size of the field, quality of radiation and the anatomical site of the irradiated skin. Depending on these variables the effects may be minimal, with complete recovery, or severe, with delayed recovery or possibly no recovery at all.

The effects of radiotherapy on the skin have been studied mostly on animal models; the most commonly selected animal is the pig because of the many structural similarities with human skin (Winter, 1972; Donovan, 1975; Van Den Aärdweg et al., 1988; Hopewell, 1990).

The cellular response to radiotherapy depends on the mitotic status of the cell and its degree of differentiation (Casarett, 1980; Ramsay, 1986). The Law of Bergonié and Tribondeau states that "the sensitivity of cells to irradiation is in direct proportion to their reproductive activity and inversely proportional to their degree of differentiation"; accordingly, basal keratinocytes which undergo regular mitotic division are radiosensitive but as they move upwards and become more differentiated they become relatively radioresistant. Non-keratinocytes also show variable responses. Melanocytes are relatively radioresistant, but in sufficient doses their enzymatic activity increases leading to increased pigmentation of the irradiated area. This increase in

activity is associated with increased cellular dimensions and an increase in the complexity of the dendritic processes (Snell, 1963). High doses of radiotherapy, however, may destroy the melanocytes resulting in decreased pigmentation (Casarett, 1980).

There are no reports about the response of Merkel cells to radiotherapy, but Merkel cell tumours (rare, locally aggressive and spreading rapidly to regional lymph nodes) appear to be radiosensitive (Ashby et al., 1989).

The response of Langerhans cells to radiotherapy is not clear; Kawase and his co-workers (1990) in a post-mortem study found that the number of Langerhans cells in recently irradiated skin is less than in non-irradiated skin of patients of the same age. Their findings were not consistent in patients who had been irradiated long before death. They concluded that radiation may temporarily decrease the density of Langerhans cells.

The epithelium of the hair follicle has similar radiosensitivity to that of the epidermis from which it is derived; hair follicles may recover following low doses of radiotherapy but with doses above 700 rads, depilation may be permanent (Casarett, 1980).

In sebaceous glands that are connected to hair follicles the basal sebocytes, the dividing cells, are radiosensitive but the cells which break down to form the secretion are relatively radioresistant (Casarett, 1980).

Sweat glands are relatively radioresistant, doses that cause destruction of hair follicles usually only inactivate

the sweat glands, and even when a dose lethal to the sweat glands is administered there is a latent period of 3 - 4 months before they totally disappear (Borak, 1936).

Irradiation produces an inflammatory reaction in the skin, the so-called radiodermatitis; it is suggested that it results from cellular damage releasing intracellular contents including proteolytic enzymes into the extracellular space (Fräki et al., 1983).

Following even a single dose of radiotherapy, the irradiated skin may appear erythematous. This erythema, known as the "brisk" reaction, is transient but it may be followed after about 10 days by further erythema which may show features of acute inflammation. In all its stages, erythema is the result of vasodilatation and vascular congestion. It is usually associated with increased endothelial permeability and oedema. With high doses, leukocytes and erythrocytes escape from the blood vessels resulting in an inflammatory picture (Casarett, 1980). In most patients these changes subside within a few weeks.

Repeated exposures to small doses of ionising radiation over a period of time either therapeutically or accidentally produce variable degrees of gross damage to the skin. After a latent period which may range from months to decades, atrophy, telangiectasia and freckling may be seen. The skin appears shiny, smooth, dry and thin. In severe cases these changes may be followed by ulceration and carcinoma (Arnold et al., 1990). In treating localised

tumours the surrounding skin is protected by a special shield.

The histological findings in the irradiated skin depend on the time since the last exposure. The microscopical effects of radiotherapy are usually described chronologically as early or late radiodermatitis; both forms have distinctive histological features.

MacKie (1984) described the damage caused by irradiation as permanent and progressive but with strict control of radiotherapy, late radiodermatitis is rarely seen nowadays.

Early radiodermatitis

Early radiodermatitis is usually seen a few weeks after exposure to a moderate therapeutic dose.

The epidermal cells show characteristic intracellular oedema "ballooning", some of the nuclei are pyknotic and the epidermal adnexa show degenerative changes. There may be flattening or loss of the rete ridges (Ramsay, 1986).

Some of the blood vessels in the upper part of the dermis may be dilated and inflammatory cells may permeate the epidermis. Deep blood vessels show oedema of their walls and endothelial proliferation, while some may be thrombosed. The fibroblasts are enlarged and contain large basophilic nuclei suggesting sarcomatous change (MacKie, 1984).

Late radiodermatitis

The epidermis is extremely irregular with atrophy in some areas and hypertrophy in other areas. Degenerative

changes may be seen particularly in the stratum spinosum; some of the cells may show individual keratinisation (dyskeratosis) and atypical nuclei. The stratum corneum may be hyperkeratotic.

The dermis shows swollen collagen bundles with new collagen formation. A pathognomonic feature is the presence of giant fibroblasts (Mehregan, 1986).

Some of the deep blood vessels may be occluded by thickening of their walls while blood vessels in the superficial part of the dermis show telangiectasia. The pilosebaceous apparatus is absent but sweat glands can still be seen.

In its severest form, complete occlusion of the blood vessels may be seen and is usually associated with sloughing of the epidermis.

THE ORAL MUCOSA

The oral cavity forms the first part of the gastrointestinal tract and is divided into two parts; the vestibule and the oral cavity proper. The vestibule is the space between the lips and cheeks on one side and the teeth and gums on the other. The oral cavity proper lies beyond the teeth and extends posteriorly to the isthmus of the fauces where it becomes continuous with the oropharynx. It is roofed by the hard and soft palates and the tongue projects from its floor.

In the oral cavity the food is moistened, chewed and tasted and the process of digestion starts. The cavity is lined by a mucous membrane which, histologically, consists of a stratified squamous epithelium resting on a layer of connective tissue known as the lamina propria below which there is another layer of connective tissue, the submucosa.

The mucous membrane shows regional variations suited to the functions of the part it lines; in parts subject to mechanical forces it resembles skin and is known as **masticatory mucosa.** On the dorsum of the tongue, the masticatory mucosa contains taste buds and is referred to as **special oral mucosa.** In areas subject to stretching and compression the mucous membrane looks like that of the oesophagus and is known as **lining mucosa.**

The cells of the stratified squamous epithelium of the oral cavity are similar to those of the skin. The keratinocytes show some structural differences in some parts, but the non-keratinocytes, i.e Langerhans cells,

Merkel cells and melanocytes which are found in all parts of the oral mucosa, are similar to those of the skin.

The rate of cell turnover is higher in the oral mucosal epithelium than in the epidermis (Squier et al., 1975) and shows a diurnal rhythm (England & Burke, 1968). It is decreased by any condition that raises the corticosteroid and adrenalin output (Squier et al., 1975). Males have a higher rate of turnover than females (Kittler & Mieler, 1969)

Masticatory mucosa:

Masticatory mucosa is present in the gingiva and hard palate. The epithelium is either ortho-keratinised (keratinocytes devoid of nuclei and organelles) or para-keratinised (pyknotic nuclei retained), stratified squamous overlying a dense connective tissue layer, the lamina propria, which in some places is firmly attached to the underlying bone. In other sites there is a submucosa which is formed of dense connective tissue that contains minor salivary glands and adipose tissue particularly in the posterior part of the hard palate.

In comparison with the epidermis of thin skin, the epithelium of the masticatory mucosa is, generally, thicker and the rete ridges are longer and more frequent. It is divided into the same four layers as the epidermis - s. basale, s. spinosum, s. granulosum, s. corneum - but the individual layers show some differences from their counterparts in the epidermis.

Stratum Basale

The stratum basale is formed of one layer of low columnar cells resting on the basement membrane and attached to it by hemidesmosomes.

Stratum spinosum

This layer is thicker than that of the epidermis with cells changing from columnar in the lower part to flattened in its upper part. The cells have prominent desmosomes with bundles of tonofilaments inserted into them. The membrane-coating granules (Odland bodies) are fewer than in the epidermis and they appear in the cytoplasm at a more basal level.

Stratum granulosum

In this layer two types of keratohyalin granules are seen in the cells; one is similar to those of the epidermis which usually blend with tonofilaments, the other type is a dense spherical granule surrounded by ribosomes and not associated with tonofilaments.

Stratum corneum

The cells of this layer have a higher lipid content than those of the epidermis. It is postulated that the lipids may play a role in preventing these cells from swelling in their wet enviroment (Farbman, 1988). The filamentous pattern of the stratum corneum is more heterogeneous than in the epidermis.

The scanning electron microscope has shown the surface of the most superficial cells of the masticatory mucosa to

be characterised by a pitted appearance or short microvilli (Cleaton-Jones, 1975; Matravers & Tyldesley, 1977).

Lining mucosa: (Fig. 10)

This mucosal type is found on the soft palate, ventral surface of the tongue, floor of the mouth, lips and cheeks. It is adapted to local functional requirements and unlike masticatory mucosa, does not normally keratinise. The thickness of lining oral mucosa is variable from thick over the cheeks to thin in the floor of the mouth. In some areas e.g. the floor of the mouth, the lining mucosa is permeable to certain substances and provides a rapid route for administration of some drugs e.g. glyceryl trinitrate.

The lining mucosal epithelium is divided into three layers: stratum basale, stratum filamentosum and stratum distendum.

Stratum basale (Fig. 11 & 56)

The cells in this layer are cuboidal or ovoid with their long axis perpendicular to the surface. The nuclei are usually round or oval and the cytoplasm contains similar organelles but fewer tonofilaments than the basal cells of keratinised epithelium (Zelickson & Hartmann, 1962; Hashimoto et al., 1966) while the intercellular spaces are wider with numerous plasmalemmal folds projecting into them. These plasmalemmal projections are different from microvilli and are called **microplicae** (Andrews, 1976). The cells are attached to the basement

membrane by hemidesmosomes and to adjacent cells by desmosomes.

Stratum filamentosum (Fig. 11 & 57)

As cells move to this layer they increase greatly in size. The cytoplasmic content of tonofilaments also increases giving this layer its name. These tonofilaments are usually free and do not form bundles (Farbman, 1988). Glycogen deposition in the cytoplasm increases as the cells move upwards (Squier et al., 1975). Membrane-coating granules appear in groups; they are circular in profile and have a dense central core (Grubb et al., 1968). There are fewer desmosomes than in keratinised epithelium. The intercellular spaces are irregular and contain abundant cement substance (Meyer & Gerson, 1964).

Stratum distendum (Fig. 11 & 58)

The cell organelles in this layer become fewer than in the underlying layer, and the nucleus becomes pyknotic. Keratohyalin granules are an inconstant finding in this layer (Silverman, 1967; Farbman, 1988).

The surface of the most superficial cells of the lining oral mucosa has been shown by scanning electron microscopy to be characterised by plasmalemmal folds called microplicae (Fig. 12). These microplicae are arranged in a parallel pattern along the length of the cell surface, but tend to branch and form a maze appearance in some areas (Matravers & Tyldesely, 1977). In certain parts of the lining oral mucosa e.g. floor of the mouth, the cell

surface presents microvilli instead of the microplicae. The width and density of the microplicae is variable between different cells and even in the same cell (Kullaa-Mikkonen, 1986). Deeper cells had also been shown to have microplicae on their surfaces (Cleaton-Jones, 1975).

The lamina propria is thick in the soft palate and thin in other parts of the lining oral mucosa with numerous short papillae. It is highly vascular and contains collagen and elastic fibres. The elastic fibres are particularly numerous in the cheeks and lips. They allow stretching of the tissues when opening the mouth and prevent the cheek from being caught between the teeth during mastication.

The submucosa is poorly developed in the ventral surface of the tongue, but in other parts of the lining mucosa it is formed of loose connective tissue firmly attached to the underlying structure. The lamina propria and the submucosa contain minor salivary glands of mucous, serous and mixed types. Sebaceous glands may be seen in the labial and buccal mucosae as small white areas called Fordyce spots.

Special oral mucosa:

On the dorsum of the tongue the mucosa is characterised by thick keratinised epithelium similar to that of the masticatory mucosa but containing taste buds in some of its papillae.

The dense lamina propria has long papillae, is highly vascular and richly innervated, especially in the region of the taste buds. It contains special minor, mostly serous,

salivary glands known as Von Ebner glands. The mucosa is bound to the epimysium of the muscles of the tongue.

The mucosae of the vermilion and the transitional zone of the lip were classified by Squier et al. (1975) as specialised mucosae as they are neither truly masticatory nor lining mucosae.

Immunohistochemistry of epithelium:

In the cytoplasm of vertebrate cells three types of filaments can be identified: actin which forms microfilaments (6 - 8 nm), tubulin forming microtubules (25 nm on the average) and the intermediate filaments (IF). The intermediate filaments consists of a group of immunologically divergent, cytoskeletal elements which are present in most vertebrate cells. Five groups of these filaments have been identified on the basis of their biochemical and immunological features: (1) cytokeratins in epithelial cells, (2) neurofilaments in most neurons, (3) glial fibrillary acidic proteins (GFA) in astrocytes, (4) desmin in muscles and, (5) vimentin in mesenchymal cells. Identification of the type of intermediate filaments present in a particular cell type gives a clue about the origin of the cell (Osborn, 1984). The functions of these filaments are not fully understood, but at least they maintain the cell shape. Because cytokeratins are specific to epithelial tissues, they will be considered in some detail.

Cytokeratins:

Cytokeratins are a group of water insoluble fibrous polypeptides with a molecular weight between 40 - 68 Kd and which form 10 nm tonofilaments (Baden et al., 1976; Inoue et al., 1976; Fuchs & Green, 1978). They are a feature of epithelial cells, being found throughout the cytoplasm, with many converging on desmosomes.

Cytokeratins differ from other intermediate filaments in their protein composition and in being formed by several different keratins rather than by one protein type (Lazarides, 1982).

There are 19 varieties of cytokeratins, divided into two "families": the more acidic "type I" and the relatively basic "type II". Each of these two families consists of a number of different cytokeratins; type I consists of 11 keratins (pH 4.5 - 5.3 and molecular weight from 40 - 57 Kd) and type II consists of 8 keratins (pH 5.5 - 7.5 and molecular weight from 52 - 67 Kd). At least one type I cytokeratin and one type II is found in every epithelial cell. Some of these keratins have the same molecular weight but they differ in the iso-electric pH. Moll et al. (1982) gave these keratins numbers according to their molecular weight and their iso-electric pH; number 1 has the highest molecular weight while number 19 has the lowest molecular weight.

Under normal conditions the composition of epithelial keratin composition varies depending on cell type (Doran et al., 1980), period of embryonic development (Banks-Schlegel & Green, 1982), stage of histological differentiation (Virdich & Sun, 1980; Woodcock-Mitchell et al., 1982), and cellular growth environment in tissue culture (Doran et al., 1980). In general, simple epithelia are characterised by few cytokeratins, stratified epithelia have many cytokeratins, and stratified keratinised epithelia e.g. epidermis, have even more.

Cytokeratins of the epidermis: (Table 1)

In the last few years several methods have been used to identify the cytokeratin content of the epidermis during different stages of development. More than 10 cytokeratins have been found in the epidermis and other keratinising epithelia, reflecting the complexity of the tissue. The type of cytokeratin present shows regional variations and, at a given site, it depends on the state of differentiation of the cell.

Relatively large and slightly basic cytokeratins (1 & 2) were found in the suprabasal layers of the epidermis from various body sites and were considered as markers for keratinisation (Baden & Lee, 1978; Franke et al., 1981; Sun et al., 1983). Cytokeratin 5 occurs in the basal layers of the epidermis and hair follicles, both of which contain cytokeratin 6 but to a lesser extent. The amount of the latter is increased in hyper-proliferative and inflammatory conditions and in cultured keratinocytes. The foot sole epidermis is particularly rich in cytokeratin 9 (Fuchs & Green, 1980), whereas cytokeratins 10 & 11 have a wider distribution in the suprabasal layers of the epidermis of most body regions and are also considered as markers for keratinisation. The relatively small and acidic cytokeratins 14, 15, 16, and 17 can be also found in the basal and suprabasal layers of the epidermis and hair follicles (Sun & Green, 1978; Fuchs & Green, 1978, 1980; Moll et al., 1982; Sun et al., 1983).

Using immunohistochemical staining for cytokeratins, Van Muijen et al. (1987) studied the distribution of different cytokeratins in fetal, new born, and adult epidermis and cultured keratinocytes. They found that cytokeratin 4 is present in the intermediate epidermal layer and the superficial layer (periderm) at 15 weeks and becomes limited to the periderm at 20 weeks before it disappears totally from the epidermis. Cytokeratin 8 was found in the basal layer and periderm at 15 and 20 weeks but not after that. The intermediate layers of the embryonic skin were positive for cytokeratin 10 by 15 and 20 weeks after birth and remained positive into adult life in the suprabasal epidermal layers. Cytokeratin 13 was positive in the intermediate layers and periderm at 15 and 20 weeks only. Cytokeratin 18 was present in the periderm up to 20 weeks. Cytokeratin 19 was present in all the layers at 15 weeks but becomes restricted to the periderm at 20 weeks before it disappears.

Cytokeratins of the oral mucosa:

As described earlier, the oral mucosa is histologically divided into keratinising masticatory and special oral mucosae and non-keratinising lining mucosa.

Similar to the epidermis, the masticatory mucosa expresses a great complexity of cytokeratins which include significant amounts of cytokeratins 1, 2, 3, 5, 6, 10, 11, 14, 16, and 17, as well as small amounts of cytokeratins 4 and 15 (Ouhayoun et al., 1985). This keratinised mucosa however, differs from the epidermis in its rich content of

the acidic type I cytokeratin 13 which is almost absent from the epidermis. Masticatory mucosa also has a higher content of cytokeratins 6 and 16 than the epidermis. The presence of cytokeratin 19 is debatable; Horima et al. (1987) found this cytokeratin in the entire basal layer of the gingiva, while Bosch et al. (1989) described it in a few samples of normal gingiva in relatively small areas of the basal layer.

The lining oral mucosa shows a simple pattern; it contains only two abundant cytokeratins, 4 and 13, in the basal and suprabasal layers, and small amounts of cytokeratins 5, 6, 14, and 17 (Moll et al., 1982; Ouhayoun et al., 1985; Cooper et al., 1985). This pattern is similar to the oesophageal epithelium.

The special oral mucosa on the dorsum of the tongue expresses significant amounts of cytokeratins 4, 5, 6, 13, 14, 16, and small amounts of cytokeratins 15, 17, 19 (Franke et al., 1981; Moll et al., 1982).

From the above description, it is concluded that it is possible to differentiate between the epidermis and the oral mucosa in its different parts on the basis of their cytokeratin pattern (Table 1). It also enables the estimation of the state of maturity of the keratinocyte which may be helpful in assessing the tissues formed during wound healing.

MATERIALS AND METHODS

This project comprises two studies:

Study I, a scanning electron microscopical investigation of the surface morphology of the cells of buccal mucosa and of radial forearm flaps transplanted into the oral cavity to reconstruct defects caused by excision of tumours and

Study II, a histological and electron microscopical investigation of structural changes in forearm skin transplanted into the oral cavity in which biopsies were taken from patients, with full ethical approval.

STUDY I

Oral biopsies:

To study the surface pattern of the most superficial cells of the epithelium of the lining oral mucosa and compare it with those seen in the swabs, five biopsies from the lining oral mucosa were taken intraoperatively from patients in Group II (see page 77) undergoing surgery for oral tumours. The patients were 2 females and 3 males, their mean age was 54 years and the range was 35 - 71 years. The samples were taken by the operating surgeon away from the tumour and immersed immediately in 3% buffered glutaraldehyde (pH 7.4) and processed for scanning electron microscopy (Appendix II). The whole surface of each specimen was screened in a similar way to that described below for oral swabs.

Oral swabs:

To study the surface morphology of desquamated mucosal cells in healthy individuals, oral swabs were taken from the buccal mucosa of eight male volunteers (students and

technicians). Four of these provided a single swab while the other four were swabbed daily for 5 successive days. One of the volunteers who gave the daily samples was also swabbed weekly thereafter for 3 weeks. All volunteers were non-smokers. The mean age was 27 years and the range was 20 - 35 years.

Using a cotton wool bud, the inner aspect of the cheek was swabbed at a point 3 cms lateral to the angle of the mouth. The swab was always taken by the same investigator applying the same pressure and turning the tip of the cotton bud three times. These swabs were used to study interindividual variation in the surface pattern of mucosal epithelial cells and the changes in pattern on successive days of swabbing.

In a further experiment aiming to study the deep surface of mucosal epithelial cells, one side of an aluminium stub was covered by double-face adhesive sellotape and applied to the inner aspects of the cheek of two volunteers.

Swabs were also collected from the surface of the radial forearm skin flaps in seven patients in Group II (see page 77), one and two weeks after surgery and during the first and second weeks of radiotherapy. The patients were one female and six males, their mean age was 56 years and the range was 42 - 71 years.

All swabs and aluminium stubs were immediately fixed in 3% buffered glutaraldehyde (pH 7.4) and processed for

scanning electron microscopy (Appendix III). The swabs were viewed on the scanning electron microscope (JEOL JSM T - 300) using 30 Kv accelerating voltage and photographs were taken on Kodak 120 technical pan black and white film.

STUDY II

The patients in this study were divided into two groups:

Group I: Consisted of 40 patients who were treated for intraoral tumours before the start of this project.

Group II: Consisted of 20 patients who were treated for intraoral tumours after the start of this project.

GROUP I:

At the start of the project the radial forearm flap technique for treating oral tumours had been in use at Canniesburn Hospital for about seven years. Forty patients previously treated in this way were selected for this study on the basis of the suitability of the flap for biopsy and their willingness to participate. These were known as Group I and consisted of 25 men and 15 women. When first seen by the investigator, a biopsy was taken from the flap of all of them and from the buccal mucosa of six of them. Twenty-five patients in this group had previously received a radical course of post-operative radiotherapy in the form of 6000 cGy in 30 fractions over 6 weeks. A further three had received a similar course of radiotherapy pre-operatively and twelve patients were treated by surgery only.

The details of the patients in this group are summarised in Table 2. With two exceptions all the patients

were diagnosed histopathologically to have squamous cell carcinoma originating from the oral epithelium. **I** - 18 had an adenocarcinoma and **I** - 32 an accinic cell carcinoma. The mean age at the time of surgery was 58 years. The youngest patient was 29 years old and the eldest was aged 81 years at the time of surgery.

The post-operative time at which the biopsies were taken ranged from 3 months to 98 months: the mean was 48 months (Table 2). A total of 46 biopsies were taken from the flaps of patients in this group.

GROUP II:

Group II was composed of 20 patients who were followed from the time of diagnosis onwards. The details of these patients are summarised in Table 3. Seven patients were later excluded from the study for the reasons shown in Table 4, which will be described in the results (page 88). The mean age of the remaining 13 patients was 55 years and their ages ranged between 35 - 75 years. There were 9 males and 4 females, all of whom were diagnosed histopathologically to have squamous cell carcinoma of the oral epithelium. Nine patients from this group received a radical course of post-operative radiotherapy in the form of 6000 cGy in 30 fractions given over a period of 6 weeks. One patient with an advanced oral tumour was treated by surgery only because he had received radiotherapy a long time previously. Three patients with early tumours were treated by surgery only (Table 3). Thirty-one biopsies were taken

from the flaps of patients in this group.

Clinical examination

During the routine visit of a patient from either group to the combined clinic at Canniesburn Hospital, he/she was examined by the investigator for any change in the appearance of the flap and any possible factors that might have brought about the change. All the observations were recorded and a colour photograph of the flap was taken whenever possible using a Nikon FG 20 camera.

When this project started, Group I patients were attending the combined clinic for follow-up at variable periods ranging from 2 to 12 months depending on the time passed since they had undergone surgery.

Group II patients were examined weekly during their post-operative hospitalisation and while they were receiving the radiotherapy. Subsequently this group attended the clinic according to the schedule described earlier (page 13). On some occasions, however, patients needed closer follow-up.

Biopsy

Biopsies were taken following examination and photography of the flap. The reason for the biopsy and the procedure involved were explained to the patient and if he/she was willing, without hesitation, to participate in this study then the patient was asked to read and sign a special consent form approved by the Ethical Committee.

Local anaesthetic (Lignocaine 1%, Phoenix Pharmaceuticals Ltd.) was infiltrated at the site to be

excised. A fusiform incision was made using a No. 15 blade, the tissue (approximately 10 x 5 x 5 mm) was excised and placed on blotting paper. Haemostasis was secured and the resulting defect closed using absorbable sutures (Ethicon, 4 - 0 chromic catgut).

In Group II patients, an intraoperative biopsy from the margin of the forearm skin flap and the lining oral mucosa away from the excised tumour were taken by the operating surgeon.

All the samples were immediately fixed in 3% glutaraldehyde in Millonig's buffer (pH 7.4) and processed for further study (Appendix IV).

Light microscopy

The biopsy samples obtained from both groups of patients were processed for study by the light microscope (Appendix IV).

Semithin resin-embedded sections were cut at 1.2 μm thickness on a Reichert-Jung 1140 Autocut or a Porter-Blum Ultramicrotome MT2, using 6 mm Latta - Hartmann glass knives cut on a LKB-7801-A knife-maker. Sections were stained by haematoxylin and eosin and azur II (Appendix V). Some sections were stained by resorcin fuchsin to show elastic fibres (Appendix VI) and others by the periodic acid/Schiff method to demonstrate the glycogen content of the epithelial cells (Appendix VII).

The degree of inflammation in the dermis and lamina propria was assessed on the following scale for all

biopsies:

Grade 0 : Occasional inflammatory cells,

Grade 1 : Few scattered inflammatory cells,

Grade 2 : Small aggregates of inflammatory cells,

Grade 3 : Large aggregates of inflammatory cells,

Grade 4 : Diffuse inflammatory reaction,

Grade 5 : The connective tissue components masked by inflammatory cells.

The dermis was also examined for the distribution of elastic and collagen fibres and the presence of epithelial adnexa.

Immunohistochemistry

Monoclonal antibodies were used in an attempt to detect markers of stratification (Anti-cytokeratin peptide 13, Clone KS - 1A3, Sigma), and markers of keratinisation (AE2, ICN Biomedicals) in skin, buccal and flap epithelia. The latter antibody detects cytokeratins 1, 2, 10 and 11. For distribution of cytokeratins in the epidermis and the oral mucosa see Table 1 and for the technique see Appendix VIII.

Transmission electron microscopy

One sample from forearm skin, and another sample from buccal mucosa were examined by transmission electron microscopy to serve as controls for comparison with the flaps transplanted into the oral cavity. Both samples were obtained from the same patient at operation.

The mucosal biopsy was also used to study the surface projections of the epithelial cells in order to

corroborate the scanning electron microscopical findings on oral swabs and mucosal biopsies.

Biopsies taken from the radial forearm flaps of three different patients were studied by transmission electron microscopy: 1) one sample from an irradiated flap that looked like normal skin 4 months after surgery, 2) two samples from an irradiated flap that had acquired a mucosal appearance but returned partially to the skin appearance 4 and 12 months after surgery respectively 3) two samples from a non-irradiated flap, one at 26 months after surgery when the flap appeared like the oral mucosa and another biopsy at 36 months when it had returned partially to the skin appearance.

Following examination of a stained semithin section, an area of epithelium, usually the narrowest, was selected for the ultrastructural studies. The block was trimmed and thin (0.6 - 0.8 μm) sections were cut on a Reichert-Jung Ultracut E ultramicrotome using a diamond knife. The thin sections were stretched using chloroform vapour and mounted on copper 200 mesh grids. The sections were stained using a double-staining technique (Appendix IX) and then viewed with JEOL 100S transmission electron microscope. Photographs were taken on Kodak EM Estar thick base film 4489, processed and then montaged.

RESULTS

STUDY I

SCANNING ELECTRON MICROSCOPY:

A) **Buccal mucosal biopsies:**

Normal buccal mucosal biopsies were obtained intraoperatively from five patients for study by scanning electron microscopy. At low magnifications, the surface appeared intact. There were considerable amounts of mucus and bacteria on all samples.

On examination at higher magnification, the surface cells showed numerous microplicae which formed several patterns. Most of the surface cells in all five biopsies had parallel microplicae of approximately similar height and thickness (Fig. 12). On some cell surfaces of all biopsies, the microplicae were extensively branched and/or arranged in whorls (Fig. 13). In all the biopsies, a few cells were seen which showed many short, regularly arranged microvilli, rather than microplicae (Fig. 14). The number of microvilli, however, varied from cell to cell. It was noted that in different cells of the same sample, the spaces between the microplicae or microvilli were variable. It was not possible to gain any useful information from the cut edges of the biopsy block.

B) **Oral swabs:** (for quantification and graphs see page 168)

a) Normal buccal mucosa:

The swabs were collected from four subjects for five consecutive days and, in one of the four subjects, weekly thereafter for three weeks. Single swabs were also obtained from another four subjects.

On examination by scanning electron microscopy the swabs were found to be covered with a blanket of cells and had minimal amounts of bacteria and mucus (Fig. 15).

(1) **Swabs taken daily**

Day 1: Most of the cells on the swabs exhibited one of three surface forms in approximately equal numbers:

i) parallel microplicae (Fig. 16),

ii) branching microplicae (Fig. 17),

iii) microvilli (Fig. 18).

The spacing of the microplicae or microvilli varied between different cells and individuals.

A smaller number of cells showed branching and anastomosing microplicae in a maze pattern (Fig. 19). In two samples, cells with microvilli wholly or partially surrounded by microplicae were occasionally seen (Fig. 20). Sometimes, both surfaces of the squamous cells were visible: in most, one of the surfaces showed microvilli while the other had a maze pattern (Fig. 21).

Day 2: In all samples, the number of cells bearing microvilli on their surfaces was markedly increased. In addition, a few cells showed a new pattern in which irregular microplicae were interrupted by microvilli (Fig. 22). Compared with Day 1, the number of cells with parallel or branching microplicae was reduced while that showing the maze pattern increased.

Where two or more cells met, the boundary was marked by either a narrow smooth zone (Fig. 23) or a raised

intercellular ridge (Fig. 12). Adjacent, adherent cells sometimes showed different surface patterns (Fig. 23 & 24).

Day 3: The commonest pattern on this day in every sample was the maze appearance (Fig. 19). Only a few cells with parallel or branching microplicae were seen in any of the four samples. Cells with microvilli surrounded by microplicae were more common than before. In two samples the relative number of cells with microvilli only was similar to Day 2, but in the other two they were rare.

Day 4: The maze appearance remained the commonest pattern. Cells showing microvilli only were seen in all individuals including those in whom they had become rare on Day 3. Other common patterns were microplicae arranged in whorls, microplicae wholly or partially surrounding microvilli and microvilli interrupting microplicae.

In two samples microridges were seen, these were taller than microplicae and had a sharper free edge (Fig. 23).

Day 5 : In all individuals the patterns were similar to those seen in Day 4. Microridges were seen in the same individuals as at Day 4 but they were now more numerous. These microridges remained absent, however, in the other two individuals. It was noted that in many cells the microvilli and microplicae were thicker and much more closely packed than before (Fig. 25 - 28).

(2) **Single and weekly swabs**

The single swabs showed patterns similar to those described above in Day 1. In the subject who provided

samples one and two weeks after five days consecutive swabbing, the surface patterns had returned to the Day 1 appearance.

(3) **Cells obtained on aluminium stubs**

Considerable numbers of cells were adherent to the sellotape in both samples. The cells lay singly or in small groups.

The patterns seen in the two samples were:

a) microvilli of variable length,

b) microplicae arranged in a maze pattern,

c) microvilli surrounded by microplicae.

The former two patterns were seen in almost equal proportion while the last form was less frequent. Parallel microplicae were not seen in any of the two samples.

It was noted that adherent cells had different patterns and that they met at raised intercellular junctions.

C) **Radial forearm flap:**

Four swabs were taken from each of seven patients one and two weeks after surgery and during the first and second weeks of radiotherapy. These swabs revealed nothing but variable amounts of mucus, bacteria and debris (Fig. 29). In some cases, because of the position of the flap, touching the adjacent mucosa with the swab was unavoidable and in such instances cells exhibiting various patterns of microplicae were seen.

STUDY II

CHANGES IN FOREARM FLAPS FOLLOWING TRANSPLANTATION TO ORAL CAVITY:

Clinical Observations:

Group I:

The forty patients in this group had received treatment up to eight years before the start of the project. When the study commenced, twenty-eight of these patients showed flaps whose surfaces appeared like normal skin (Fig. 30). The remaining twelve showed flaps with an appearance resembling adjacent buccal mucosa in whole or in part; in some the change was more extensive than in others (Fig. 31 & 35). Eighteen of the unchanged and seven of the changed flaps had received postoperative radiotherapy, the remaining fifteen patients were treated by surgery only.

	SURGERY ONLY	SURGERY+RADIOTHERAPY	TOTAL
UNCHANGED	10	18	28
CHANGED	5	7	12
TOTAL	15	25	40

The unchanged flaps looked like normal skin in both colour and texture and retained this appearance throughout the 30 months of the study. In only one patient did a change in the appearance occur during this period. This patient (**I - 20**) had been treated by surgery only in June, 1982; the flap remained unchanged until April, 1990 when its colour became similar to that of the oral mucosa (Fig. 32 & 33).

Hairs could still be seen in some of the unchanged non-irradiated flaps (Fig. 34). They were also seen in the unchanged part of some of the partially changed non-irradiated flaps (Fig. 35); they were not seen in any part of the irradiated flaps.

Seven of the twelve changed flaps showed only partial changes. Of those flaps which were partially changed, some showed the appearance of oral mucosa towards the periphery of the flap while others showed it in more central regions. In three patients (**I** - 2, **I** - 26, **I** - 29), two non-irradiated and one irradiated, parts of the flap acquired a silver-white colour during the study (Fig. 37). During the course of the study it was noted that, of the changed flaps, three irradiated and two non-irradiated flaps showed a partial return to the appearance of normal skin. The newly keratinised areas formed anywhere on the flap (Fig. 36).

When the flap was completely changed to the colour of buccal mucosa it was sometimes difficult to distinguish its boundaries. By using good lighting, however, the edges of the flap could be identified as a fine linear scar (Fig. 31). In a number of changed flaps, however, the site could be recognised because the surface had uneven contours resulting from the operation (Fig. 38).

Candidiasis was occasionally diagnosed and promptly treated (Fig. 39 & 40). On resolution of the infection, the flap returned to its appearance before infection.

Group II:

Group II consisted of 20 patients whose treatment for oral tumours commenced after the start of the project. Seven of these patients, however, were later excluded from the study (Table 4): one would not attend for follow-up, two showed necrosis of the flap and in the other it was not possible to take a biopsy either because the flap was inaccessible or because the patient had severe and prolonged trismus.

During the patients' stay in hospital immediately following surgery, the flaps were inspected frequently. It was sometimes difficult or impossible to inspect flaps in the retromolar area because of their position and the tenderness and swelling in the mouth. The surfaces of those flaps which could be inspected did not show any changes in the post-operative period in which the patients were hospitalised (Fig. 41).

It was only possible to take biopsies from four patients before their discharge.

Of the 13 patients followed from the start of treatment, 4 had Stage I tumours and received surgery only, the others had more advanced disease and received radiotherapy as well as surgery.

<u>The appearance of the flap in patients treated by surgery only.</u>

The flaps of the four patients treated by surgery only retained the appearance of skin throughout the study (Fig. 42).

The appearance of the flap in patients receiving post-operative radiotherapy.

In the days following radiotherapy, the irradiated skin of the neck and lower part of the face became red and more sensitive, the acute reaction. The onset, severity and the duration of this reaction varied from patient to patient. To minimise these effects the patients were advised not to expose the irradiated areas to strong sun or to apply soap. The transplanted flap behaved in a similar way; it became pink in colour and remained so after the skin reaction subsided. One flap regained normal skin colour in less than 8 weeks but others were still pink at the end of the study more than 16 months later.

During treatment by radiotherapy, some patients developed oral ulcers and/or infection. The patients were advised not to smoke and to avoid spicy foods, citrus fruits and spirits. Frequent mouth washes with sodium bicarbonate were recommended to ensure good oral hygiene.

The appearance of the flap following radiotherapy.

I) **First visit**

Six to eight weeks after the completion of the course of radiotherapy, when the patients returned to the combined clinic, the brisk reaction was still obvious in the flap of all but one of the nine individuals. Biopsies were taken from the patient in whom the brisk reaction had subsided and from another four individuals whose flaps were pinker in colour, resembling oral mucosa. In the remaining four,

however, the reaction was of sufficient severity to make biopsy unethical. As in Group I, all irradiated flaps were devoid of hair.

In the four patients from whom a biopsy was not taken, the flaps and the surrounding oral mucosa were red and inflamed. Two of these patients (**II - 7** & **II - 19**) had some degree of trismus while the other two (**II - 12** & **II - 13**) had Candida infections which were treated by Diflucan (Pfizer). One of these patients (**II - 13**) had oedema of the tongue, attributable to radiotherapy; this made speech and swallowing very difficult; the oedema settled later and a biopsy was taken.

II) **Subsequent visits**

The flap which showed early recovery from radiotherapy and appeared as normal skin at the first visit continued to have this appearance throughout the study.

In subsequent visits following radiotherapy, those flaps which had initially been pink and resembled buccal mucosa developed, in whole or in part, a silver-white surface (Fig. 37). This appearance also developed in the patients who had had trismus and fungal infection, once these problems settled, but was not seen in any of the non-irradiated patients in Group II. In some patients this had formed by the second visit and in others it took longer; in two cases this change progressed and the flaps again acquired the appearance of skin.

Four of the remaining 13 patients in Group II could not be followed until the end of the period of the project.

After 16 months of follow-up, patient **II** - 1 who had been treated by surgery and radiotherapy, developed a recurrence of tumour and underwent further surgery. Patient **II** - 9 had fat surgically excised from the flap after 12 months of follow-up. Patients **II** - 15 and **II** - 16 died eight and fourteen months after surgery respectively.

The appearance of the flaps of both Group I and II patients at the conclusion of the study.

The table below shows the number of patients with changed and unchanged flap at the conclusion of the study. As described above, the flap of three patients in both groups I and II returned to the appearance of skin during the study; two had received radiotherapy and the other had received surgery only.

Appearance of flaps in patients in both Groups I and II at the conclusion of the study

	SURGERY ONLY	SURGERY+RADIOTHERAPY	TOTAL
UNCHANGED	13	22	35
CHANGED	6	12	18
TOTAL	19	34	53

Those registered as changed included those showing partial recovery. Statistical analysis by Chi-Squared test showed no significant difference in the number of changed flaps following radiotherapy compared with surgery only. fewer patients showed changes at the conclusion of the study than had shown them earlier since 3 irradiated flaps

returned to the skin appearance. Taking these into consideration, there was still no statistically significant association between radiotherapy and change of the flap to mucosal form.

Histology:

(1) **Normal forearm skin** (Fig. 7)

Thirteen forearm skin biopsies from Group II patients obtained intraoperatively were examined by light microscopy. These samples showed the general appearance of thin skin described earlier (see page 24). In the epidermis, no mitotic figures could be seen in the stratum basale of any sample, the stratum spinosum was 3 - 5 layers thick and glycogen was not present in any stainable amounts, the stratum granulosum was formed of 2 - 3 layers and the stratum corneum formed a thin layer.

In the dermis, occasional inflammatory cells, mainly lymphocytes, were seen (Grade 0). Hair follicles, sweat glands and their ducts were seen in all samples but sebaceous glands were seen in three samples only. In the reticular dermis collagen bundles were irregular and closely packed while in the papillary dermis they were less dense. In two samples skeletal muscle fibres were seen in the deep part of the dermis.

(2) **Normal buccal mucosa** (Fig. 10)

Nineteen mucosal biopsies from both groups were examined by light microscopy. One sample was from masticatory mucosa the histological features of which were similar to those described earlier (see page 63). The remaining eighteen samples were from the lining buccal mucosa.

The epithelium of the lining oral mucosa was thicker than that of the skin, the middle and superficial layers

had high glycogen content, and the cells of the most superficial layer retained pyknotic nuclei (parakeratosis). The stratum granulosum was not seen in any sample.

In the lamina propria, a few inflammatory cells, mainly lymphocytes and neutrophils, were seen in some samples (Grade 0 - 1). The collagen and elastic fibres were irregularly arranged and less closely packed than in the dermis of forearm skin. In some samples the deep part showed accessory salivary glands.

(3) **Intraoral radial forearm flaps**

In total 77 biopsies were obtained from the flaps of patients in Groups I and II. On the basis of their gross appearance the flaps could easily be classified as changed or unchanged. The histological appearance of the following groups of flaps will be described separately:

A) In post-operative period and before radiotherapy

B) Unchanged; irradiated and non-irradiated

C) Changed; irradiated and non-irradiated

D) Showing partial recovery; irradiated and non-irradiated. For data on epithelial thickness see page 184.

A) <u>Samples obtained in the post-operative period and before radiotherapy.</u>

Four biopsies were taken from different patients: one at 18 days, one at 19 days and two at 20 days following surgery and before referral for radiotherapy. At that time all flaps looked like normal skin, and a few absorbable sutures were still seen at their margin.

Histologically (Fig. 43), the four samples showed increased epithelial thickness which was marked in the two samples at 20 days and moderate in the other two samples.

Epidermis:

Stratum basale: Few mitotic figures were seen in the stratum basale of any sample, the cells were widely separated.

Stratum spinosum: This layer was increased in thickness relative to that of normal skin. The "spines" of the cells in the deep part of this layer were prominent, and the intercellular spaces appeared widened. The nuclei of some cells showed 2 - 3 nucleoli. In the superficial layers the cells were more closely packed and had higher glycogen content than those in the deep layers.

Stratum granulosum: This layer was markedly increased in thickness in the samples taken at 20 days after operation, reaching 11 cells in thickness in some parts. In the sample taken at 19 days, it was diminished or even absent in some parts. In the sample taken at 18 days it appeared similar to the forearm skin of the same patient.

Stratum corneum: In the three samples taken at 19 and 20 days after surgery the stratum corneum was increased in thickness. In one of the samples taken at 20 days keratohyalin granules and ghosts of nuclei were seen in the horny cells. In the sample taken at 18 days, however, the stratum corneum was normal.

Dermis:

The epidermal-dermal interface was highly undulating in all samples with deep rete ridges and high dermal papillae.

The papillary dermis of all samples showed a mild degree of inflammatory reaction (Grade 1), inflammatory cells were mainly lymphocytes with a few plasma cells. In the deep part of the reticular dermis, the capillaries appeared dilated and were surrounded by a few inflammatory cells.

Hair follicles and sweat glands were always present but sebaceous glands were not seen in any sample.

B) Unchanged flaps. (Fig. 44)

All unchanged flaps were found to have a similar histological structure. A total of 36 biopsies, from both Group I and II, were taken from these flaps; a biopsy from each of 18 irradiated patients and another 18 biopsies from 15 non-irradiated patients.

Epidermis:

Stratum basale: Apart from occasional mitotic figures in some of the flaps that had shown increased epithelial thickness, no abnormality was detected in this layer.

Stratum spinosum: Compared with normal thin skin, this layer showed a slight increase in thickness in some samples in which glycogen was present in stainable amounts in its upper part.

Stratum granulosum: This layer was present in all samples and resembled that of thin skin in being 2 - 3 layers thick.

Stratum corneum: Mild hyperkeratosis was noted in a few samples.

Dermis:

The dermal-epidermal interface was normal except in 4 previously irradiated flaps (Patients **I** - 5, **I** - 31, **I** - 35, **I** - 38) in which dermal papillae and rete ridges were flattened.

Dermal fibres: In the dermis of some of the irradiated flaps collagen bundles were less closely packed and elastic fibres were increased in number, thickened, curved and randomly arranged compared with the dermis of normal forearm skin.

Dermal cells: Fibroblasts were characteristically enlarged in the irradiated flaps. All the flaps showed mild or no inflammatory reaction in the dermis (Grade 0 - 2). In the reticular dermis, inflammatory cells, when seen, were mostly in perivascular positions. They were mainly lymphocytes.

Dermal vessels: Blood and lymphatic capillaries were dilated in all samples and endothelial swelling was seen in the blood capillaries of the irradiated samples.

Epidermal adnexa: Hair follicles were absent in the irradiated category but they were seen in biopsies from 7 of the 15 non-irradiated patients. Sweat glands and/ or ducts were seen in all biopsies from the non-irradiated flaps but were seen in only 12 biopsies from the 18 irradiated flaps; because of difficulties in access, the biopsies from some of

the irradiated flaps may not have been deep enough to show sweat glands. Sebaceous glands were not seen in any sample.

C) Changed flaps.

Twenty patients out of 53 from both Groups I and II showed changes in their flaps; fifteen of these patients received post-operative radiotherapy and five were treated by surgery only. One biopsy was taken from each flap.

Epidermis:

In biopsies of changed flaps, the stratified squamous epithelium was markedly increased in thickness compared with that of normal thin skin, had deeper rete ridges and a less distinct stratum corneum (Fig. 45 & 46). A few lymphocytes and neutrophils had infiltrated the epithelium.

Stratum basale: In the stratum basale, mitotic figures were more numerous than in normal thin epidermis and in the unchanged flaps, about 2 - 4 were observed per high-power field. The basal keratinocytes were widely separated.

Stratum spinosum: The stratum spinosum was markedly increased in thickness in all flaps. Occasional mitotic figures were seen in the immediate suprabasal part of this layer. In the deep part adjacent cells made contact at prominent spines projecting into wide intercellular spaces (spongiosis). The cells of the more superficial layers of the stratum spinosum were more closely packed than the deeper ones, to the extent that the spaces between them were almost absent. These cells were enlarged and had a higher glycogen content than the cells in the lower layers. Vesicles were noted in the upper part of the stratum

spinosum in the flaps that had been recently irradiated (Fig. 46 & 47).

Stratum granulosum: The stratum granulosum was variable, absent in some parts and diminished in thickness in others.

Stratum corneum: Parakeratosis was a prominent feature of these flaps and a few nucleated cells were seen apparently separated from the surface. In some flaps, both irradiated and non-irradiated, thin flakes of anucleate cornified cells were seen in some parts. Vesicles were also noted in this layer in the recently irradiated flaps.

Dermis:

The dermal-epidermal junction of some of these flaps showed a highly undulating interface with high connective tissue papillae. The basement membrane appeared intact in sections stained with PAS technique.

Dermal fibres: Collagen and elastic fibres were normal in non-irradiated flaps but in some irradiated flaps elastic fibres were abundant and randomly distributed, thickened and curved (Fig. 48).

Dermal cells: Variable grades of inflammatory reaction were seen in the dermis (Grade 2 - 5), which in some flaps was so severe that it masked the dermal constituents (Fig. 45). The inflammatory cells were mainly plasma cells and lymphocytes (Fig. 49); many were in perivascular positions. In the deeper parts of the dermis, occasional mast cells were seen. In three samples, two recently irradiated and one

non-irradiated, large amounts of extravasated red blood cells were seen in the reticular dermis. The deep part of the reticular dermis of the irradiated flaps appeared more cellular than the non-irradiated ones. Giant fibroblasts were seen in the irradiated samples (Fig. 50).

Dermal vessels: Blood and lymphatic capillaries appeared dilated in all flaps. The endothelial cells of some blood capillaries were swollen.

Epithelial adnexa: The pilosebaceous apparatus was not seen in the irradiated flaps but hair follicles were present in two of the non-irradiated flaps. Sweat glands and or their ducts persisted in all non-irradiated flaps, but they were seen in only nine irradiated flaps; in four of them they showed degenerative changes (Fig. 51).

D) Flaps that showed partial recovery.

During the routine follow-up of the patients, a number of flaps which had changed in whole or in part to the appearance of oral mucosa showed subsequent changes which indicated a return to the appearance of skin. Some of these flaps showed diminution in the changed areas while others showed a silver-white appearance.

i) Flaps which showed reduction in size of changed area

Four biopsies were taken from flaps that had shown partial recovery, since a previous biopsy, at the same site. Three of these biopsies were taken from irradiated patients and one from a non-irradiated patient. Biopsy material came from the changed areas of these flaps.

The histology was generally similar to that described

for changed flaps; there were, however, some differences:

1) Although still increased in thickness compared with thin skin, the epithelium was thinner than in the previous biopsies (Fig. 52).

2) In the stratum basale, mitotic figures appeared fewer than in the earlier biopsies.

3) In the earlier biopsies of two of these flaps the stratum granulosum had been absent but was present in the later biopsies.

4) In some of the later biopsies the stratum corneum had become well-formed (Fig. 53).

5) Dermal inflammation was reduced in the biopsies of three of the four flaps. Two of the irradiated ones showed Grade 4 at the first biopsy but Grade 3 at the second. The non-irradiated flap had originally shown Grade 5 reaction but the later biopsy showed Grade 4.

ii) Flaps that showed a silver-white appearance

Thirteen biopsies were taken from the flaps which had acquired a silver-white appearance: eleven were from irradiated flaps and two were from non-irradiated ones. The two non-irradiated and one irradiated specimens were collected from Group I patients, the other ten irradiated specimens came from Group II patients two of whom had had repeated biopsies. An earlier biopsy, before the silver-white colour developed, had been taken from some of these patients allowing comparison of the flap histology before and after the development of the silver-white appearance.

The histology of the flap showing the silver-white appearance was generally similar to that described earlier for the changed flaps. Some differences were observed, however:

1) The epithelium was increased in thickness compared with thin skin but for those flaps from which a previous sample was available, it had become thinner since the earlier biopsy.

2) The stratum corneum remained parakeratotic, but it was noted that many parakeratotic cells were seen loosely attached to the surface, either as single cells or in clusters (Fig. 54). The silver-white appearance was attributed to these features which resembles the lesions of psoriasis.

3) In the six individuals with previous biopsies, mitotic figures appeared less frequent in the stratum basale of the biopsies with the silver -white appearance.

4) In the six flaps previously biopsied, the later biopsies of five showed reduction of dermal inflammatory reaction.

While the histology of the flaps showing a reduction of the changed areas and the flaps with a silver-white appearance both suggested a return towards the morphology of thin skin, nothing was observed histologically to explain why some flaps developed the silver-white appearance while others did not.

Immunohistochemistry:

Material previously processed for semithin sections was used in this part of the study.

The two antibodies, AE2 and anticytokeratin 13, were used for immunohistochemistry on normal thin skin, normal buccal mucosa and on biopsies of flaps. Despite many attempts using reagents at different concentrations the staining obtained was non-specific and inconsistent. This was attributed to the material having been fixed in glutaraldehyde and processing for resin histology.

The technique was modified in attempt to achieve the proper reaction. Sodium borohydride ($NaBH_4$)1% was used to reduce the glutaraldehyde (Kosaka, 1986); the background staining was diminished but the epithelial reaction was not improved. Deosmication with 1% periodic acid instead of 1% sodium metaperiodate was also without effect.

Transmission Electron Microscopy:

The epithelium of one sample from normal forearm skin (Fig. 55) and another sample from normal lining buccal mucosa (Fig. 56 - 58) were examined by transmission electron microscopy. These samples showed the typical appearances described earlier in the introduction. In addition, the surface of the buccal epithelial cells was characterised by projections which were short on the superficial layers but became branching and more complicated in deeper layers. The projections from neighbouring cells were joined by desmosome-like junctions. These projections are the microplicae, microvilli and microridges of the scanning electron microscopy study.

Five flap samples were selected from three different patients for ultrastructural study of the epithelium; one sample from a flap which was irradiated and had not changed (Patient **II** - 3), two samples from a flap which was irradiated (Patient **II** - 1) and had changed completely at the time of the first biopsy but returned partially to the skin appearance at the time of the second biopsy, and another two samples were taken from a non-irradiated flap (Patient **I** - 1) which had changed partially at the time of the first biopsy but whose changed area had become smaller when the second biopsy was taken. The two second biopsies both came from areas with the appearance of mucosa.

(1) **Unchanged irradiated flap**

A single biopsy was taken from patient **II** - 3, three

months after she had finished radiotherapy (6 months after surgery). On gross examination the flap had the appearance of skin. On electron microscopical examination, the epithelium could not be distinguished in any way from the epidermis of normal thin skin (Fig. 59).

(2) **Changed flaps**

Two samples were examined by transmission electron microscopy; one from irradiated (Patient **II** - 1)(Fig. 60 & 61) and the other from non-irradiated (Patient **I** - 1) (Fig. 62 & 63) changed flaps. The samples were taken 4 and 26 months after surgery respectively. The epithelium of both samples showed almost the same appearance and they will be described together.

The basement membrane appeared intact in the area examined. Basal keratinocytes were separated by a wide intercellular space and their cytoplasm showed bundles of tonofilaments inserted into desmosomes and hemidesmosomes. Lymphocytes and neutrophils were occasionally seen in the intercellular spaces.

In the deeper part of the stratum spinosum, cells were separated by intercellular spaces that appeared narrower than those in the basal layer. Tonofilaments formed smaller bundles than those in the basal keratinocytes and normal thin skin. In both samples melanosomes appeared as small oval or rounded strongly electron-dense bodies in some cells. A few glycogen granules were seen in this layer. The nuclei of some cells had 2 - 3 nucleoli.

In the upper part of the stratum spinosum cells were

larger and more closely packed than in deeper layers to the extent that the intercellular space was almost absent. Tonofilaments were diffuse in the cytoplasm and no bundles could be seen in any of the cells at this level. In both irradiated and non-irradiated flaps, glycogen granules appeared as fine electron dense granules, which were more numerous in the non-irradiated sample.

In the most superficial part of the stratum spinosum a few vacuoles appeared in the cytoplasm of some cells and the nuclei were partially surrounded by a space.

The stratum granulosum was absent from the non-irradiated specimen in the area examined but was present in the irradiated one.

The cells of the stratum corneum retained pyknotic nuclei (parakeratosis). Large numbers of electron-opaque membrane-limited structures of variable size were seen; these were probably fat droplets. Some of these structures appeared empty.

A few desquamated parakeratotic cells were also seen in both specimens.

(3) **Flaps that showed partial recovery**

Two samples were taken from the same above patients when their flaps showed partial return to the skin appearance, 14 months after surgery in the irradiated patient (Fig. 64) and 36 months after surgery in the non-irradiated patient (Fig. 65).

The epithelium of both samples was thinner than the

previous biopsies but showed basically the same structure with the following differences:

1) Tonofilaments formed small bundles in the cells of the stratum spinosum. Some of these bundles were inserted into desmosomes while others were scattered in the cytoplasm of the cell. In the previous biopsies no bundles were seen in the cytoplasm of the upper spinous layers.

2) Keratohyalin granules appeared in the region of stratum granulosum of the non-irradiated flap but they were fewer than in normal skin.

3) Parakeratosis was less evident in both flaps and a well-formed stratum corneum was seen in both samples, although the horny cells contained large amounts of fat droplets.

DISCUSSION

STUDY I - SCANNING ELECTRON MICROSCOPY:

Microplicae and microvilli are well-documented surface features of stratified squamous non-keratinised epithelia. The variation in the appearance of these surface features in normal and pathological conditions has been the subject of many investigations (Cleaton-Jones, 1975; Andrews, 1976; Matravers & Tyldesley, 1977; Robinson et al., 1981; Dourov, 1984; Robertson et al., 1987).

In the present study the following patterns were seen in biopsies and swabs from the buccal mucosa: parallel and branching microplicae, microvilli, microplicae in a maze pattern, microvilli partially or completely surrounded by microplicae, microridges twisted into whorls and microvilli interspersed between microplicae. The frequency of their appearance was variable in different individuals and on different days of swabbing.

All the above patterns were reported by Robertson et al. (1987) in patients receiving radiotherapy but the appearance of each pattern and its frequency, in a general way, was claimed to depend on the dose of the treatment. These workers suggested that the changes might be useful in monitoring the dosage of radiotherapy. However, they were uncertain as to whether radiotherapy had altered the cellular structure or whether some of the cell surface patterns described may have belonged to deeper cells exposed as a result of the treatment.

One of the aims of this project was to document the different surface patterns of normal buccal epithelial

cells and to study their variation within and between healthy individuals.

Repeated swabbing at a defined site allowed examination of the surface features of more deeply placed cells. Having documented the normal surface features of the epithelial cells, it might then be possible to identify changes in the surface pattern specifically associated with radiotherapy.

The surface appearances of the most superficial cells as seen in buccal mucosal biopsies were characterised by parallel microplicae that tended to branch and form whorls in some areas; a few cells showed only microvilli. These appearances confirm the reports of other workers (Matravers & Tyldesley, 1977; Bancozy et al., 1980; Kullaa-Mikkonen, 1986). Matravers and Tyldesley (1977) described the surface of normal non-keratinising oral epithelium of the floor of the mouth to be characterised by a fine granular pattern with a tendency to form parallel microplicae, while in the buccal mucosa the surfaces of the cells were characterised by parallel microplicae and maze patterns.

Bancozy et al. (1980) described the microplicae on the surface of buccal mucosal cells as parallel, branching or ring-shaped protrusions, while Kullaa-Mikkonen (1986) described the same patterns but added that the width of microplicae and the furrows between them were variable from cell to cell and even in the same cell. Matravers and Tyldesley (1977) and Kullaa-Mikkonen (1986) noted that the

surface of keratinised oral epithelium showed a pitted appearance.

The appearance of more complicated patterns in later swabs of the present study e.g. the maze pattern and the microvilli that were surrounded by or interspersed between microplicae, was probably due to exposure of deeper cells.

Microvilli were seen in increasing numbers on successive days of swabbing and on one surface of some cells in which the other surface showed microplicae. These findings suggest that microvilli were either present on the undersurface of the cells or were present on immature deeper cells that had been exposed by repeated swabbing. Microvilli were reported in fetal tongue mucosa (Boshell et al., 1980), on the deep surface of cells of the dorsum of the adult tongue (Dourov, 1984) and in pre-irradiated and irradiated buccal mucosa (Robertson et al., 1987). Following treatment with trypsin, they were seen on the cells of the stratum spinosum in keratinised oral epithelium of vervet monkeys (Cercopithecus aethiops) but the cells acquired a pitted appearance in the stratum corneum (Cleaton-Jones, 1975).

The indication from the scanning electron microscopy study that the surface patterns of deeper cells were more varied and more complicated than superficial cells was confirmed during Study II, by transmission electron microscopy, which showed surface projections on both surfaces of the cell that became more complicated in deeper layers. This technique also showed that the surface

projections of one cell attach to those on adjacent cells by desmosome-like junctions.

Variations in the pattern of microplicae are also reported in the stratified non - keratinising epithelia of the normal oesophagus and vagina (Robinson et al., 1981; Hafez & Kenemans, 1982), and in different pathological disorders affecting the oral mucosa (Matravers & Tyldesley, 1977; Bancozy et al., 1980; Dourov, 1984).

Robinson et al. (1981), in their study of the oesophageal mucosa, found that the arrangement of microplicae was variable from specimen to specimen and from cell to cell within the same specimen. They attributed the variation in pattern to the maturity of the cells, so that when surface cells are shed, the underlying less differentiated cells do not necessarily show the same pattern. This view was supported by Dourov (1984), in his study on lingual mucosa, who also found a difference in the microplical pattern on different surfaces of the same cell.

Parallel microplicae and maze patterns were seen on the surface of human vaginal cells and shown to vary with the cyclical changes that occur in the vagina (Hafez & Kenemans, 1982).

In oral leukoplakia the surface pattern of the affected cells ranged from smooth to covered with irregular microplicae. There is also considerable variation in the surface morphology of malignant cells but so far no changes specific for malignancy have been identified by scanning

electron microscopy (Matravers & Tyldesley, 1977; Bancozy et al., 1980; Dourov, 1984).

The raised intercellular ridges seen between some cells were reported by Traynor and his co-workers (1990) between superficial cells of normal oesophageal mucosal epithelium. They considered these raised ridges as extracellular matrix. Similar raised ridges were reported by Robertson et al. (1987) at high doses of radiotherapy.

The cell surface patterns observed in healthy individuals in this study were similar in every way to those reported by Robertson et al. (1987) in patients receiving radiotherapy. They regarded their most important observation to be the increase in variety of cell-surface patterns during treatment. They thought these variations might be dose related, and due to an irradiation-induced change in the internal structure of the cells, expressed on the cell surface. As an alternative explanation, they suggested that irradiation had increased the rate of exfoliation of the surface cells, resulting in exposure of deeper cells with different microplical patterns.

The present finding, that pattern variations tentatively attributed by Robertson et al., (1987) to the effects of radiation, are all to be seen in swabs from the buccal epithelium of normal individuals, clearly invalidates the use of surface morphology in evaluating dosage of radiotherapy applied to oral mucosa. The present finding that repeated swabs of buccal epithelium at the same site in normal individuals revealed reasonably

consistent changes in surface pattern of the cells, is in conformity with their suggestion that deeper cells are exposed following radiotherapy.

The differences in the microplical pattern on the different surfaces of the cells made it impossible to differentiate with certainty between the upper and lower surfaces of the cells on swabs.

Finally, the absence of cells with identifiable surface markings on swabs from the surface of skin flaps in the group of patients, and the highly variable surface patterns of cells in oral swabs from healthy volunteers indicate that scanning electron microscopy is unlikely to be useful in monitoring the progress of radiotherapy.

CONCLUSIONS:

Scanning electron microscopy of buccal epithelial cells showed their surfaces to have a variable morphology. The results indicate that the surface morphology depends on the position of the cell within the epithelium; those nearer the surface show a pattern of parallel microplicae or microvilli. At deeper levels, the cell surface morphology showed more complex patterns such as irregular microplicae, the maze appearance and a mixture of microplicae and microvilli. Frequently adjacent cells showed different patterns and superficial and deep surfaces of the same cell consistently showed different appearances. In particular all the appearances described by Robertson *et al.* (1987) following radiotherapy were noted in the normal subjects in the present study. It is unlikely that scanning electron microscopy of oral swabs will be of any use in monitoring the progress of radiotherapy.

STUDY II - INTRAORAL RADIAL FOREARM FLAPS:

The "mucosa-like" appearance observed clinically in many of the free radial forearm flaps of the patients treated for oral tumours suggested that the flap epithelium had changed to the oral form, which is contradictory to what is known about the behaviour of the epidermis when it is transplanted with its underlying dermis to ectopic sites.

The clinical observations in patients with oral tumours treated by surgical excision and reconstruction by a radial forearm flap with or without radiotherapy were correlated with the light and electron microscopical findings in an attempt to explain the behaviour of these flaps and in particular to answer the following questions:

1) Had the epidermis of free radial forearm flaps of the Canniesburn patients survived in the oral cavity, or had it been replaced by regenerating oral epithelium?

2) Can the apparent change to mucosa be confirmed histologically?

3) Is the observed alteration to a mucosal appearance a genuine or a reactive change?

4) What is the aetiology of any morphological change in the flap?

Flap survival

In the immediate post-operative period, all patients underwent regular clinical examination to assess the viability of the flap and the presence of adequate circulation. Failure of the flap to bleed following a pin

prick or the presence of necrotic tissue on the surface of the flap indicate ischaemia; should these occur, the blood vessels of the flap are explored.

Experience at Canniesburn Hospital has shown that if the flap survives the first two weeks after surgery it is unlikely to slough thereafter. Past experience in the unit has also shown that small defects caused by sloughing heal by secondary intention, larger ones require further surgery possibly using another flap to replace the sloughed area (Soutar, 1991). Two patients were excluded from the study because of flap necrosis, one before and one after radiotherapy.

Identification of unchanged flaps in all patients was easy because of the distinct contrast between the appearance of the skin of the flap and the surrounding oral mucosa. In addition, hairs were present in many of the non-irradiated flaps.

In the changed category, although the colour was similar to that of the oral mucosa, the position of the flap was frequently obvious because of its uneven surface contours resulting from the operation, which involved excision of the tumour and surrounding tissue. Many flaps showed only partial changes; the presence of skin allowed easy recognition. Some of the non-irradiated flaps bore hairs. On examination in good light, each changed flap showed a fine linear scar marking its borders.

In some of the changed flaps, the pink colour was

first seen in the central parts of the flap and spread towards the periphery; this is unlikely to be compatible with invasion and replacement of the flap epithelium by adjacent oral epithelium.

There was, therefore, no doubt about the survival of the flap in all the patients included in this study.

Features of the flaps suggesting a change to oral mucosa

a) **Gross features**

Those flaps which were said to have "changed", either partially or completely, had been categorised solely on the basis of their colour which was similar to that of the oral mucosa and persisted for some time during the follow-up of the patients.

The surface texture of the changed flaps was variable, ranging from a smooth texture similar to the oral mucosa to a rougher form similar to that of the skin. More than half of the flaps whose colour resembled oral mucosa had a rough surface texture.

b) **Light microscopy**

Apart from the gross appearance of the changed flaps, some of the histological features of these flaps closely resembled those of oral mucosa.

For convenience the findings will be discussed under two headings, epidermal and dermal findings:

EPIDERMAL FINDINGS:

Parakeratosis and changes in the stratum granulosum.

Parakeratosis, the presence of pyknotic nuclei in the

cells of the stratum corneum, is a normal feature of buccal epithelium but not of normal epidermis. This phenomenon was seen in all the "changed" flaps regardless of whether or not they had been irradiated.

In normal buccal epithelium there is no stratum granulosum. It is well documented that, in epidermis, parakeratosis is associated with disturbance of the stratum granulosum (Jarrett, 1973; MacKie, 1984). It was noted that beneath the parakeratotic cells of the changed flaps the stratum granulosum was frequently either thinner than in epidermis or absent. In some flaps which subsequently regained the appearance of skin, the stratum granulosum was thickened. It has been reported by others (Scothorne & Scothorne, 1953; Jarrett, 1973; MacKie, 1984) that the stratum granulosum may be thickened in grafted and healing skin.

Although not a feature of normal epidermis, parakeratosis is seen in a wide variety of dermatological disorders, inflammatory (e.g. psoriasis) and neoplastic (e.g. squamous cell carcinoma), but it is not pathognomonic of any particular disease and may simply indicate accelerated cell turnover. This phenomenon was extensively investigated in psoriasis by Jarrett (1973) who described the stratum granulosum as a transitional zone separating the viable from the non-viable parts of the epidermis; in the stratum granulosum many hydrolytic enzymes can be detected by histochemical methods. Release of these autolytic lysosomal enzymes kills the keratinocytes before they are

transformed into the stratum corneum. Jarrett concluded that parakeratosis in psoriasis results from failure of organised lysosomal release in the stratum granulosum and consequently the nuclei and some cellular organelles are carried into the stratum corneum. This view was confirmed by electron microscopy (Haber & Symmers, 1980).

Increased epithelial thickness.

In many of the flaps, both irradiated and non-irradiated, the epidermis showed cellular hypertrophy and hyperplasia and became as thick as normal buccal epithelium. This change, however, was not confined to changed flaps but was also seen in some of the unchanged flaps but to a lesser extent. In a few changed flaps, this thickening exceeded the thickness of normal buccal epithelium. The thickened epithelium closely resembled that of the buccal mucosa.

The increase in thickness mainly affected the stratum spinosum (acanthosis) and was due to cellular hyperplasia and hypertrophy. The former is evidenced by the deep rete ridges, mitotic figures in the stratum basale, and prominent multiple nucleoli in cells of the stratum spinosum. On the other hand, cellular hypertrophy is evidenced by the widening of the distance between the nuclei of two adjacent cells.

The mechanism by which acanthosis occurs in skin disorders is not fully understood. It may be associated with lysosomal changes (Jarrett, 1973) and is likely to

have many causes (MacKie, 1984).

Increased glycogen content.

Increased glycogen content was observed in all the epithelia that had increased in thickness, whether they were changed or not. Glycogen deposition was confined to the stratum spinosum, especially the superficial part, and was absent in the stratum basale and stratum corneum. The deposition was most marked in those flaps which showed the greatest degree of change. On the other hand the flaps that had retained their normal thickness were similar to normal thin skin in that intracellular glycogen was minimal.

Scothorne and Scothorne (1953), working on full thickness, free skin grafts of the lower limb and therefore in a dry environment, also noted accumulations of glycogen in the grafted epidermis, which also showed hyperplasia, hypertrophy, and disturbances in keratinisation.

It is well known that glycogen is found in the non-keratinised stratified squamous epithelia of mouth, oesophagus and vagina. Glycogen is almost absent in normal adult epidermis but it is present in fetal epidermis (Lyne & Hollis, 1972). Glycogen accumulates in hyperproliferative states such as squamous cell carcinoma (Goltz, 1958), psoriasis (Voorhees & Duell, 1971; Mehregan, 1986), following physical trauma (Freinkel, 1983) and after exposure to ultraviolet irradiation (Ohkawara et al., 1972); its presence in these conditions is a non-specific response resulting from disturbance of cellular metabolism (Freinkel, 1983) which is the most likely explanation for its

accumulation in the flaps in which the epidermis had increased in thickness.

DERMAL FINDINGS

Inflammatory changes.

In the majority of flaps the connective tissue beneath the epithelium showed variable degrees of chronic inflammation. The inflammatory cells were mostly small lymphocytes and plasma cells. These inflammatory reactions could have a wide variety of causes, which are difficult to assess, but certainly include the surgical trauma involved in tissue transplantation in the immediate post-operative period and radiotherapy.

Inflammatory reactions in the oral mucosa often contain many plasma cells (Mehregan, 1986), these cells are less frequently seen in inflamed skin. The presence of large numbers of plasma cells in many of the flaps of the present study suggests that the flap is behaving as a part of the oral lining.

Although plasma cells are infrequent in the normal dermis, they are found in a number of skin disorders such as mycosis fungoides, solar keratosis and chronic deep folliculitis; their role in these disorders is unclear (Lever & Schaumburg-Lever, 1983).

c) **Immunocytochemistry**

When this study started, another project (Ogunbekun, 1990) at Canniesburn Hospital was approaching its end. It aimed to document changes in the pattern of keratinisation

in intraoral radial forearm flaps, before and after radiotherapy using the 1 - dimensional SDS-PAGE method of Laemmli (1970).

Ogunbekun (1990) found an increase in the 48 kd keratin which is considered one of the markers of stratification, together with a decrease in the 65-67 kd keratins which are known as markers of keratinisation. In her study, she did not find any significant difference in keratin pattern between irradiated and non-irradiated flaps.

These findings suggested that the epithelium of the intraoral flap had changed from the keratinised form of the epidermis to the more proliferative type of mucosal epithelium.

Attempts to study the distribution of markers of keratinisation and stratification in this study were unfortunately unsuccessful, presumably because of destruction of antigenicity of the tissues by the technique used in their processing.

d) **Electron microscopy**

A small sample of biopsies was examined by electron microscopy. The appearance of an unchanged flap was similar to that of normal thin epidermis. In the changed flaps, on the other hand, the ultrastructural study confirmed the light microscopical findings and also showed the following unusual feature.

Disturbed organisation of the tonofilaments.

In the changed flaps, keratinocytes in the deeper parts

of the epidermis were found to have smaller bundles of tonofilaments than in normal thin epidermis. In the more superficial cells of the stratum spinosum, the tonofilaments were scattered in the cytoplasm of the cells. Tonofilaments are known to form bundles in the epidermal keratinocyte cytoplasm but are diffuse in buccal epithelial cells; many cells, therefore, showed a tonofilament distribution resembling that in oral epithelium.

Earlier in this discussion, skin diseases were described in which disturbance in the stratum granulosum was associated with abnormal keratinisation. In tumours of the basaloid type, poor keratinisation is associated with disturbance of the organisation of tonofilaments, which are found to be dispersed in the cytoplasm of the relatively small cells (Lever & Schaumburg-Lever, 1983).

Features of the flap suggesting that the change from thin skin was incomplete or reactive

a) **Gross features**

Return to skin morphology.

During follow-up of the patients, it was noted that some of the flaps, irrespective of whether or not they had had radiotherapy, returned to the appearance of normal skin.

Hairs.

Despite their mucosa-like appearance, some of the partially changed non-irradiated flaps showed hairs in the areas that retained the appearance of skin (Fig. 35). No

hairs were seen in the irradiated flaps, even after they returned to the skin appearance; depilation was permanent at the dose given to these patients (6000 cGy).

b) **Light microscopy**

Although many flaps had acquired features in common with buccal mucosa, they still possessed a number of characteristics indicative of thin skin rather than buccal mucosa.

EPIDERMAL FINDINGS.

Stratum corneum

Although parakeratosis was a prominent feature of biopsy sections from changed flaps, thin flakes of cornified cells were occasionally seen in localised areas. They were recognised by their eosinophilic cytoplasm and the absence of nuclei. A stratum corneum similar to that of thin skin (orthokeratosis) reappeared in some of the subsequent biopsies taken from the same site. This observation indicates that this partial deviation from skin morphology was temporary in many flaps.

Stratum granulosum

A stratum granulosum was seen in the epithelium of a number of changed flaps. This layer is absent from the normal non-keratinised oral mucosa (Squier & Hill, 1985). Its presence indicated that those flaps still possessed characteristics in common with normal epidermis.

In some biopsies of the flaps, the stratum granulosum was absent but reappeared in subsequent biopsies from the

same site. Its re-emergence may indicate that the skin is recovering.

Intercellular oedema.

Intercellular oedema (spongiosis) was seen in all the changed flaps, particularly those showing higher grades of inflammatory reaction in the dermis. This spongiosis was noted in the basal and immediate suprabasal layers, while cells became more closely packed in the upper part of the stratum spinosum. Lymphocytes were occasionally encountered in the intercellular spaces. Keratinocyte spines were prominent in the space due to stretching of the desmosomal contacts between cells. These findings were not seen in normal skin and mucosal biopsies.

The presence of spongiosis suggests that the epithelium was responding to an underlying pathology; it is seen in dermatitis caused by different stimuli (MacKie, 1984). The intercellular fluid is probably extravasated from dermal blood vessels responding to radiotherapy or an inflammatory reaction in the dermis. It is believed that the exudate enters the epithelium through temporary breaks in the basement membrane.

DERMAL FINDINGS

Inflammatory cells.

In many of the changed flaps, both irradiated and non-irradiated, large numbers of inflammatory cells were found in the papillary dermis and masked much of the dermal structure. In contrast, unchanged flaps and biopsies of normal buccal mucosa showed either low grade or no

inflammation in the connective tissue.

All the flaps which had shown marked (Grades 4 & 5) inflammatory reactions in the dermis were changed. This abnormal finding and its association with the changed appearance of the flap raise the question of whether the dermal inflammation might be the stimulus for the epidermal changes.

It was not possible to take a biopsy from the normal oral mucosa nearby the flap to see if the inflammatory changes in the flap were part from a more generalised reaction involving the oral cavity.

Dermal fibres and cells.

In all the flaps, both changed and unchanged, the connective tissue beneath the epithelium was dense and irregular, similar to that of the dermis and quite different from that of the corium of buccal mucosa. In some changed flaps the fibres and fibroblasts of the papillary dermis were obscured by the inflammatory cells but they were clearly seen in the reticular part of the dermis.

Even in the irradiated flaps, connective tissue was clearly of the dermal form, the only observed changes being an increase in the development of elastic fibres and the presence of giant fibroblasts.

Epidermal adnexa.

The presence of epidermal adnexa in some of the flaps indicated the persistence of "skin" features.

Sweat glands and/or their ducts were seen in most of

the changed flaps regardless of whether or not they had been irradiated. In those biopsies in which these structures were not seen, it may have been that, for technical reasons, the sample was not large enough to include them.

No hair follicles were seen in the irradiated flaps as radiotherapy at the dose given produces permanent depilation. On the other hand, hair follicles were occasionally seen in the dermis of non-irradiated flaps regardless of whether or not the flap had changed. No sebaceous glands were seen in any flap, although they were observed in association with hair follicles of normal forearm skin.

c) **Transmission electron microscopy**

Tonofilament bundles.

Transmission electron microscopy has shown the tonofilaments collected into bundles in the basal keratinocytes but in the changed flaps they became dispersed in the cytoplasm as the cell moved upwards.

In later biopsies taken from the same patients, when the flap showed partial recovery, tonofilaments were collected into small bundles dispersed in the cytoplasm of the cells of the stratum spinosum. These bundles were smaller than in normal thin epidermis. Their appearance, however, may indicate that the flaps were recovering.

Appearance of fat droplets.

Membrane-limited vesicles were noted in large numbers in the cells of the stratum corneum of changed flaps and the flaps that showed partial recovery, both irradiated and non-

irradiated. These vesicles were not seen in the normal epidermis of forearm skin or the unchanged flap.

The appearance of lipid droplets in the stratum corneum has been observed in a variety of dermatological disorders e.g. psoriasis (Swanbeck & Thyresson, 1962; Bowser & Gray, 1978) and in acne vulgaris (Knuston, 1974). Their appearance is indicative of disturbance in cellular metabolism (Lavker & Leyden, 1979) which is the most likely explanation for their presence in large amounts in changed flaps.

The aetiology of the change in the flaps

Changes similar to those described in this study have been reported by other workers using skin flaps for intraoral reconstruction (Lawson, 1985; Wei et al., 1989) and were described earlier in the introduction (page 55).

In the present project, variable grades of inflammation were seen in most of the flaps. It was noted that all the flaps with severe inflammatory reactions were changed. This observation suggested a causative relation between inflammation and the occurrence of the change.

A number of factors may be responsible for the inflammation in the flap. Intraoral skin flaps are exposed to different types of trauma in their new site in addition to the surgical trauma involved in tissue transplantation.

1) **Radiotherapy**

Before the present project began, the structural basis of the apparent change to a mucosa-like appearance in the intraoral radial forearm flaps was poorly documented but

the "change" was believed to be associated with radiotherapy. The present study shows that this is not the case; 6 out of 19 non-irradiated patients showed changes in the flaps to a mucosal appearance at some time during the project. Although more patients were treated by surgery with radiotherapy than by surgery alone, a similar percentage of patients showed changes with each treatment (surgery + radiotherapy: 12/34 = 35 %; surgery only: 6/19 = 32 %). No significant difference in the incidence of the flap change occurred between the two forms of treatment.

The results of the study suggest, however, that radiotherapy accelerates the onset of the change. The changes observed in 8 patients in Group II occurred shortly after radiotherapy, suggesting a failure to recover from the "brisk" reaction. Recovery of the flap from radiotherapy was early in one patient in Group II; in another two patients the flap took a longer time to recover. In these three patients the flap returned to the skin appearance. In the remaining patients the flaps had not completely recovered when this study finished.

The site of the excised tumour which was covered by the flap received most of the dose of radiotherapy (6000 cGy) while the skin of the neck and lower part of the face received only fractions of the dose. This is probably why the flap took longer to recover than did the skin.

During the treatment with radiotherapy and for some time thereafter, patients developed variable grades of oral

discomfort. This may have adversely affected the oral hygiene and given more chances for infection, particularly candidiasis.

2) **The wet environment of the mouth and its microbiological flora**

The cells of the oral mucosa, but not those of the skin, are adapted to the wet environment of the oral cavity (Jarrett, 1980). The normal bacterial flora of the mouth may become pathogenic because of alterations in the environment caused by trauma and poor oral hygiene (Nolte, 1982). This in turn causes inflammatory reactions in the oral tissues of which the flap now forms a considerable part. In some patients, irregularities in the anatomy of the oral cavity and the restricted tongue movements lead to collections of food which form a good medium for bacterial and fungal proliferation.

In the patient who underwent surgery in 1982 (**I - 20**), in whom the flap remained unchanged until April 1990, candidiasis was diagnosed clinically when the change was noticed (Fig. 32 & 33).

In another patient (**I - 30**) a lot of debris was found on the flap and when this debris was cleaned the flap was noted to be changed (Fig. 39 & 40), a biopsy was taken at that time and a mouth rinse revealed heavy candidal growth. The patient was started on antifungal treatment and at the next visit the flap looked like skin again. This patient developed fungal infection on a second occasion and the flap "changed" once again.

Four of the changed flaps in Group I were tested for Candida and found positive at some time following surgery or radiotherapy.

3) **Dentures**

Poorly fitting dentures are known to cause leukoplakia of the oral mucosa through chronic irritation (Haber & Symmers, 1980; Arnold et al., 1990). In some patients dentures were in contact with the flap, and if they were poorly fitting then the chronic pressure and irritation would result in inflammation. This problem was common in those patients in whom parts of the gums had been excised during surgery.

4) **The trauma caused by ingested substances**

Friction during chewing and swallowing may traumatise the flap surface. Hot and spicy foods may be difficult to avoid and can irritate the tissues.

5) **Smoking and drinking**

Smoking and drinking habits were difficult to assess with certainty in some patients; several denied any alcohol intake although it could be smelled readily on their breath during their visit to the clinic. Smoking and drinking are known predisposing factors in oral cancer. Smoking is also an important aetiological factor in oral leukoplakia.

CONCLUSIONS:

Twenty of the fifty-three patients in this study showed a gross change in the appearance of the flap from the morphology of skin towards that of oral mucosa at some stage in the period of observation. There was no significant difference in the incidence of flap change between irradiated and non-irradiated patients. This change frequently followed radiotherapy and, in non-irradiated patients, occured at any time after surgery. In three of these patients, all of whom received radiotherapy, the changed appearance reverted to the original skin form after variable intervals and some others showed partial recovery.

Histologically, biopsies of the changed flaps differed from normal thin skin in that the epithelium was increased in thickness and showed parakeratosis and increased glycogen content. These findings were confirmed by electron microscopy which also showed that the tonofilaments were dispersed in the cytoplasm of the cells. While these changes resembled normal buccal epithelium, the underlying connective tissue retained the structure of dermis and contained sweat glands but showed marked inflammatory changes in many specimens. These features, together with intercellular oedema in the epithelium, suggested a reactive change. The histological changes in the flap epithelium were similar in both irradiated and non-irradiated patients although some differences could be recognised in the dermis.

It is clear that, while radiotherapy might be important

in initiating the change, other factors must also be important. In a few patients changes in the appearance were associated with infection with Candida albicans and in one patient the flap returned to the appearance of normal skin following treatment of the infection. The relative roles of environmental changes and of radiotherapy, infection and other forms of trauma in the aetiology of the observed changes in the flaps remain obscure. The changes, however, would appear to be reactive and related to the degree of inflammation which occurs in the dermis.

Finally, the findings in this study support those described earlier in the introduction that regional variations in epithelial pattern are inherently determined by the underlying connective tissue and although the epidermis changes appearance in response to various stimuli this change is reversible once the stimulus is removed.

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APPENDICES

APPENDIX I

TNM CLASSIFICATION

T - Staging

Tx	Primary tumour can not be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ
T1	Tumour 2 cm or less in greatest dimension
T2	Tumour more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumour more than 4 cm in greatest dimension
T4	Tumour invades adjacent structures, e.g. through cortical bone, into deep (extrinsic) muscles of tongue, maxillary sinus, skin

N - Staging

Nx	Regional lymph nodes can not be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in diameter
N2	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension, or in multiple ipsilatertal lymph nodes, none more than 6 cm in greatest dimension, or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N2a	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm

in greatest dimension

N2b Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension

N2c Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension

N3 Metastasis in a lymph node more than 6 cm in greatest dimension

M - Staging

Mx Presence of distant metastasis can not be assessed

M0 No distant metastasis

M1 Distant metastasis

Stage grouping

Stage 0	Tis N0 M0
Stage I	T1 N0 M0
Stage II	T2 N0 M0
Stage III	T3 N0 M0
	T1,T2, or T3 N1 M0
Stage IV	T4 N0 M0
	Any T- stage, N2 or N3 M0
	Any T-stage, any N-stage, M1

APPENDIX II

Preparation of mucosal biopsies for S.E.M.

All biopsies were immediately fixed in 3% buffered glutaraldehyde (pH 7.4) for 24 hours.

- Millonig's buffer 4 hours
- Osmic tetraoxide 1% in Millonig's buffer 1 hour
- Millonig's buffer (pH 7.4) 4 hours
- 70% acetone 4 hours
- 90% acetone 4 hours
- 100% acetone 2 changes X 4 hours

The biopsies were then critical point dried (Polaron, England), mounted on aluminium stubs using double face sellotape, and then gold-coated.

APPENDIX III

Preparation of oral swabs for S.E.M.

The swabs were fixed in 3% buffered glutaraldehyde (pH 7.4) for 1 hour.

- Millonig's buffer (pH 7.4)	30 minutes
- 1% OsO_4 in Millonig's buffer	20 minutes
- Millonig's buffer (pH 7.4)	30 minutes
- 50% acetone	30 minutes
- 70% acetone	30 minutes
- 90% acetone	30 minutes
- 100% acetone	2 changes X 30 minutes

Swabs were then critical point dried (Polaron, England), mounted on aluminium stubs using double face sellotape, and then gold-coated.

APPENDIX IV

Preparation of tissues for semithin and thin sections

Tissue was fixed in 3% glutaraldehyde overnight, and then washed in Millonig's buffer (pH 7.4). It was then osmicated for one hour in 1% OsO_4, washed again in buffer and processed as the follows:

50% acetone	6 hours
70% acetone	6 hours
90% acetone	6 hours
100% acetone	several changes over 3 days
Propylene oxide (P.O)	20 minutes
= =	20 minutes
P.O./ Spurrs 1:1	2 days
P.O./ Spurrs 1:2	2 days
Fresh Spurrs	2 days
Fresh Spurrs	2 days

Embedded in low viscosity Spurr's resin, incubated in oven 60 °C for 24 hours.

APPENDIX V

Haematoxylin and eosin staining

1. Deresinate sections in ethanolated NaOH.
2. Hydrate through descending concentration of ethanol.
3. Wash in water.
4. Stain with haematoxylin.
5. Wash in water.
6. Differentiate in acid alcohol.
7. Wash in water.
8. Blue in Scott's solution, check differentiation.
9. Wash in water.
10. Eosin.
11. Wash in water.
12. Differentiate eosin in 70% ethanol.
13. Dehydrate through graduated alcohol.
14. Clear in xylene. Check staining.
15. Mount in "Histomount".

Results: Nuclei: blue

Other tissues : pink / red

APPENDIX VI

Resorcin fuchsin / Van Gieson staining

1-3. As in Appendix V

4. 0.25% Potassium permanganate in distilled water
5 minutes

5. Rinse in water.

6. 1% Oxalic Acid until sections are clear
5% Oxalic Acid in water.

7. Wash in tap water (5 minutes) place in distilled water.

8. Resorcin-Fuchsin 24 hours

Resorcin-Fuchsin stock	10 ml
Alcohol 70%	100 ml
HCl (Conc)	2 ml

9. Wash in tap water 10 minutes

10. Rinse in distilled water.

11. Van Gieson 1 minute

12. Dehydrate and mount.

Results:

Elastic fibres	: blue-black to black
Nuclei	: blue to black
Collagen	: pink to red
Other tissues elements	: yellow

APPENDIX VII

Periodic acid / Schiff staining:

1 - 3. as in Appendix I.

4. Oxidise in 1% periodic acid - 5 minutes.

5. Wash in running water - 5 minutes.

6. Schiff reagent - 10 minutes.

7. Wash in running water - 10 minutes.

8. Counter-stain in Weigert's haematoxylin.

9. Rinse in water.

10. Blue in Scott's solution, check differentiation.

11. Wash in water.

12. Dehydrate, clear in xylene, check and mount in "Histomount".

Results:

Glycogen : purple.

APPENDIX VIII

Immunohistochemistry technique:

Sections on slides

- sodium ethanolate 40 minutes
- absolute ethanol 3 changes X 5 minutes
- distilled water 2 changes X 5 minutes
- 1% sodium metaperiodate 7 minutes
- distilled water 2 changes X 5 minutes
- phosphate buffer saline (PBS) 2 changes X 10 minutes
- dilute blocking serum 20 minutes
- drain off serum
- primary antiserum in blocking solution overnight
- PBS rinse 10 minutes
- biotinylated second antibody solution 60 minutes
- PBS rinse 10 minutes
- ABC reagent 60 minutes
- PBS rinse 10 minutes
- phosphate buffer (PB) (0.1M, pH 7.4) 5 minutes
- DAB + 1% H_2O_2 to conc of 0.01% (until brown)
- PB 2 changes X 5 minutes
- PB 0.01% OsO_4 5 minutes
- distilled water 3 changes X 3 minutes
- dehydrate, clear and coverslip.

APPENDIX IX

Ultrathin section staining

The double staining technique used in this study for staining thin sections was described by Echlin (1964) :

1) Saturated solution of uranyl acetate in 40% ethanol for 1 minute,

2) Wash in water,

3) Alkaline solution of lead citrate for 1 minute,

4) Wash in water, allow to dry in air.

When staining with lead citrate, crystals of sodium hydroxide were present in the Petri dish to react with any CO_2 present which otherwise would react with the lead citrate, producing lead carbonate, which would form an insoluble contaminant on the surface of the section.

TABLES

Table 1

Distribution of major cytokeratins in different normal stratified squamous epithelia*

C/K	M.W	pH	Epid	Sole	H/F	S/G	Tongue	Epigl.	Oesoph.
1	68	7.8	+	+					
2	65.5	7.8	±	±					
3	63	7.5							
4	59	7.3				+	+	+	+
5	58	7.4	+	+	+	+	+	+	+
6	56	7.8		+	+	+	+	+	±
7	54	6.0							
8	52.5	6.1							
9	64	5.4		+					
10	56.5	5.3	+	+		±			
11	56	5.3	+	+		±			
12	55	4.9							
13	54	5.1					+	+	+
14	50	5.3	+	+	+	+	+	+	±
15	50	4.9	±		±	±	±	+	±
16	48	5.1		+	+		+	+	±
17	46	5.1			+	±	±	±	±
18	45	5.7							
19	40	5.2							

* Modified from Moll et al., (1982).
(C/K = Cytokeratin, M.W = Molecular weight(Kd), Epid = Epidermis, Sole = Foot sole epidermis, H/F = Hair follicle outer sheath, S/G = Sebaceous glands, Epigl = Epiglottic epithelium, Oesoph = Oesophageal epithelium.)
+ Components always observed in substantial amounts.
± Components present in minor or variable amounts.

Table 2
Summary of patients in Group I

NO.	INITIALS	AGE	SEX	DTE	TUMOUR SITE	STAGE
I - 1*	J.B	51	M	26,36	Floor of the mouth	T1 N0 M0
I - 2	R.S	69	M	82	Floor of the mouth	T3 N0 M0
I - 3	J.C	39	M	88	Retromolar trigone	T3 N0 M0
I - 4*	D.M	67	M	9	Lower alveolus	T1 N0 M0
I - 5	J.T	61	M	52	Tongue	T2 N1 M0
I - 6	J.A	73	M	24,30	Floor of the mouth	T1 N1 M0
I - 7	V.H	54	M	51	Floor of the mouth + Palate + Tongue	T4 N1 M0
I - 8	J.M	56	M	64	Floor of the mouth + Tongue	T4 N1 M0
I - 9#	M.R	62	F	6	Tonsillar fossa	T2 N0 M0
I - 10	J.M	73	M	64	Lower alveolus	T4 N1 M0
I - 11	E.M	39	F	27	Tongue	T1 N1 M0
I - 12#	J.L	81	F	49	Floor of the mouth	T2 N0 M0
I - 13*	J.W	43	M	81	Floor of the mouth	T2 N1 M0
I - 14	W.D	52	M	26	Tongue	T2 N2 M0
I - 15	L.M	70	F	56	Floor of the mouth + Tongue	T4 N1 M0
I - 16	J.D	71	M	64	Floor of the mouth	T3 N0 M0
I - 17	M.T	72	F	11,15	Floor of the mouth	T2 N0 M0
I - 18¥	J.G	52	F	22	Floor of the mouth	T1 N1 M0
I - 19	A.D	64	F	11,13	Cheek	T3 N3 M0
I - 20*	M.C	73	F	84	Tongue	T1 N1 M0
I - 21	I.M	35	F	76	Floor of the mouth + Tongue	T4 N0 M0
I - 22	T.D	52	M	82	Floor of the mouth + Tongue	T2 N1 M0
I - 23*	H.M	55	M	23	Floor of the mouth + Gum	T2 N0 M0
I - 24	W.D	58	M	10	Lower alveolus	T4 N0 M0

(continued Table 2)

I - 25	E.G	61	F	39,47	Tongue	T2 N1 M0
I - 26*	R.M	40	M	76	Cheek	T3 N0 M0
I - 27*	C.C	54	F	79	Cheek	T1 N0 M0
I - 28*	M.M	69	F	69	Floor of the mouth	T2 N0 M0
I - 29*	C.C	50	M	82	Floor of the mouth	T1 N0 M0
I - 30#	J.R	57	M	15	Floor of the mouth	T2 N1 M0
I - 31	A.R	72	M	59	Tongue	T3 N0 M0
I - 32*	J.M	29	M	3	Floor of the mouth + Cheek + Palate	T4 N0 M0
I - 33	F.M	50	F	68	Tongue	T1 N1 M0
I - 34*	A.W	61	M	84	Floor of the mouth	T1 N0 M0
I - 35	J.B	61	M	15	Retromolar trigone	T2 N1 M0
I - 36	R.F	56	M	93	Floor of the mouth	T1 N1 M0
I - 37*	M.N	53	F	53	Floor of the mouth	T1 N0 M0
I - 38	J.H	58	M	6	Floor of the mouth	T2 N1 M0
I - 39	E.R	54	F	81	Retromolar trigone	T2 N1 M0
I - 40	J.C	63	M	98	Lower alveolus	T4 N1 M0

DTE = Post-operative time at which the biopsy was taken (in months).
* Patient did not receive radiotherapy.
# Patient received radiotherapy long before surgery.
¥ The flap was not included in the field of radiotherapy.

Table 3
Summary of patients in Group II

NO.	INITIALS	AGE	SEX	TUMOUR SITE	STAGE
II - 1	G.C	42	M	Retromolar trigone	T4 N0 M0
II - 2@	J.F	72	M	Floor of the mouth	T4 N1 M0
II - 3	M.M	67	F	Retromolar trigone + Cheek + Tongue	T3 N1 M0
II - 4*	A.M	75	F	Floor of the mouth + Tongue	T1 N0 M0
II - 5@	D.P	54	M	Retromolar trigone	T4 N1 M0
II - 6@	T.C	79	M	Tongue	T3 N1 M0
II - 7	J.W	64	M	Floor of the mouth + Tongue	T2 N0 M0
II - 8*	L.R	35	F	Floor of the mouth	T1 N0 M0
II - 9#	E.H	59	F	Floor of the mouth	T4 N0 M0
II - 10@	R.M	63	M	Tongue	T3 N0 M0
II - 11@	A.W	38	M	Retromolar trigone	T3 N2 M0
II - 12	J.H	49	M	Floor of the mouth	T3 N0 M0
II - 13	J.S	44	M	Floor of the mouth + Tongue	T3 N2 M0
II - 14	W.M	71	M	Tongue	T1 N0 M0
II - 15*	J.C	50	M	Floor of the mouth + Tongue + Alveolus	T4 N0 M0
II - 16	J.H	53	M	Floor of the mouth	T4 N0 M0
II - 17@	J.M	59	F	Tongue	T2 N1 M0
II - 18	W.M	57	M	Tongue	T2 N0 M0
II - 19	J.M	55	M	Tongue	T2 N0 M0
II - 20@	W.M	54	M	Floor of the mouth	T3 N0 M0

@ Patient excluded from the study for the reason shown in Table 3.
* Patient did not receive radiotherapy.
# Patient received radiotherapy long before surgery.
All patients offered sequential biopsies except patients number **II** - 4 and **II** - 9.

Table 4
Patients excluded from the study

NO.	REASON
II - 2	Post-operative necrosis of the flap
II - 5	Severe trismus, impossible biopsy
II - 6	Did not attend for follow up
II - 10	Deep flap, impossible biopsy
II - 11	Severe trismus, impossible biopsy
II - 17	Deep flap, impossible biopsy
II - 20	Post-radiotherapy partial necrosis of the flap

STATISTICAL ANALYSIS

AND GRAPHS

The percentage of different surface patterns seen in normal human volunteers in Study I.

DAY 1

NO.	PARALLEL MICROPLICAE	BRANCHING MICROPLICAE	MICROVILLI	MAZE	MICROVILLI & MICROPLICAE
1	32	29	30	7	2
2	39	30	25	6	–
3	37	32	28	3	–
4	34	34	25	7	–

DAY 2

NO.	MICROVILLI	PARALLEL MICROPLICAE	BRANCHING MICROPLICAE	MAZE	MICROVILLI & MICROPLICAE
1	49	12	15	20	7
2	35	12	20	25	5
3	48	13	16	18	5
4	37	17	14	20	8

DAY 3

NO.	MICROVILLI	PARALLEL AND BRANCHING MICROPLICAE	MAZE	MICROVILLI & MICROPLICAE
1	11	16	39	29
2	30	19	35	25
3	48	19	48	22
4	38	10	38	18

DAY 4

NO.	MICROVILLI	WHORLS	MICRORIDGES	MAZE	MICROVILLI & MICROPLICAE
1	23	15	6	35	20
2	25	17	5	35	18
3	18	22	-	36	17
4	26	21	-	42	16

DAY 5

NO.	MICROVILLI	WHORLS	MICRORIDGES	MAZE	MICROVILLI & MICROPLICAE
1	27	14	9	32	18
2	24	15	11	33	17
3	22	22	-	38	18
4	26	21	-	41	12

The percentage of cells in swabs of normal buccal mucosa showing a maze pattern surface on 5 successive days of swabbing.

Table of means for maze pattern

	N	Mean	SEM	95% Confidence for mean	
patient					
a	5	26	±5	23.35	29.48
b	5	27	±5	23.95	30.44
c	5	25	±6	22.55	29.05
d	5	31	±7	28.35	34.85
day					
one	4	5	±0	2.11	9.38
two	4	20	±1	17.11	24.38
three	4	40	±3	35.86	43.13
four	4	37	±1	33.36	40.63
five	4	36	±2	32.36	39.63
Total	20	27.8		26.17	29.42

Analysis of Variance for maze pattern

Source	SS	d.f.	Mean sq	F	P
MAIN EFFECTS	3399	7	485	43	<.001
day	3298	4	824	74	<.001
patient	101	3	33	3	<.700
RESIDUAL	189	12	15		
TOTAL	3565	19			

Multiple range analysis for maze pattern by patient

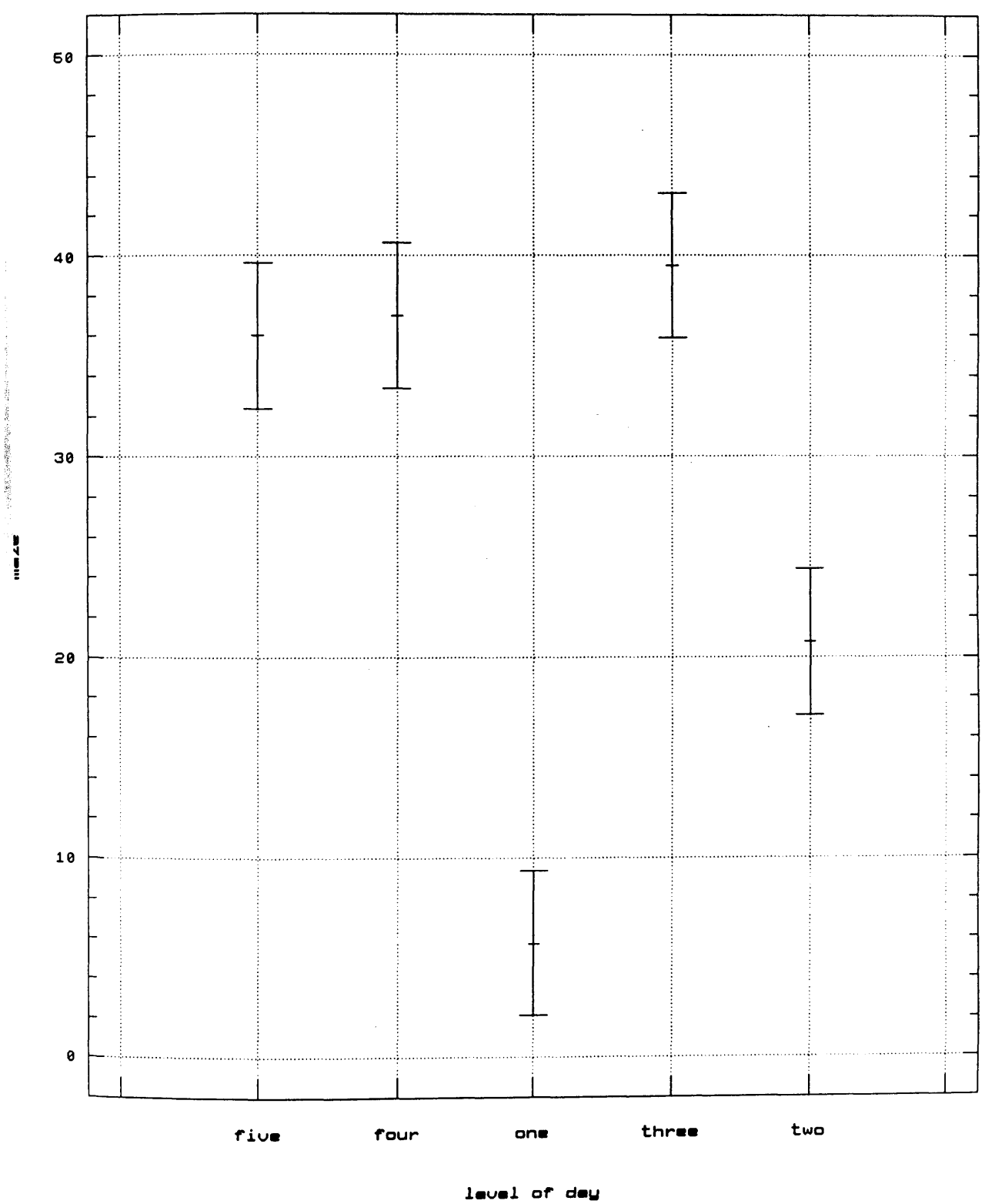
Method: 95 Percent Confidence Interval

Level	N	Mean	Homogeneous Groups
a	5	26.60	*
b	5	27.20	*
c	5	25.80	*
d	5	31.60	*

Multiple range analysis for maze pattern by day

Method: 95 Percent ConfidenceInterval

Level	N	Mean	Homogeneous Groups
one	4	5.75	*
two	4	20.75	*
three	4	39.50	*
four	4	37.00	*
five	4	36.00	*


95 Percent Confidence
Intervals for Factor Means
50
40
30
20
10
0
five
four
one
three
two
level of day

The percentage of cells in swabs of normal buccal mucosa showing a parallel microplicae pattern surface on 5 successive days of swabbing.

Table of means for parallel microplicae

Level	N	Mean	SEM	95% Confidence for mean	
day					
one	4	35.50	±2	33.21	37.79
two	4	15.50	±2	13.21	17.79
three	4	7.75	±1	5.46	10.04
four	4	-	-	−2.29	2.29
five	4	-	-	−2.29	2.29
patient					
a	5	10.40	±6	8.35	12.45
b	5	13.60	±7	11.55	15.65
c	5	11.80	±7	9.75	13.85
d	5	11.20	±6	9.15	13.25
Total	20	11.75	.47	10.73	12.77

Analysis of Variance for parallel microplicae

Source	SS	d.f.	Mean sq	F	P
MAIN EFFECTS	3509	7	501	113.49	<.001
day	3481	4	870	197.04	<.001
patient	28	3	9	2.09	<.154
RESIDUAL	53	12	4		
TOTAL	3562	19			

Multiple range analysis for parallel microplicae by patient

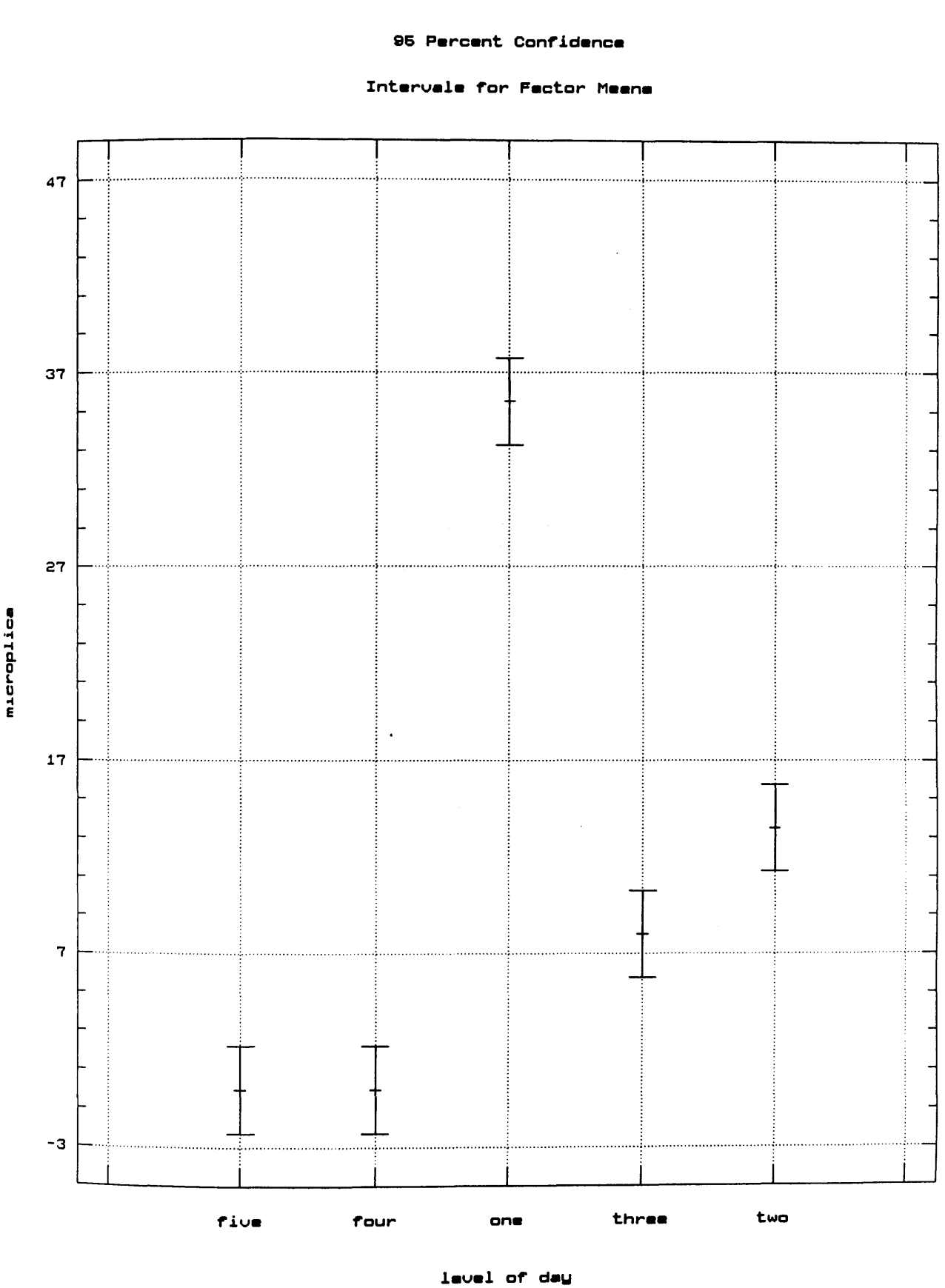
Method: 95 Percent Confidence Interval

Level	N	Mean	Homogeneous Groups
a	5	10.40	*
b	5	11.20	*
c	5	11.80	*
d	5	12.20	*

Multiple range analysis for parallel microplicae by day

Method: 95 Percent Confidence Interval

Level	N	Mean	Homogeneous Groups
one	4	35.50	*
two	4	13.50	*
three	4	8.00	*
four	4	-	*
five	4	-	*


95 Percent Confidence
Intervals for Factor Means
47
37
27
17
7
-3
microplica
five
four
one
three
two
level of day

The percentage of cells in swabs of normal buccal mucosa showing a microvilli pattern surface on 5 successive days of swabbing.

Table of means for microvilli

	N	Mean	SEM	95 % Confidence for mean	
day					
one	4	27.00	±1	21.45	36.35
two	4	41.50	±3	35.95	51.60
three	4	23.25	±3	17.70	41.10
four	4	23.00	±1	17.45	28.55
five	4	24.75	±1	19.20	30.30
patient					
a	5	28.40	±4	23.43	30.30
b	5	25.40	±2	20.43	28.55
c	5	29.20	±5	24.23	32.55
d	5	28.60	±2	23.63	47.05
Total	20	29.75	±1	25.57	33.93

Analysis of Variance for microvilli

Source	SS	d.f.	Mean sq	F	P
MAIN EFFECTS	1008.70	7	144.10	5.56	<.004
patient	43.40	3	14.47	.56	<.652
day	965.30	4	241.3	9.31	<.001
RESIDUAL	311.10	12	25.93		
TOTAL	1319.80	19			

Multiple range analysis for microvilli pattern by patient

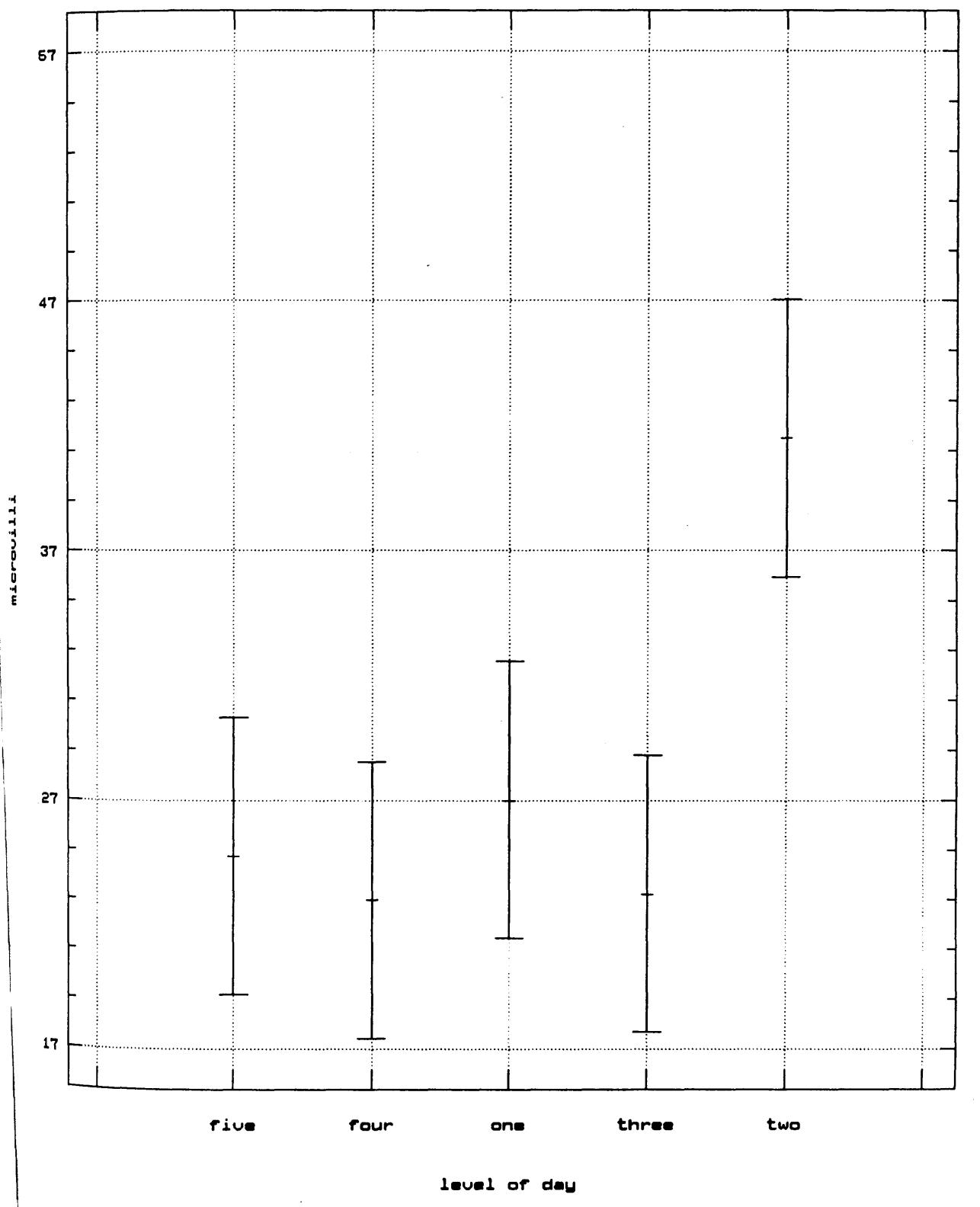
Method: 95 Percent Confidence Interval

Level	N	Mean	Homogeneous Groups
a	5	28.40	*
b	5	25.40	*
c	5	29.20	*
d	5	28.60	*

Multiple range analysis for microvilli pattern by day

Method: 95 Percent Confidence Interval

Level	N	Mean	Homogeneous Groups
one	4	23.00	*
two	4	41.50	*
three	4	23.25	*
four	4	23.00	*
five	4	24.75	*


95 Percent Confidence
Intervals for Factor Means
57
47
37
27
17
microvilli
five
four
one
three
two
level of day

The percentage of cells in swabs of normal buccal mucosa showing a branching microplicae pattern surface on 5 successive days of swabbing.

Table of means for branching microplicae

Level	N	Mean	SEM	95% Confidence for mean	
day					
one	4	30.50	±2	28.19	32.81
two	4	16.25	±1	13.94	18.56
three	4	7.25	±1	4.94	9.56
four	4	-	-	−2.31	2.31
five	4	-	-	−2.31	2.31
patient					
a	5	9.80	±5	7.73	11.87
b	5	11.80	±5	9.73	13.87
c	5	11.00	±6	8.93	13.07
d	5	10.60	±6	8.45	12.67
Total	20	10.80	.49	9.76	11.83

Analysis of Variance for branching microplicae

Source	SS	d.f.	Mean sq	F	P
MAIN EFFECTS	2665.10	7	380.73	72.97	<0.001
day	2654.70	4	663.68	127.17	<0.001
patient	10.40	3	3.47	.69	<0.5738
RESIDUAL	54.10	12	4.51		
TOTAL	2719.20	19			

Multiple range analysis for branching microplicae by patient

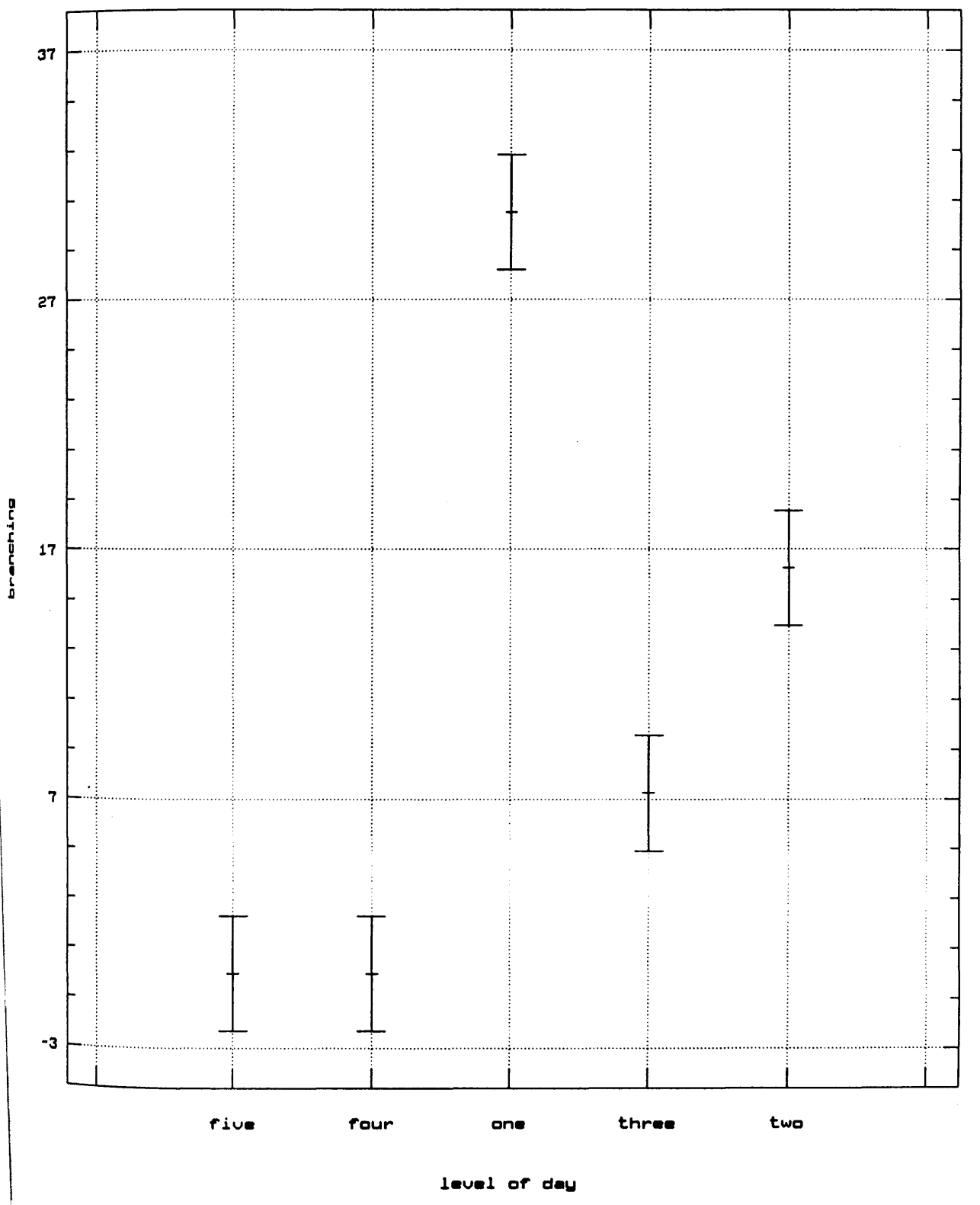
Method: 95 Percent Confidence Interval

Level	N	Mean	Homogeneous Groups
a	5	9.8	*
b	5	11.80	*
c	5	11.00	*
d	5	10.60	*

Multiple range analysis for branching microplicae by day

Method: 95 Percent Confidence Interval

Level	N	Mean	Homogeneous Groups
one	4	30.50	*
two	4	16.25	*
three	4	7.25	*
four	4	0.00	*
five	4	0.00	*


95 Percent Confidence
Intervals for Factor Means
37
27
17
7
-3
branching
five
four
one
three
two
level of day

The percentage of cells in swabs of normal buccal mucosa showing mixed microplicae and microvilli pattern surface on 5 successive days of swabbing.

Table of means for mixed microplicae and microvilli

Level	N	Mean	SEM	95% Confidence for mean	
patient					
a	5	15.80	±4	13.46	18.14
b	5	13.20	±5	10.86	15.54
c	5	12.00	±4	9.66	14.34
d	5	10.80	±3	8.46	13.14
day					
one	4	1.25	±1	−1.37	3.87
two	4	6.25	±1	3.63	8.87
three	4	23.25	±3	20.63	25.87
four	4	17.75	±1	15.13	20.37
five	4	16.25	±1	13.64	18.87
Total	20	12.95	.58	11.88	14.12

Analysis of Variance for mixed microplicae and microvilli

Source	SS	d.f.	Mean sq	F	P
MAIN EFFECTS	1286.85	7	183.84	27.541	<.0001
patient	64.15	3	21.38	3.203	<.0621
day	1222.70	4	305.68	45.794	<.0001
RESIDUAL	80.10	12	6.68		
TOTAL	1366.95	19			

Multiple range analysis for microplicae & microvilli by patient

Method: 95 Percent Confidence Interval

Level	N	Mean	Homogeneous Groups
a	5	15.80	*
b	5	13.20	**
c	5	12.00	**
d	5	10.80	*

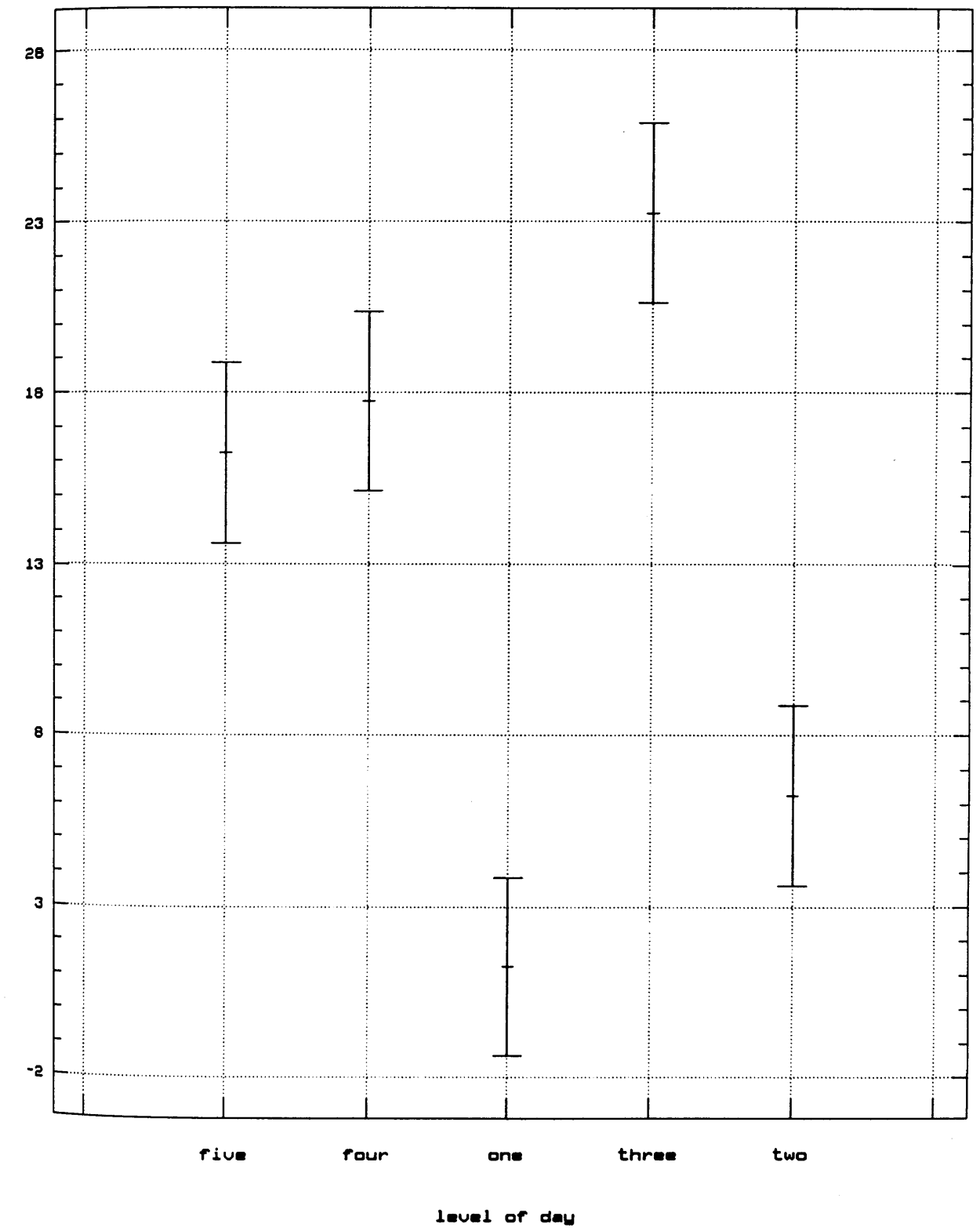
Multiple range analysis for microplicae & microvilli by day

Method: 95 Percent Confidence Interval

Level	count	Mean	Homogeneous Groups
one	4	1.25	*
two	4	6.25	*
three	4	23.25	*
four	4	17.75	*
five	4	16.25	*

95 Percent Confidence

Intervals for Factor Means



The percentage of cells in swabs of normal buccal mucosa showing whorls pattern surface on 5 successive days of swabbing.

Table of means for whorls pattern

Level	N	Mean	SEM	95% Confidence for mean	
day					
four	4	18.75	±2	17.67	21.23
five	4	18.00	±2	16.92	19.98
patient					
a	2	14.50	±1	12.98	16.03
b	2	16.00	±1	14.48	17.52
c	2	22.00	±0	20.48	23.52
d	2	21.00	±0	19.48	22.52
Total	8	18.37	.51	17.61	19.14

Analysis of Variance for whorls pattern

Source	SS	d.f.	Mean sq	F	P
MAIN EFFECTS	85.50	4	20.63	45.00	<.0001
patient	81.38	3	27.13	59.18	<.1071
day	1.13	1	1.13	2.46	<.0001
RESIDUAL	1.38	3	.45		
TOTAL	83.88	7			

Multiple range analysis for whorls pattern by patient

Method: 95 Percent Confidence Interval

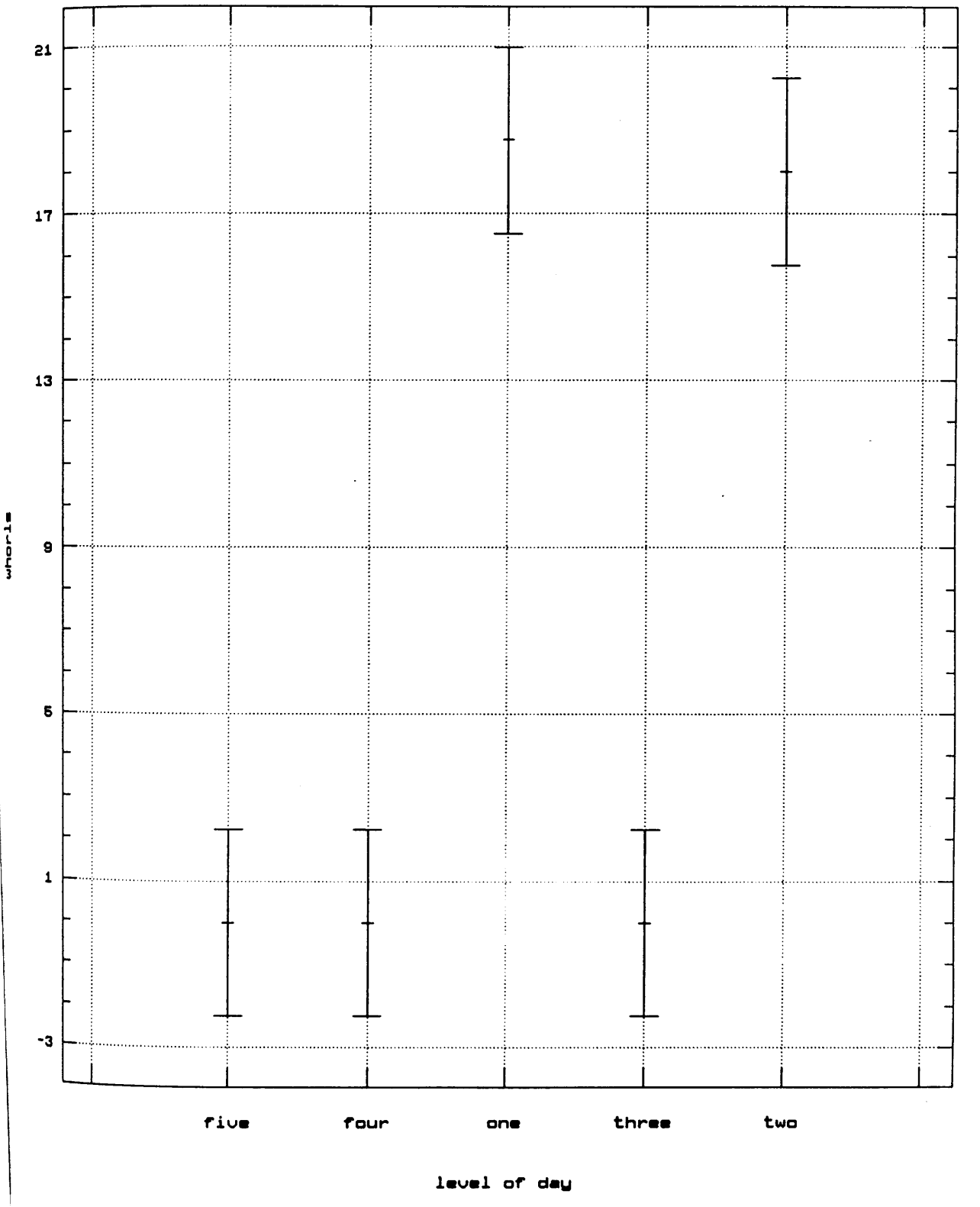
Level	N	Mean	Homogeneous Groups	
a	2	14.50	*	
b	2	16.00	*	
c	2	22.00		*
d	2	21.00		*

Multiple range analysis for whorls pattern by day

Method: 95 Percent Confidence Interval Level	count	Mean	Homogeneous Groups
four	4	18.75	*
five	4	18.00	*

95 Percent Confidence

Intervals for Factor Means



The percentage of cells in swabs of normal buccal mucosa showing microridges pattern surface on 5 successive days of swabbing.

Table of means for microridges pattern

Level	N	Mean	SEM	95% Confidence for mean	
day					
four	4	5.00	±2	−.18	6.18
five	4	3.00	±3	1.82	8.18
patient					
a	2	8.00	±2	3.50	12.50
b	2	8.00	±2	3.50	12.50
c	2	-	-	−4.50	4.50
d	2	-	-	−4.50	4.50
Total	8	4.00	.70	1.75	6.25

Analysis of Variance for microridges pattern

Source	SS	d.f.	Mean sq	F	P
MAIN EFFECTS	136.00	4	34.00	8.50	<.054
day	8.00	3	8.00	2.00	<.252
patient	128.00	1	42.67	10.67	<.041
RESIDUAL	12.00	3	4.00		
TOTAL	148.00	7			

Multiple range analysis for microridge pattern by patient

Method: 95 Percent Confidence Interval

Level	N	Mean	Homogeneous Groups
a	2	8.00	*
b	2	8.00	*
c	2	0.00	*
d	2	0.00	*

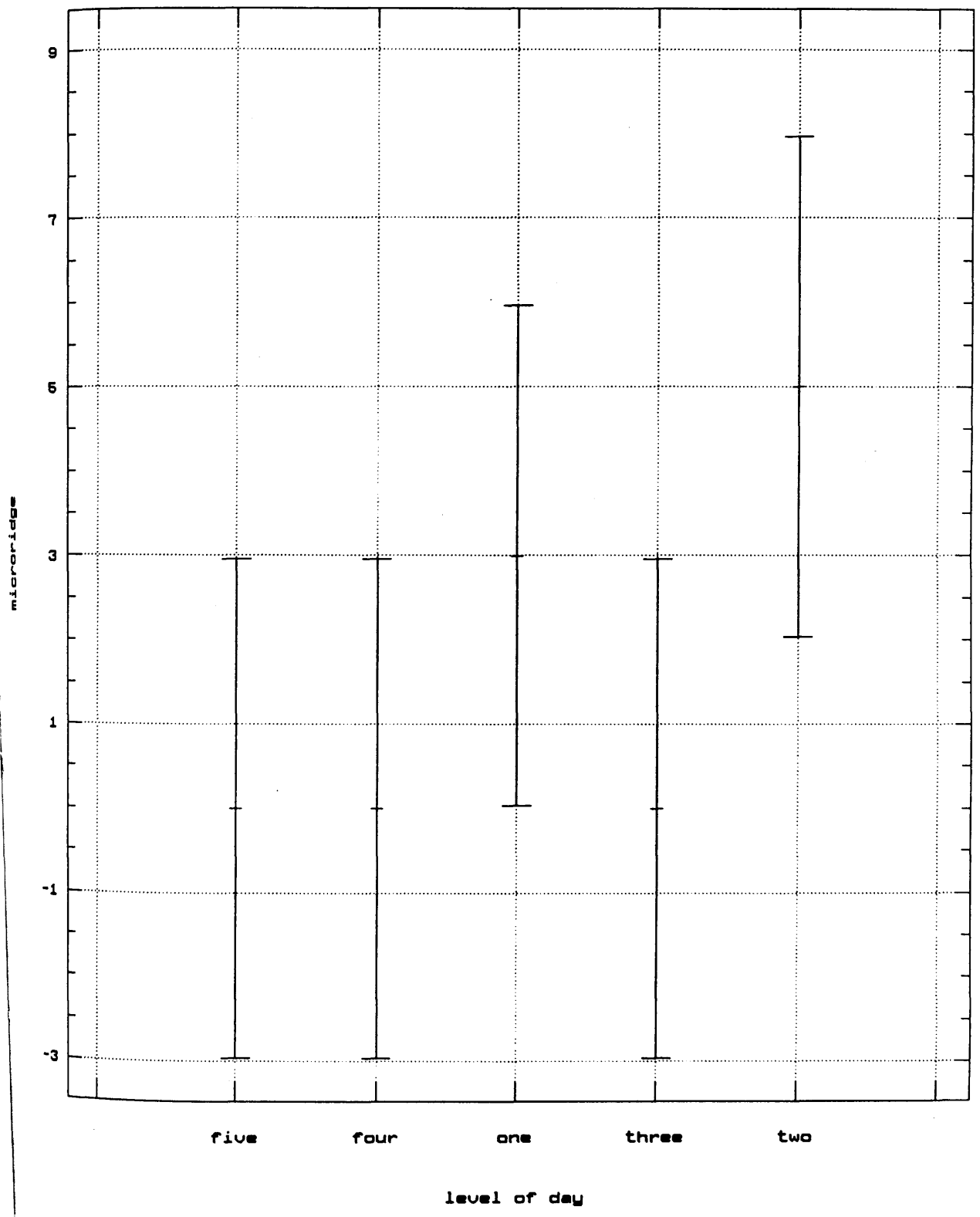
Multiple range analysis for microridge pattern by day

Method: 95 Percent Confidence Interval

Level	N	Mean	Homogeneous Groups
four	4	3.00	*
five	4	5.00	*

95 Percent Confidence

Intervals for Factor Means



EPITHELAL THICKNESS OF UNCHANGED FLAPS*

PATIENT	A	B	V
I–3	98	195	146.5
4	49	439	244
5	73	122	97.5
6	48	146	97
	73	134	103.5
7	49	122	85.5
10	48	134	91
12	61	317	189
13	73	341	207
14	122	220	171
15	61	280	170.5
16	73	232	152.5
18	73	329	201
20	85	232	158.5
22	49	98	73.5
23	49	207	128
25	49	126	87.5
	49	134	91.5
27	49	134	91.5
28	61	171	116
31	73	146	109.5
32	73	488	280.5
33	61	195	128
34	73	122	97.5
35	85	134	109.5
36	98	207	152.5
37	122	366	244
38	49	171	110
39	49	98	73.5
40	73	122	97.5
II–3	98	220	159
4	122	463	292.5
8	49	146	97.5
	49	171	110
9	73	244	158.5
15	146	780	463

* Figures in microns and based on measuring 10 sections per biopsy.

EPITHELIAL THICKNESS OF POST–OPERATIVE SAMPLES (UNCHANGED)*

II–3	242	878	560
16	134	402	268
18	280	1146	713
19	171	390	280.5

* Figures in microns and based on measuring 10 sections per biopsy.

EPITHELIAL THICKNESS OF CHANGED FLAPS*

PATIENT	A	B	V
I–1	159	707	433
2	85	280	182.5
8	85	171	128
9	98	244	171
11	122	220	171
17	280	439	329.5
19	244	365	305
21	183	646	414.5
24	134	244	189
26	122	488	305
29	122	293	207.5
30	122	585	353.5
II–1	159	402	280.5
7	439	1195	817
12	207	512	359.5
13	195	317	256
14	146	232	189
16	220	496	358
18	439	537	488
19	220	549	384.5

* Figures in microns and based on measuring 10 sections per biopsy.

EPITHELIAL THICKNESS OF FLAPS THAT STARTED TO RECOVER*

PATIENT	A	B	V
I–17	123	268	195.5
19	110	232	171
26	134	293	213.5
II–7	171	732	451.5
	146	412	279
12	85	305	195
	110	195	152.5
14	110	220	165
	142	195	168.5
16	134	402	268
18	122	293	207.5
	122	230	176
19	171	549	360
I–1	97	866	481.5
II–1	110	341	225.5
13	98	244	171
19	110	230	170

* Figures in microns and based on measuring 10 sections per biopsy.

EPITHELIAL THICKNESS OF SKIN, ORAL MUCOSA AND FLAP IN GROUP II PATIENTS*

No.	SKIN A	SKIN B	SKIN V	ORAL MUCOSA A	ORAL MUCOSA B	ORAL MUCOSA V	FLAPS A	FLAPS B	FLAPS V
1	073	146	109.5	171	244	207.5	159	402	280.5(C)
							110	341	225.5(R)
3	049	195	122	146	220	183	242	878	560 (P)
							098	220	159 (U)
4	049	146	097.5	171	341	256	122	463	292.5(U)
7	098	195	146.5	146	244	195	439	1195	817(C)
							171	732	451.5(S)
							146	412	279 (S)
							171	549	360 (S)
8	049	146	097.5	488	683	585.5	049	146	097.5 (U)
							049	171	110 (U)
9	049	293	171	146	439	292.5	073	244	158.5 (U)
12	073	220	146.5	098	195	146.5	207	512	359.5 (C)
							085	305	195 (S)
							110	195	152.5(S)
13	085	207	146	122	256	189	195	317	256 (C)
							098	244	171 (R)
14	073	195	134	146	220	183 #	146	232	189 (C)
							110	220	165 (S)
							142	195	168.5(S)
15	085	329	207	293	390	341.5	146	780	463 (U)
16	073	293	183	268	463	365.5	134	402	268 (P)
							220	496	358 (C)
							134	402	268 (S)
18	073	207	140	085	220	152.5	280	1146	713 (P)
							439	357	398 (C)
							122	293	207.5(S)
							122	230	176 (S)
19	085	220	152.5	098	500	299	171	390	280.5(P)
							220	549	384.5(C)
							110	230	170 (R)

* figures in microns and based on measuing 10 sections per biopsy.

A thinnest part measured.

B thickest part measured.

V mean of A + B.

\# masticatory mucosa.

(C) changed

(R) partially recovered

(P) post-operative

(U) unchanged

(S) silver-white

Analysis of variance

Variation	SS	d.f.	Mean sq	F	P
Between groups	201033	3	67011	20.37	<0.001
Within groups	240165	73	3289		
Total	441199	76			

Table of means for thickness by appearance

Level	Count	Average	Stnd. Error (internal)	99 Percent intervals	Confidence for mean
change	20	189	±22	155	222
normal	36	74	± 4	48	99
postop	4	206	±33	130	282
recover	17	123	± 5	86	160
Total	77	121	6	104	139

Multiple range analysis for thickness by appearance

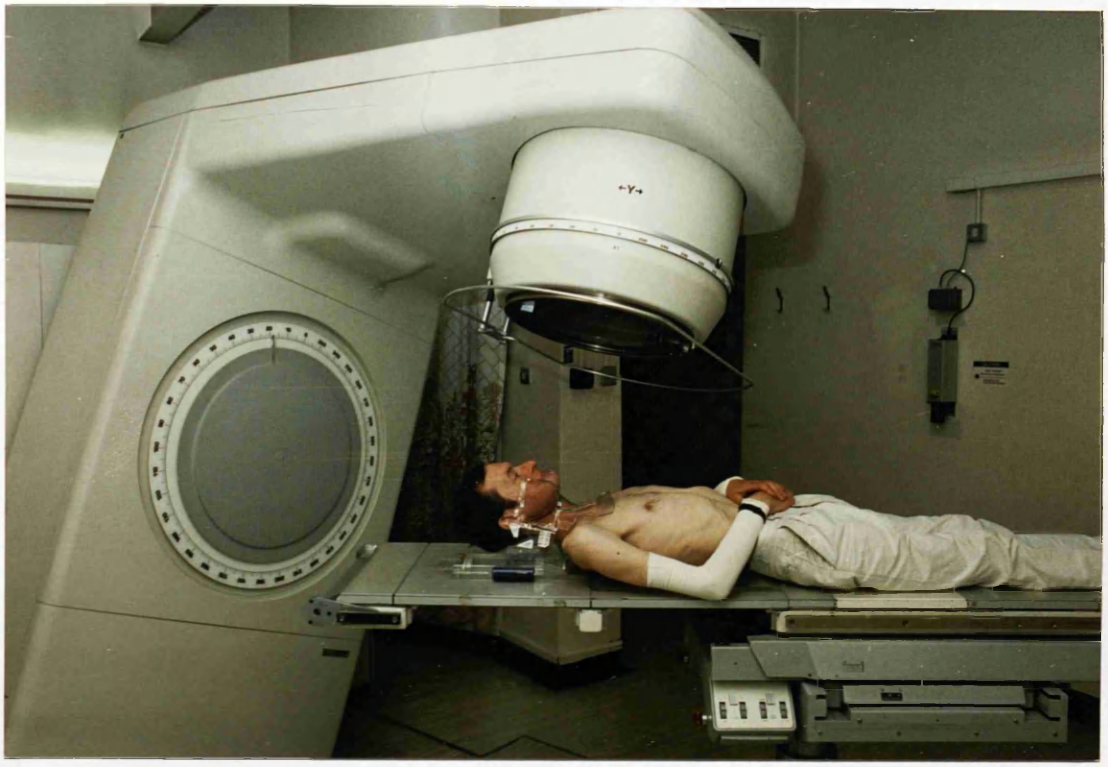
Method: 95 Percent Confidence Intervals

Level	Count	Average	Homogeneous Groups
normal	36	74	*
recover	17	123	*
change	20	189	*
postop	4	206	**

PLATES

Fig. 1. The position of the patient during radiotherapy. The machine used is a Philips SL75, 5MV linear accelerator (Courtesy of Dr. A. G. Robertson).

Fig. 2. Close-up of the patient in Fig. 1 showing the beam directing shell outlining the field of radiotherapy (Courtesy of Dr. A. G. Robertson).



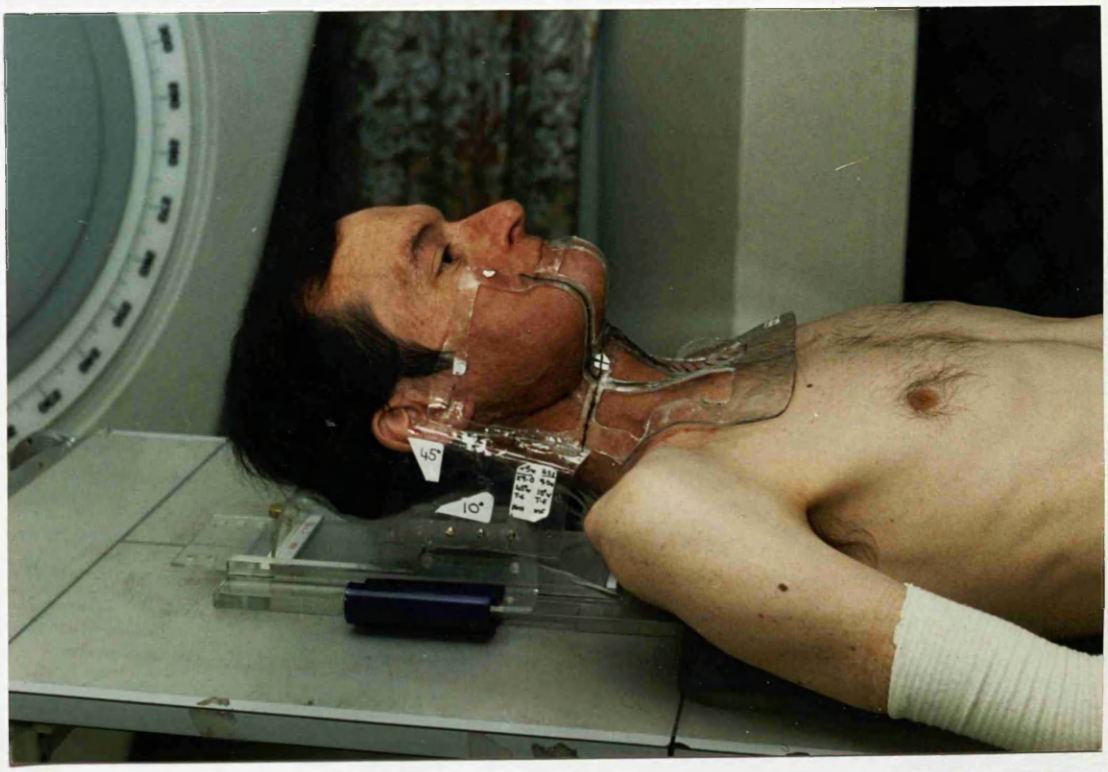

45°
10°

Fig. 3. Sketch showing the cutaneous distribution of the radial and ulnar arteries in the forearm (adapted from Cormack & Lamberty, 1986).

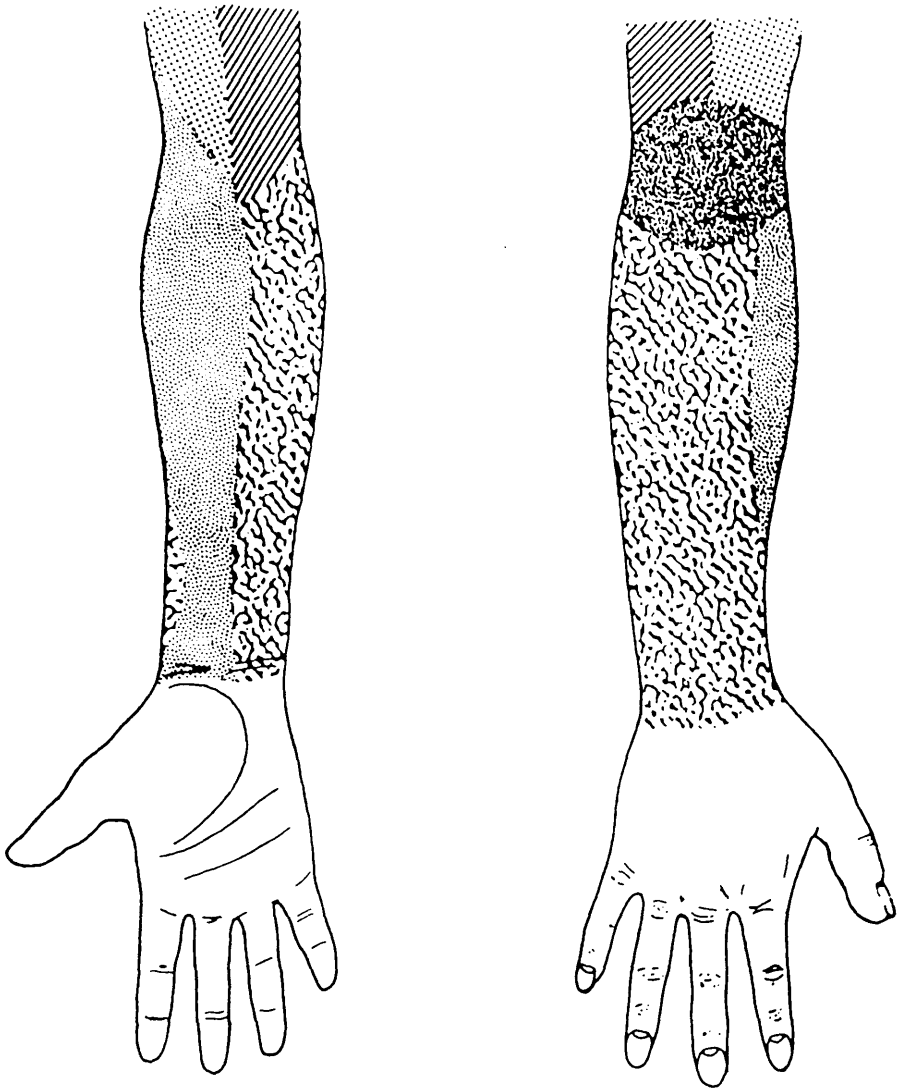
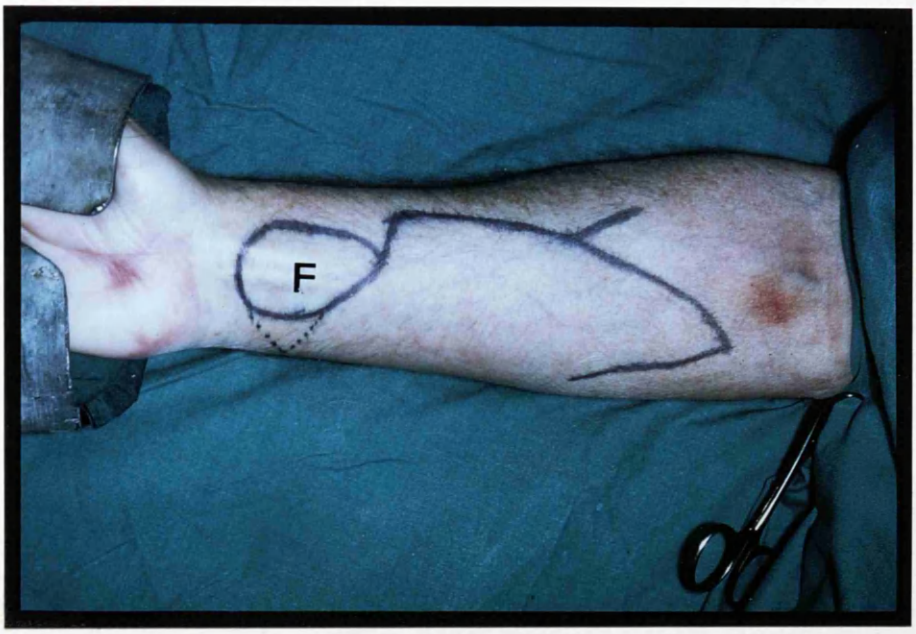

Ulnar artery
Brachial artery
Brachial artery
Profunda brachii artery
Olecranon anastomosis

Fig. 4. The design of the free radial forearm flap (F) and the ulnar based transposition flap to close the donor defect. (Courtesy of Mr. D. S. Soutar).

Fig. 5. Raising of the free radial forearm flap (Courtesy of Mr. D. S. Soutar).


F

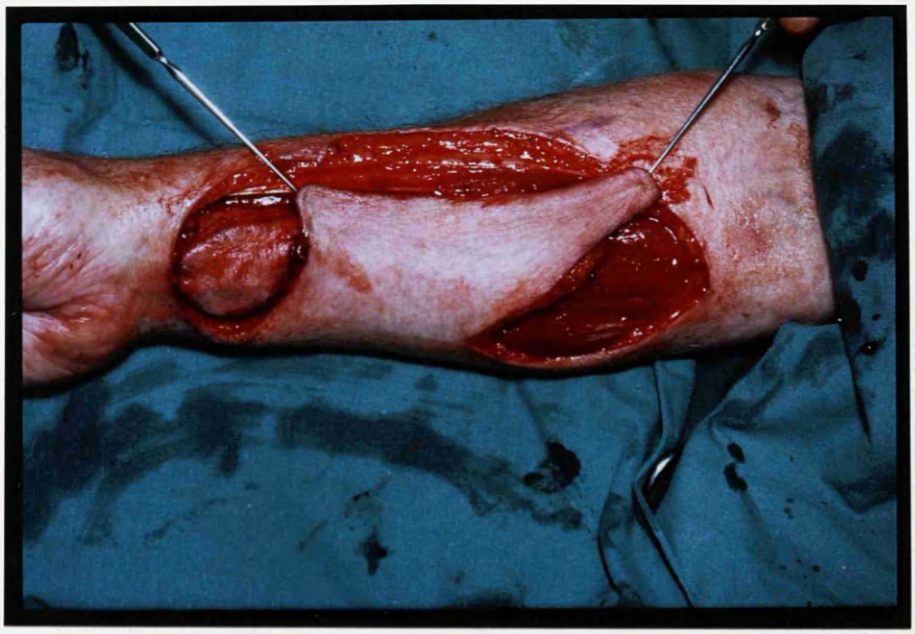


Fig. 6. The appearance of the forearm 2 years after closing the donor defect by a transposition flap based on the ulnar artery (Courtesy of Mr. D. S. Soutar).

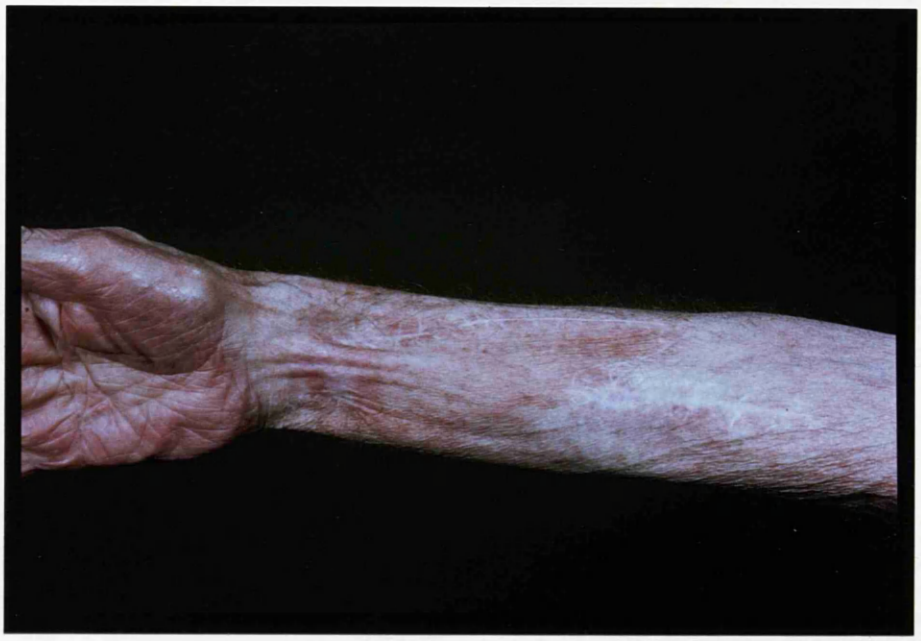
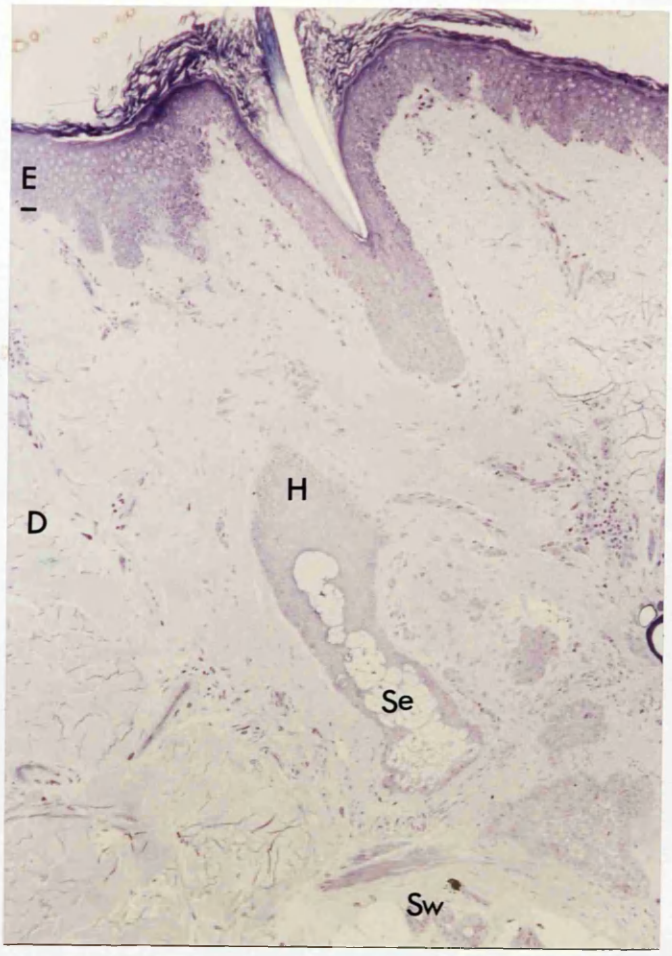


Fig. 7. Normal forearm skin.
E = Epidermis
D = Dermis
H = Hair follicle
Se = sebaceous gland
Sw = Sweat gland
Azur II. X 80.

Fig. 8. The epidermis of normal forearm skin in Fig. 7.
b = Stratum basale
s = = spinosum
g = = granulosum
c = = corneum
Azur II. X 360.


E
H
D
Se
Sw

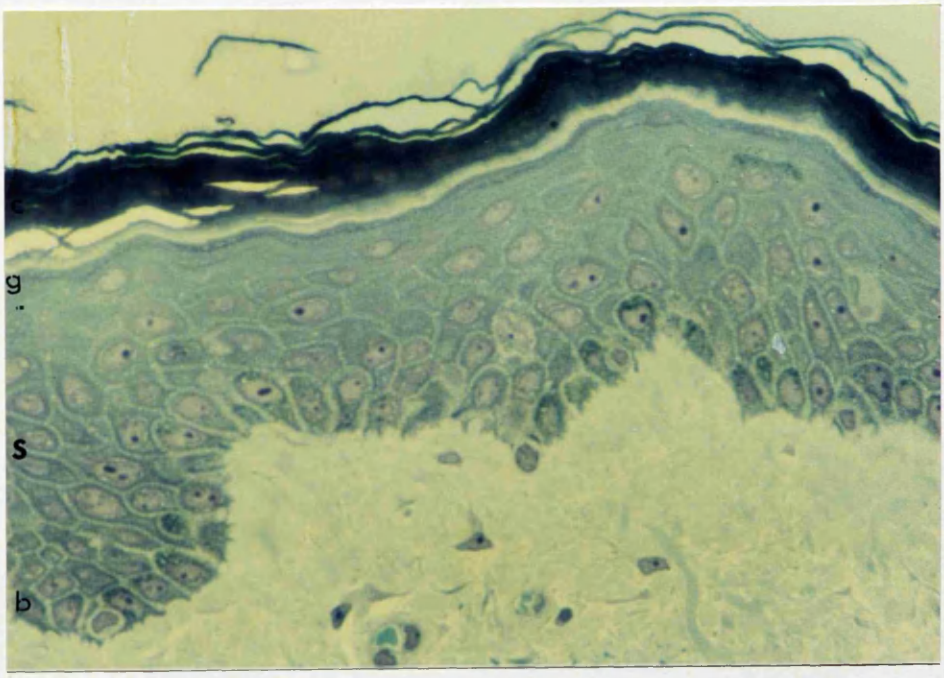

c
g
s
b

Fig. 9. Sketch of the epidermal-melanin unit showing relationship between a melanocyte (M) and keratinocytes (K) in the epidermis (adapted from Jimbow et al., 1976).

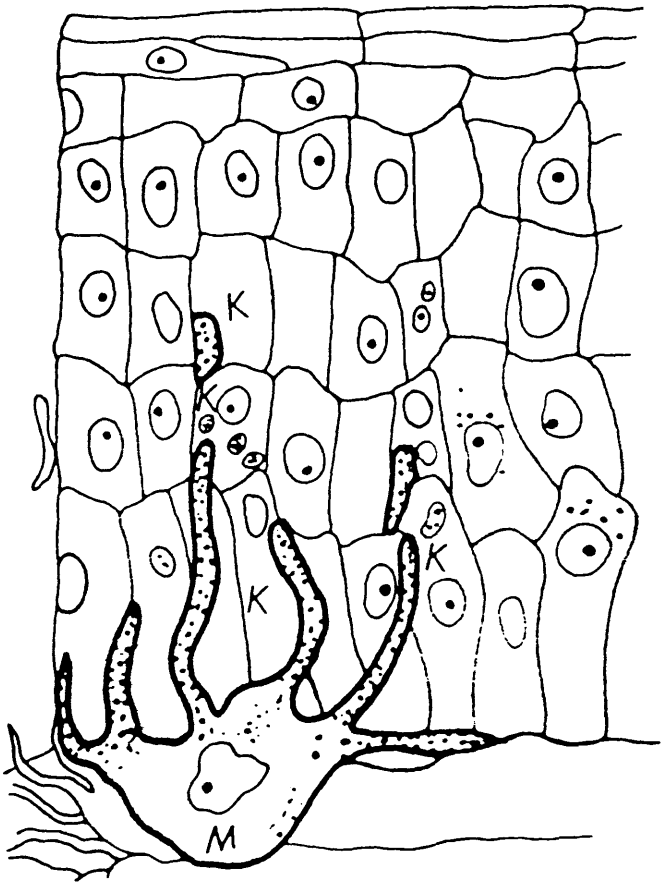
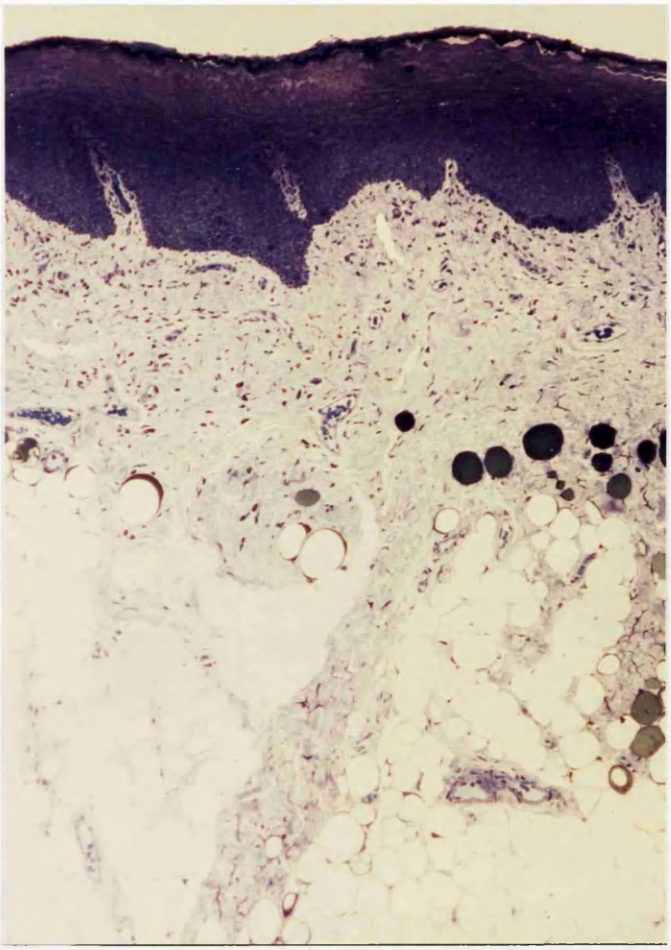

K
K
K
K
M

Fig. 10. Normal lining (buccal) oral mucosa.
Azur II. X 70.

Fig. 11. The lining mucosal epithelium in Fig.10.
b = Stratum basale
f = Stratum filamentosum
d = Stratum distendum
Azur II. X 240.



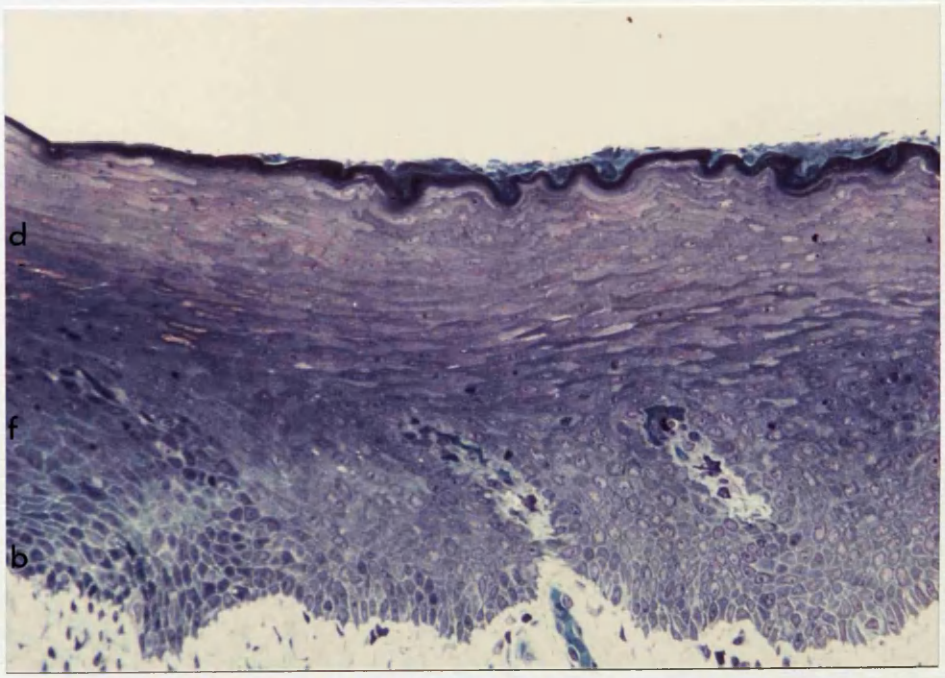
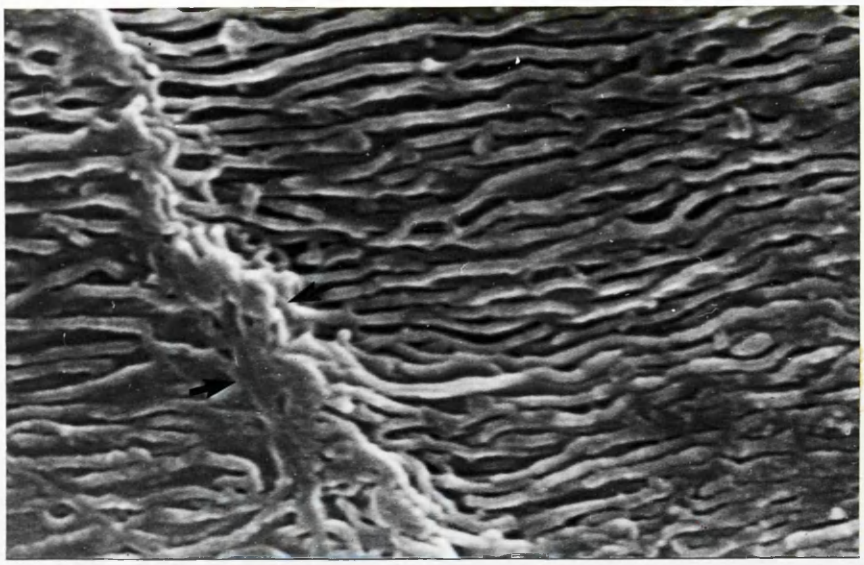

d
f
b

Fig. 12. Scanning electron micrograph of a buccal mucosal biopsy showing two adjacent cells with parallel microplicae separated by a raised intercellular ridge (arrows) X 7030.

Fig. 13. Scanning electron micrograph of a buccal mucosal biopsy, the cell surface shows microplicae which are extensively branched and arranged in whorls in some areas. X 5000.



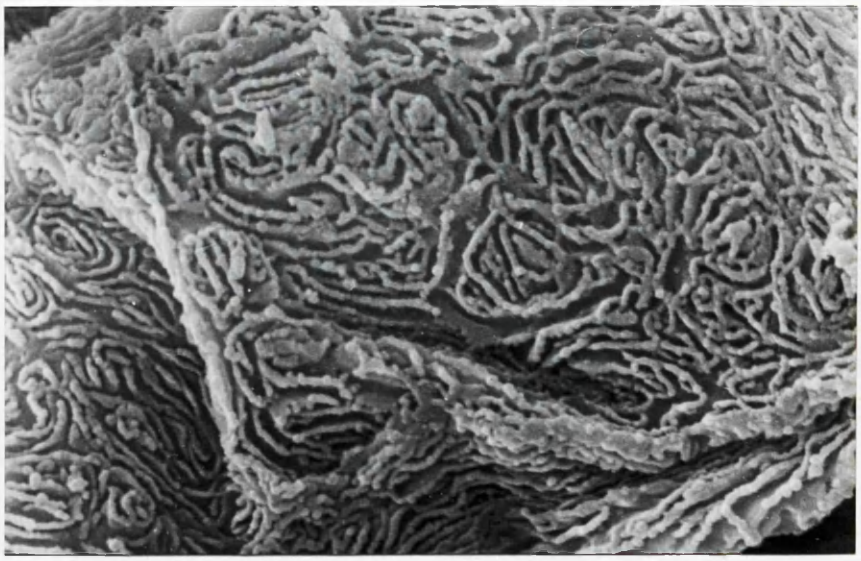
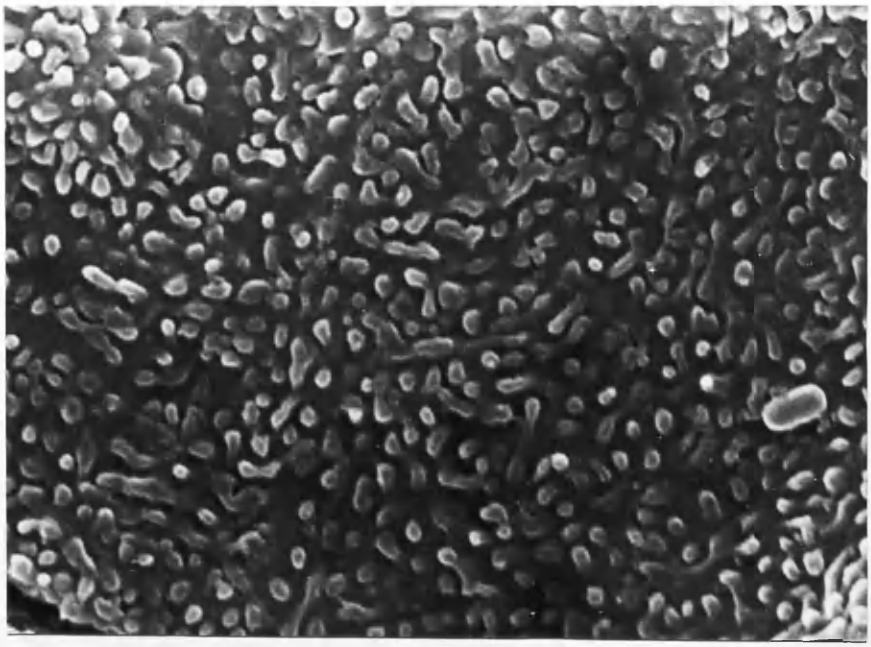


Fig. 14. Scanning electron micrograph of mucosal biopsy, the cell surface shows microvilli. X 5000.

Fig. 15. Scanning electron micrograph of a buccal swab covered with cells. X 50.



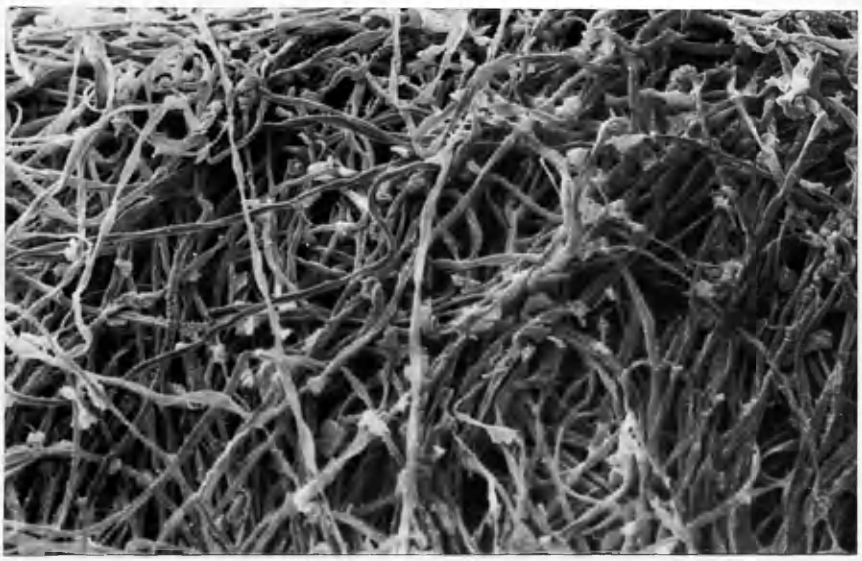
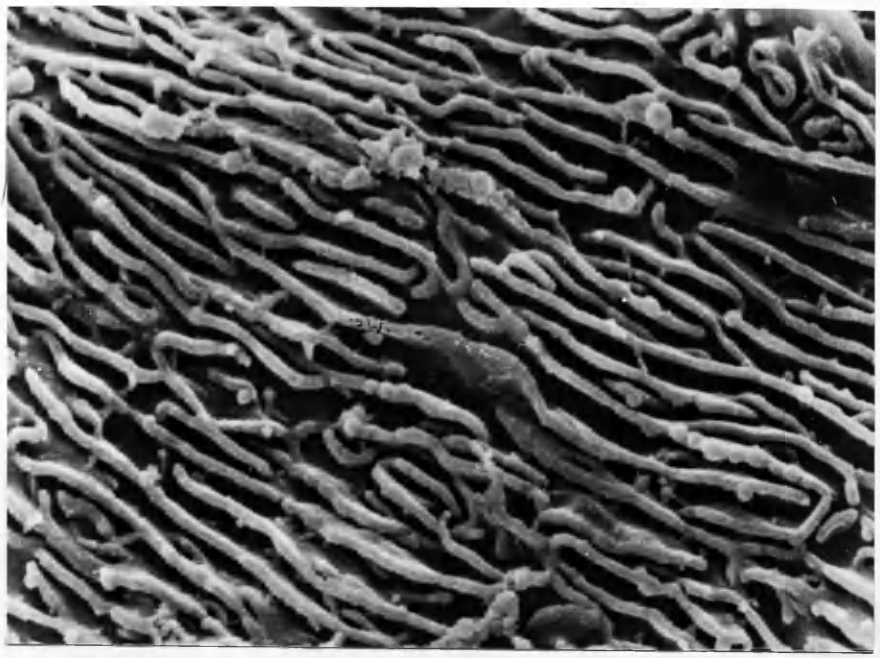


Fig. 16. Scanning electron micrograph of a buccal swab, the cell surface shows parallel microplicae. X 7030.

Fig. 17. Scanning electron micrograph of a buccal swab, the cell surface shows branching microplicae. X 7030.



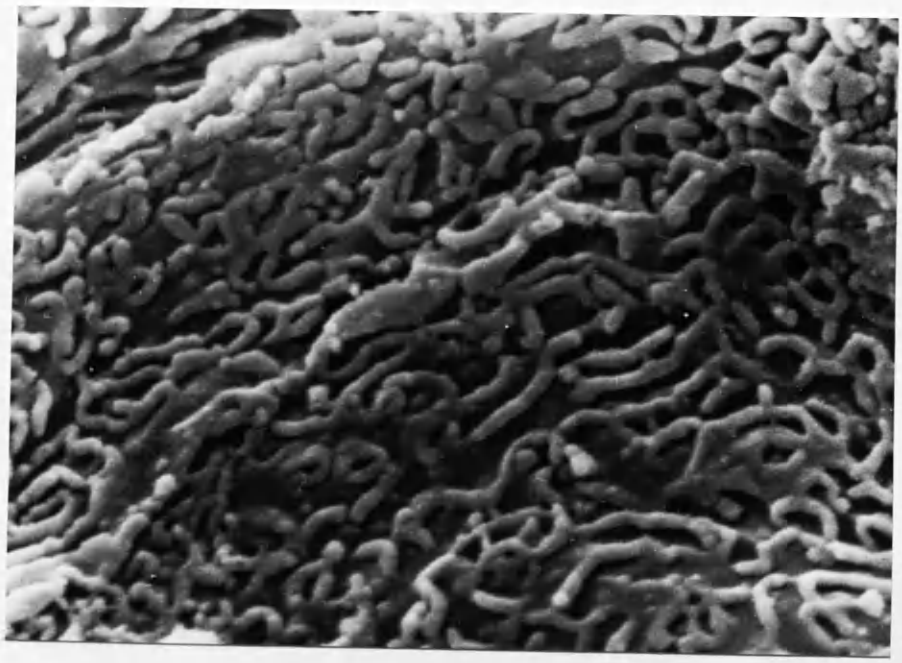
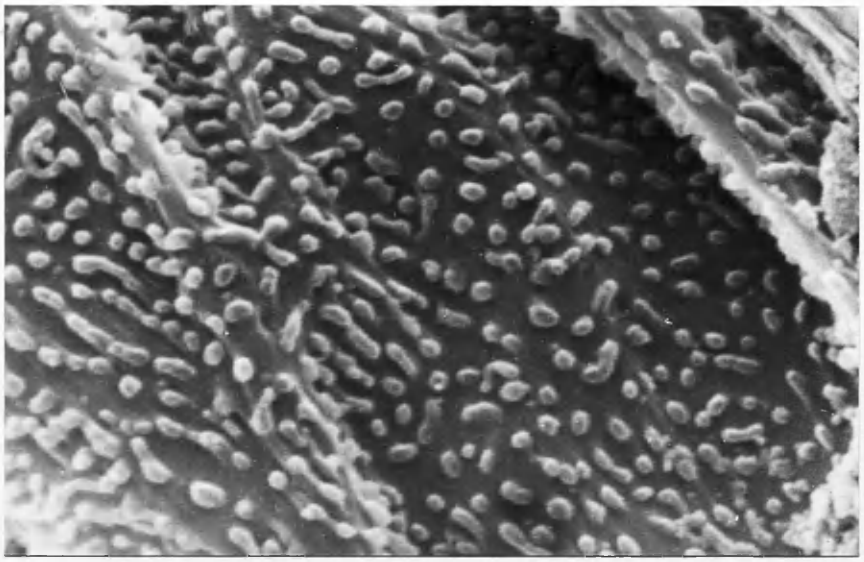


Fig. 18. Scanning electron micrograph of buccal swab showing the surface of a cell with widely-spaced microvilli. X 7030.

Fig. 19. Scanning electron micrograph of a buccal swab, showing a cell with maze appearance X 7030.



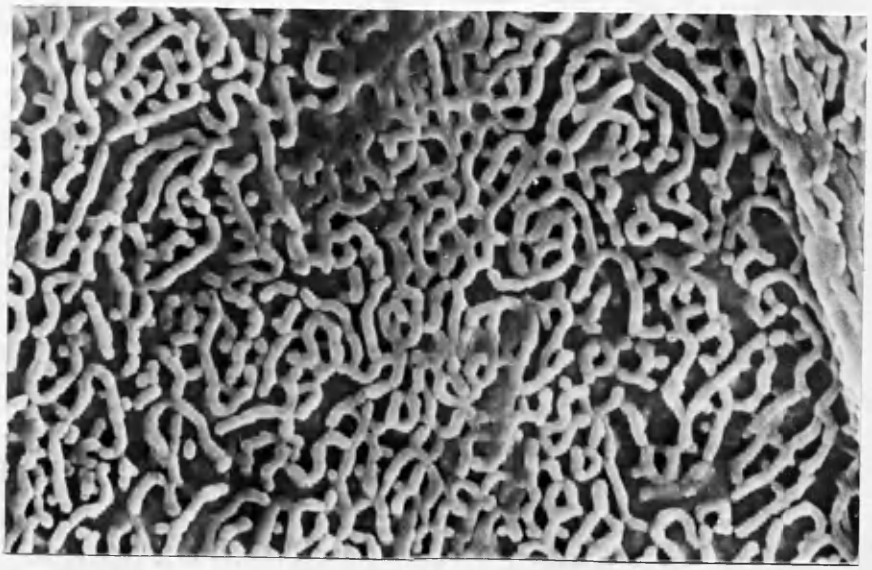
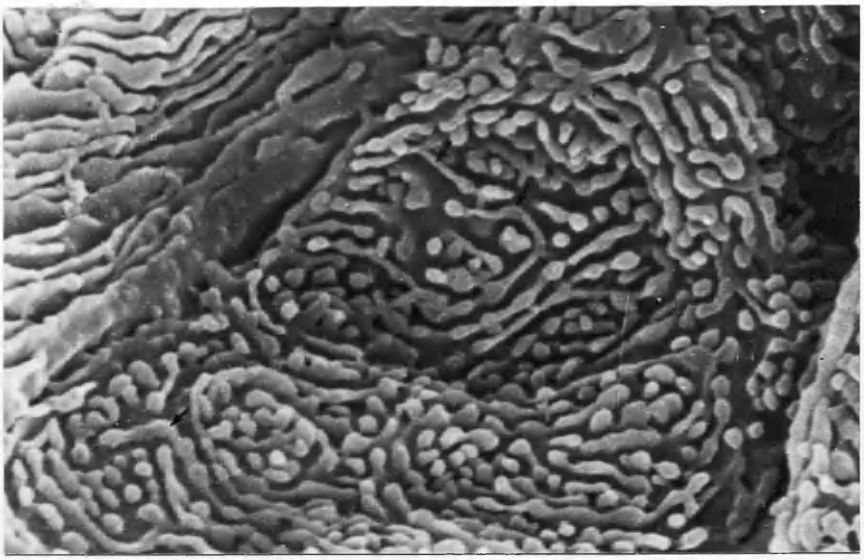


Fig. 20. Scanning electron micrograph of a buccal swab showing the surface of a cell with microvilli surrounded by microplicae (arrows). X 7030.

Fig. 21. Scanning electron micrograph of buccal swab showing two surfaces of the same cell. 1. Microvilli. 2. Maze pattern. X 7030.



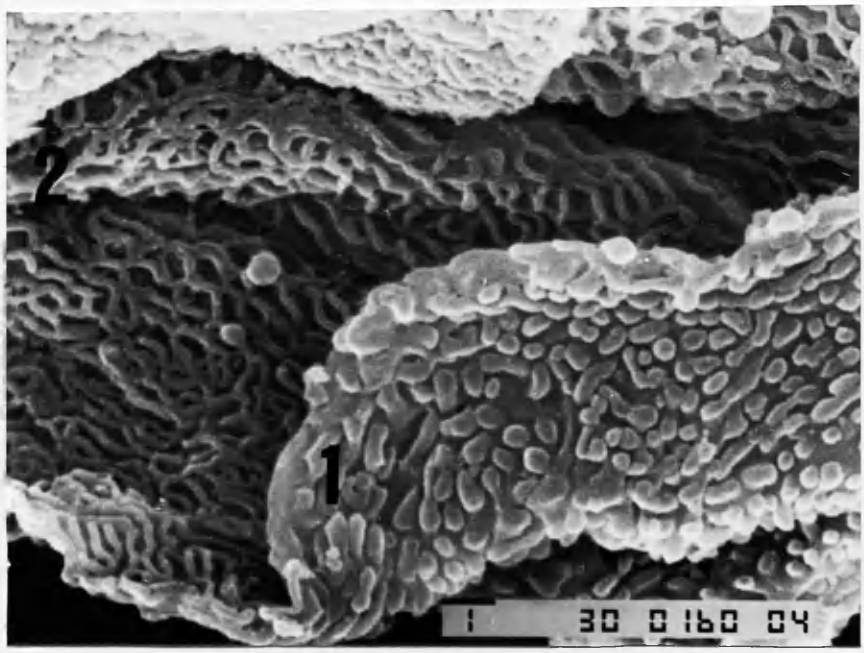
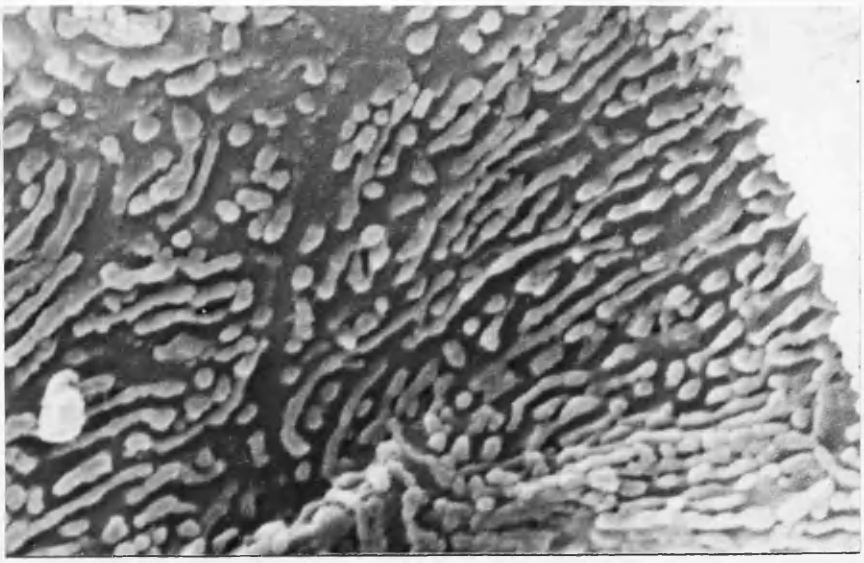

2
1
1 30 0160 04

Fig. 22. Scanning electron micrograph of buccal swab showing the surface of a cell with microvilli interspersed between microplicae. X 7030.

Fig. 23. Scanning electron micrograph showing two adjacent cells separated by a smooth area (arrows); the two cells have different surface patterns. 1. Microvilli interspersed between microplicae. 2. Microridges. X 4750.



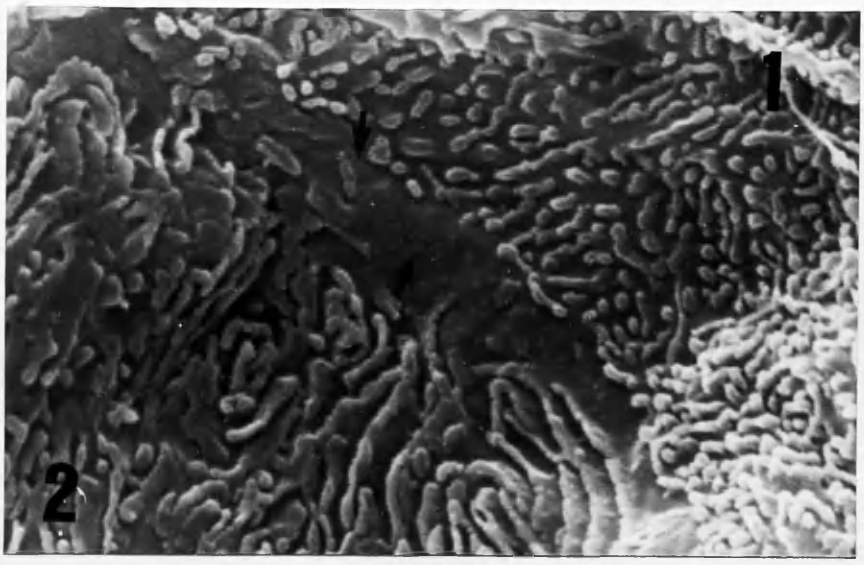
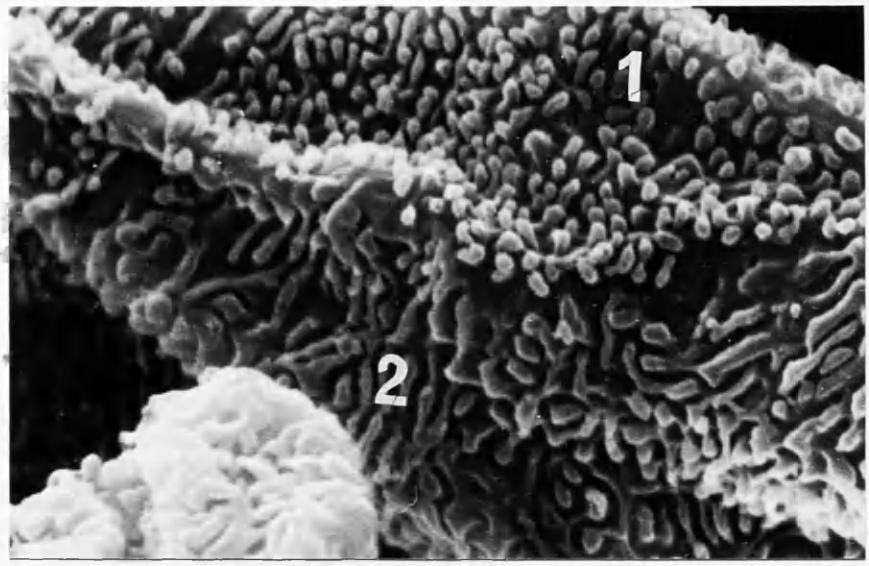

1
2

Fig. 24. Scanning electron micrograph of buccal swab showing two adherent cells with different surface patterns. 1. Microvilli. 2. Microplicae. X 7030.

Fig. 25. Scanning electron micrograph of buccal swab showing a group of adherent cells with different surface patterns. 1. Maze pattern. 2. Microplicae and microvilli. 3. Whorls. X 4750.


1
2

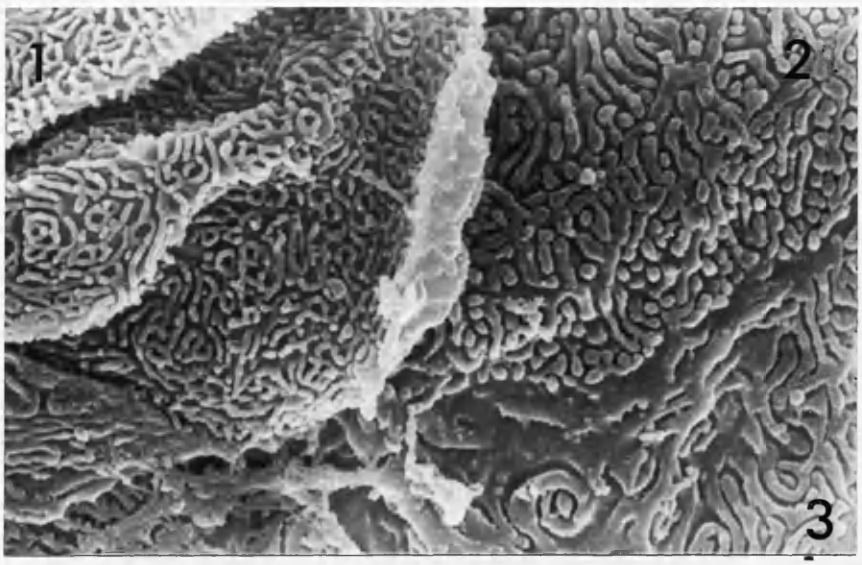

1
2
3

Fig. 26. Scanning electron micrograph showing the surface of a cell with closely packed microplicae. X 5000.

Fig. 27. Scanning electron micrograph of buccal swab showing the surface of a cell with closely packed microvilli. X 7030.

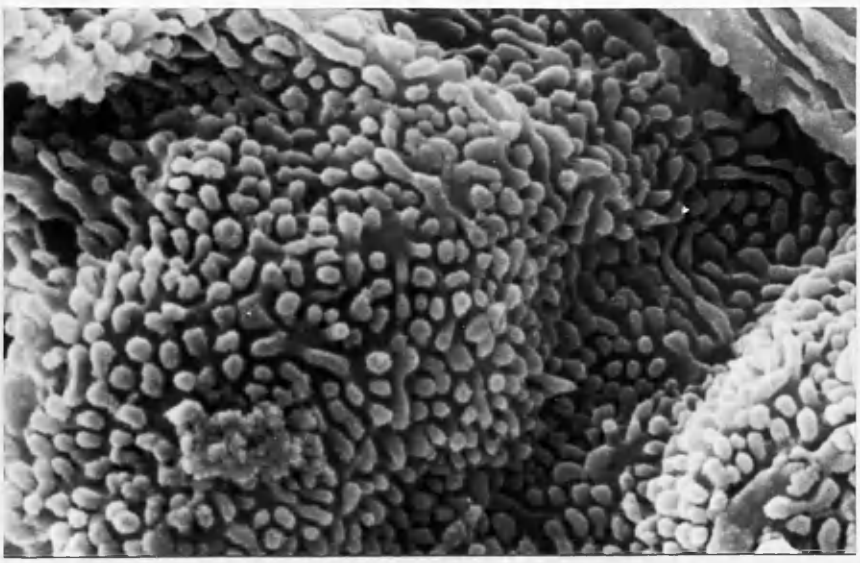
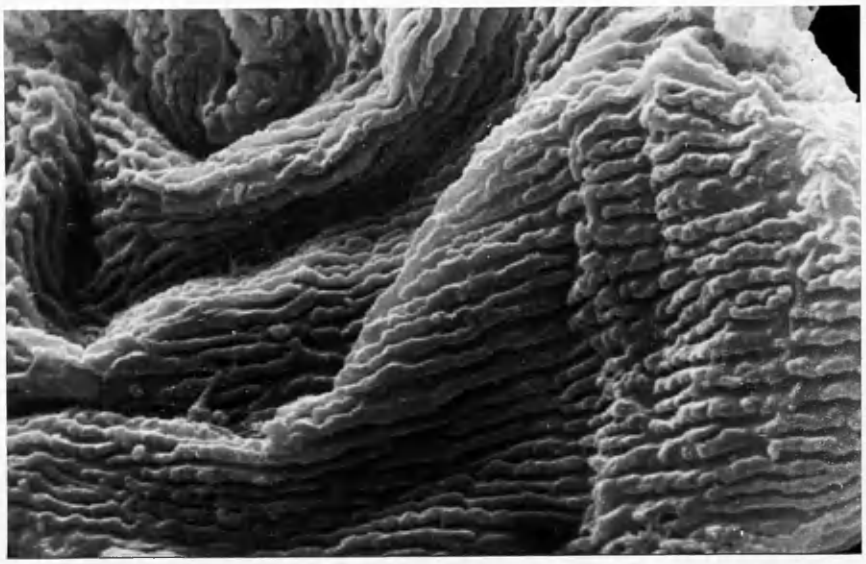


Fig. 28. Scanning electron micrograph of a buccal swab showing the surface of a cell with thickened closely packed microplicae. X 5000.

Fig. 29. Scanning electron micrograph of a swab from the surface of an intraoral radial forearm flap taken two weeks after the patient started to receive radiotherapy. The swab is covered with mucus and cells with no surface features. X 200.



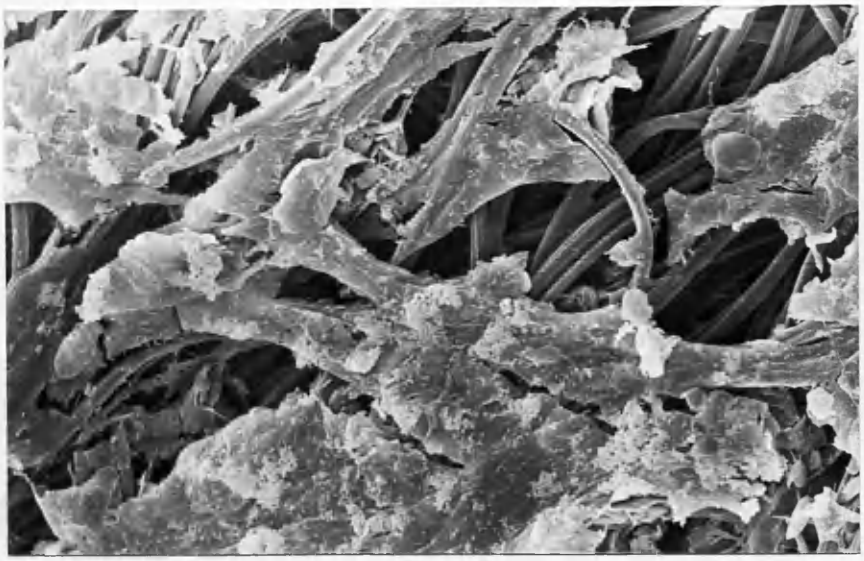
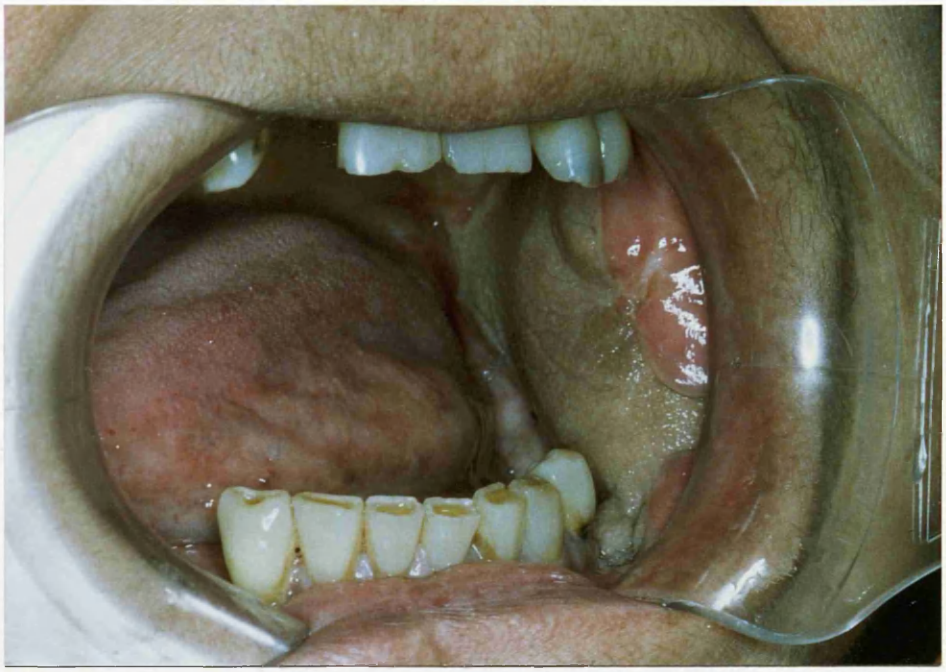


Fig. 30. The appearance of an unchanged non-irradiated flap 5 years after surgery (Patient I - 27).

Fig. 31. The appearance of a changed flap 1 year after radiotherapy. Arrows point to the boundaries of the flap (Patient I - 19).



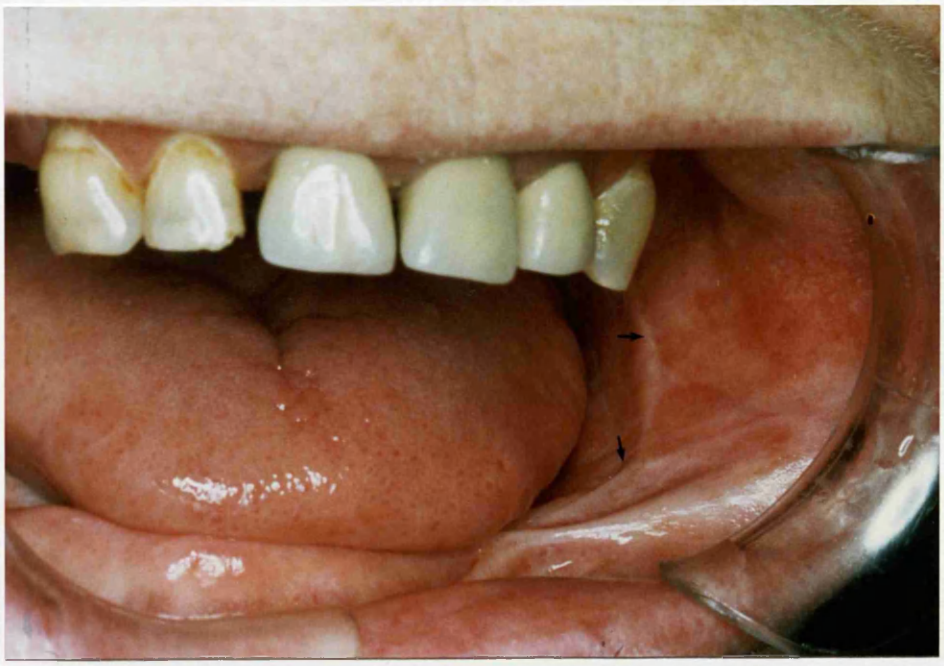
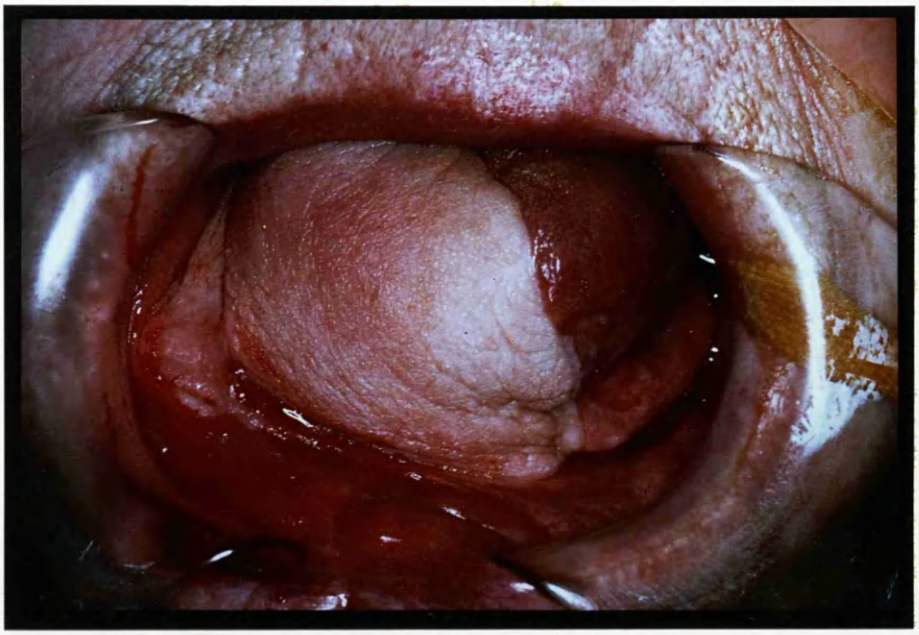


Fig. 32. The appearance of an unchanged non-irradiated forearm flap 7 years after surgery (Patient I - 20).

Fig. 33. The appearance of the flap in Fig. 32, six months later by which time it showed change to mucosal form (F = Flap).



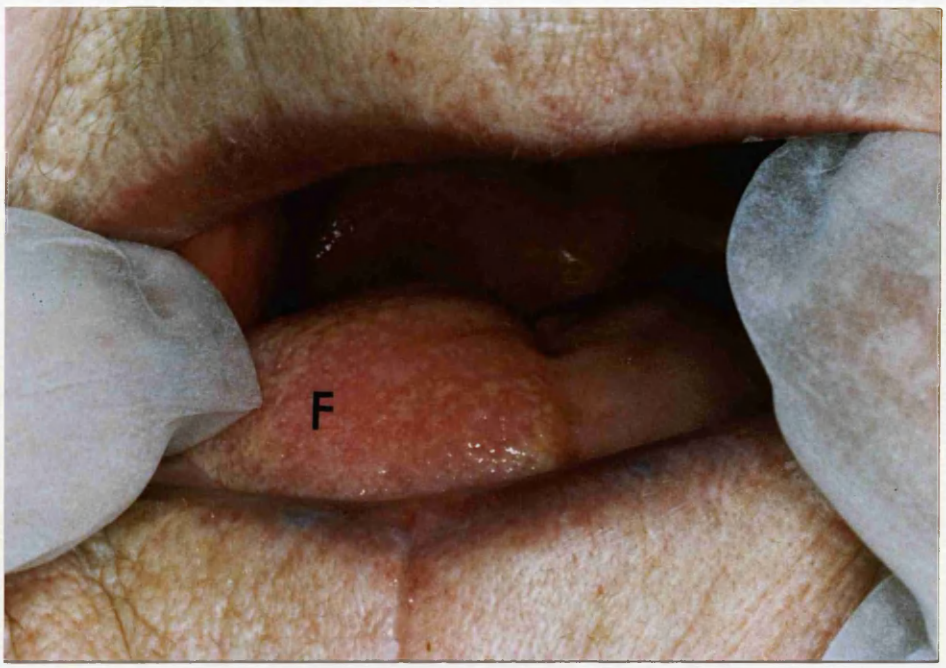

F

Fig. 34. The appearance of an unchanged non-irradiated forearm flap, hairs are seen at the margin of the flap 2 years after surgery. (Patient I - 25)

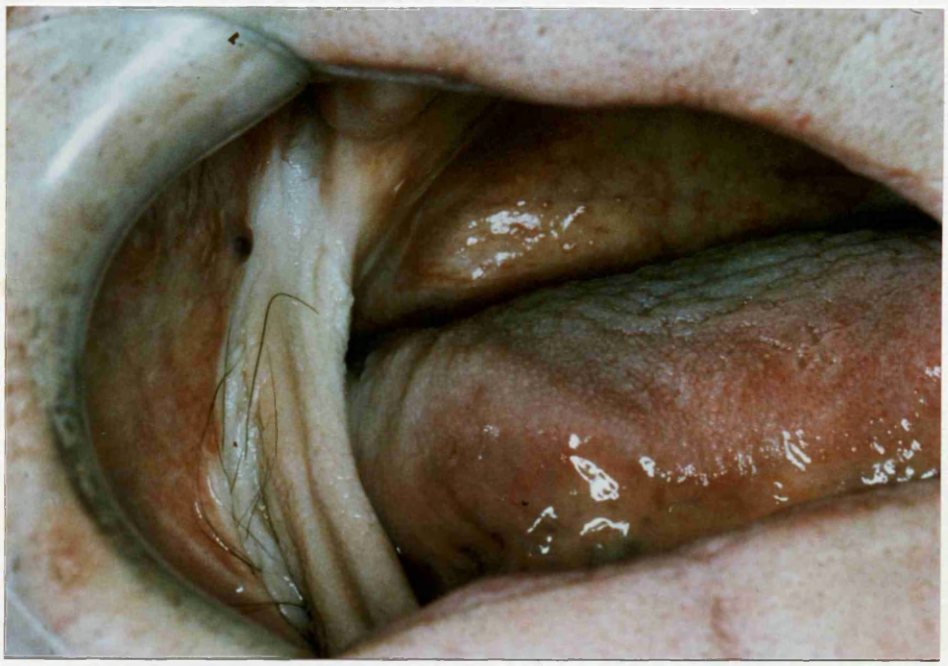
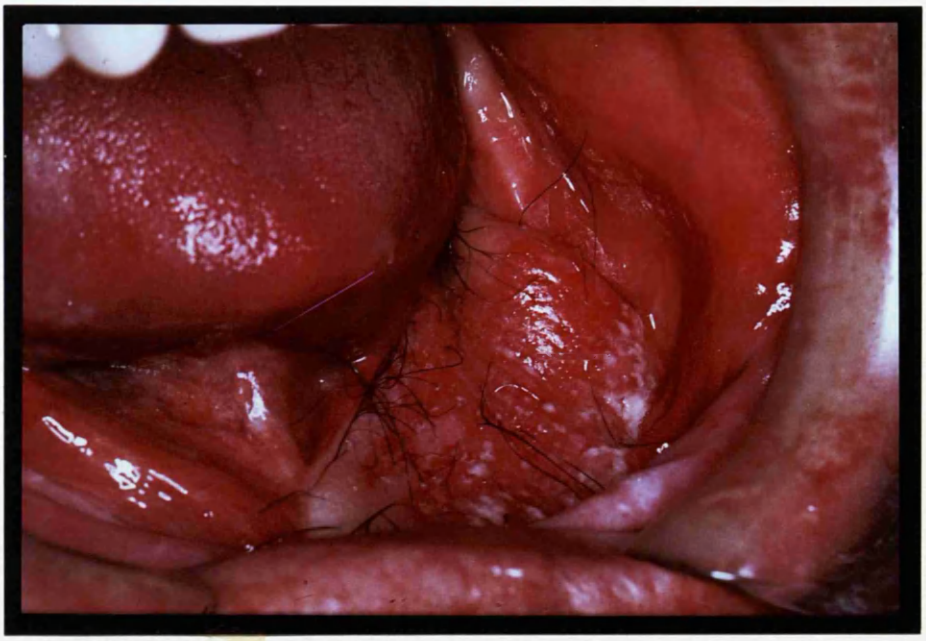


Fig. 35. Non-irradiated flap 2 years after surgery showing partial change in colour towards that of mucosa. Hairs are seen in the non-changed part (Patient I - 1).

Fig. 36. The same flap in Fig. 35, three years after surgery, the changed area appears smaller.



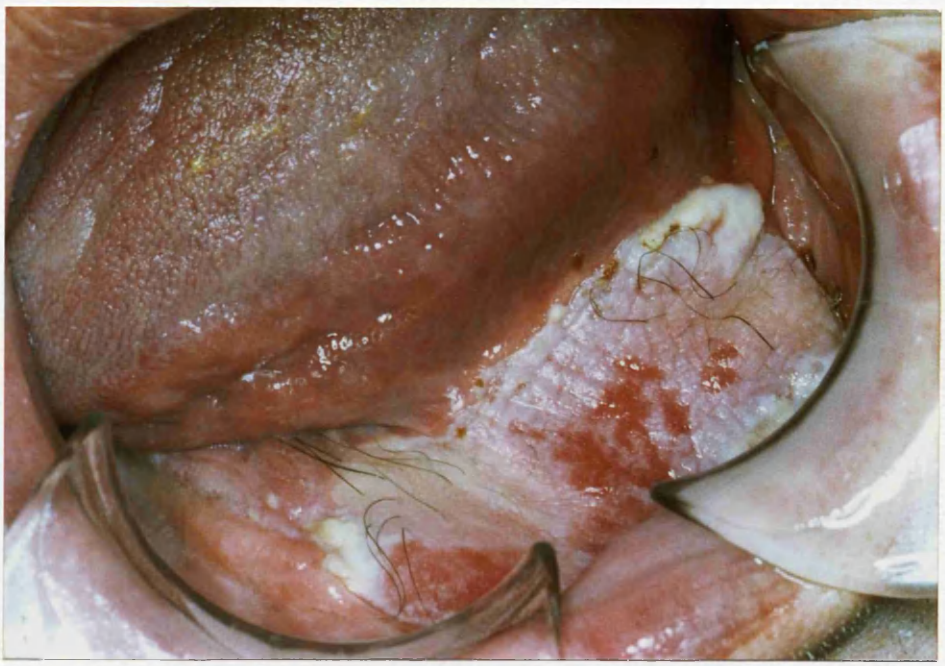
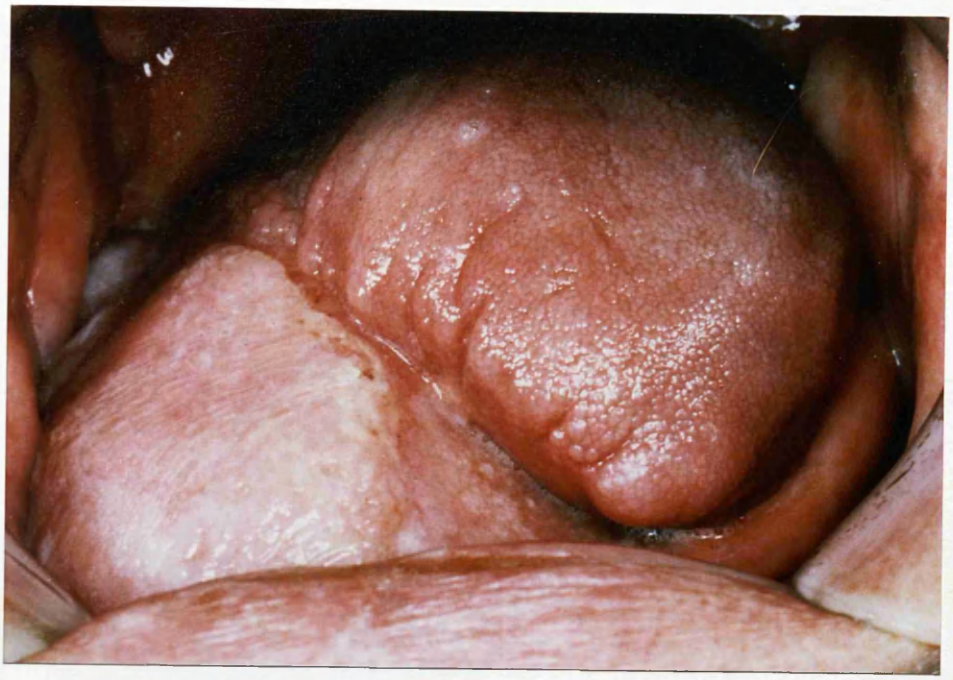


Fig. 37. The silver-white appearance of the flap (Patient II - 7) six months after radiotherapy.

Fig. 38. A radial forearm flap (F) having mucosal appearance and bulging from its site in the cheek.



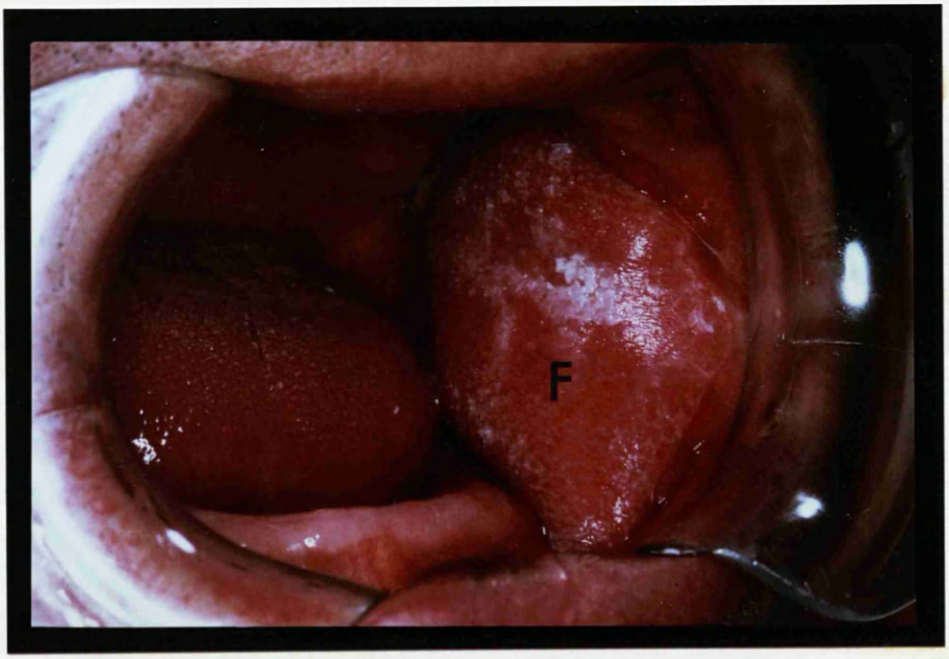
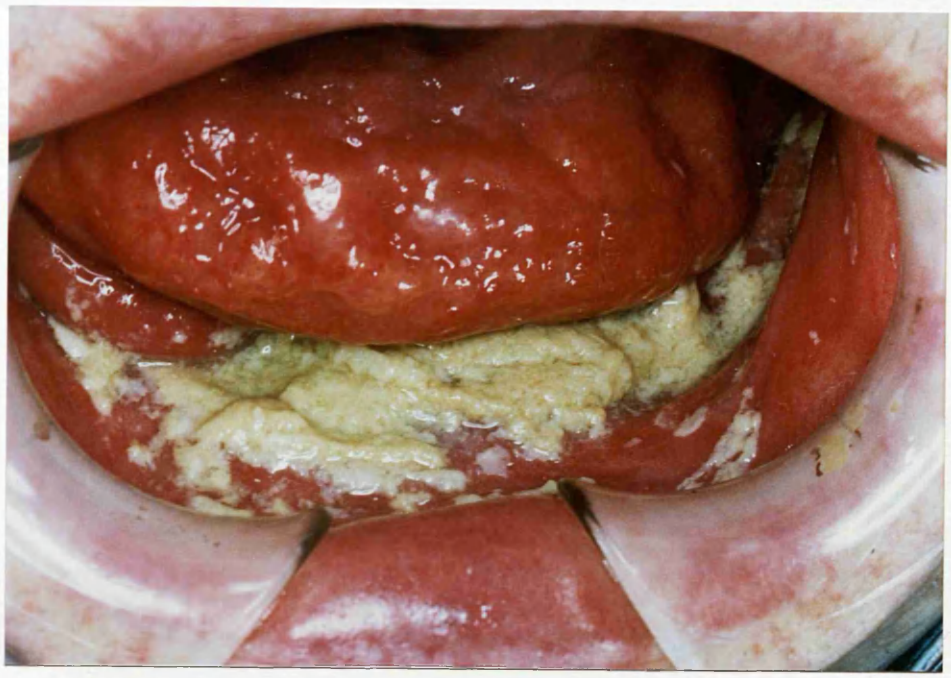

F

Fig. 39. Debris on the surface of the flap (sublingual), mouth rinse was positive for Candida albicans (Patient I - 30).

Fig. 40. The same flap In Fig. 39 after cleaning the surface.



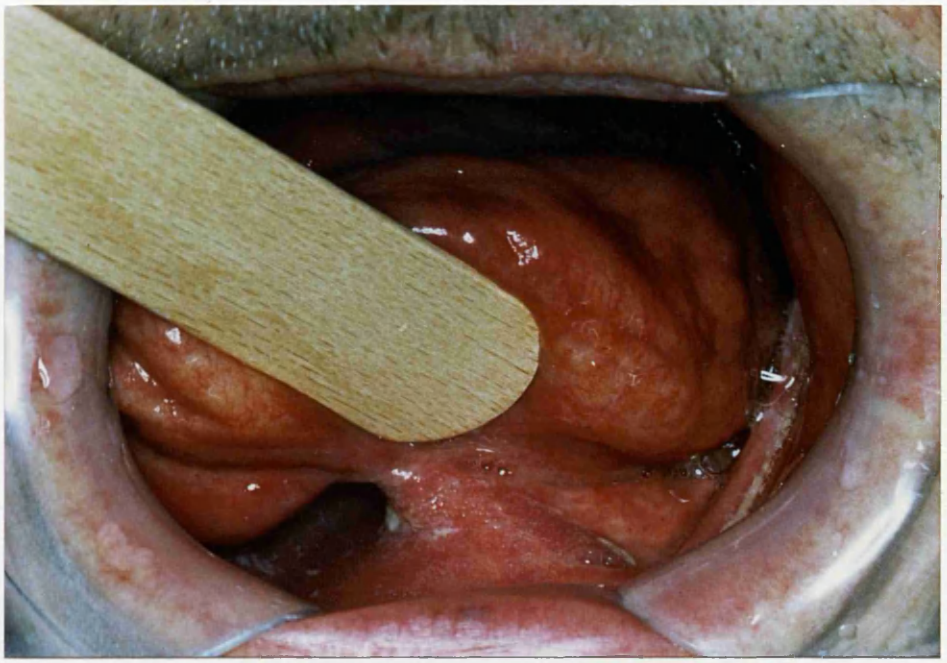
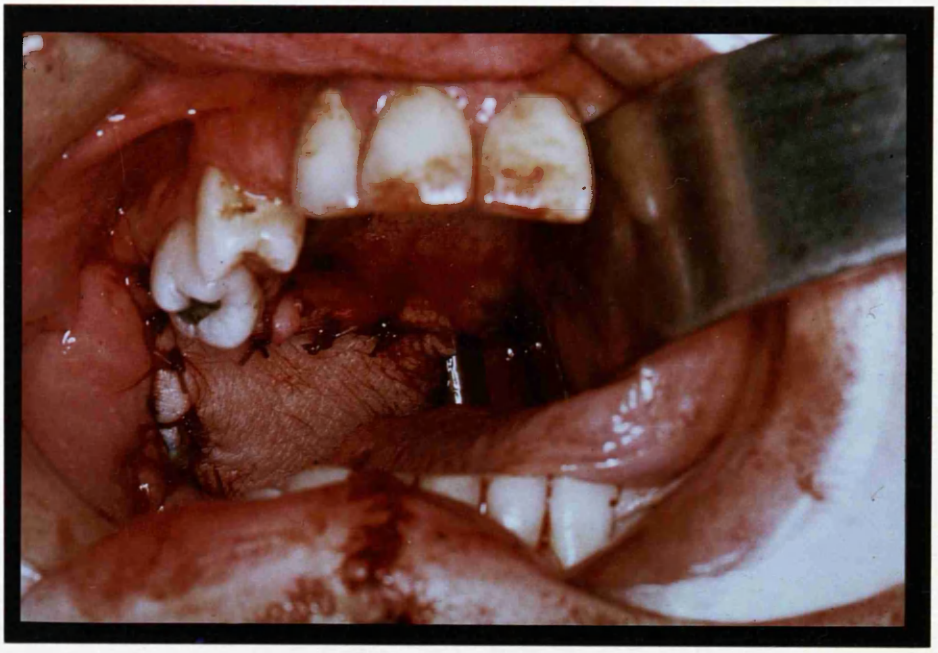


Fig. 41. The appearance of the radial forearm flap 6 days after surgery; absorbable sutures are still in place.

Fig. 42 The appearance of a non-irradiated free radial forearm flap 1 year after surgery (Patient **II** - 8).



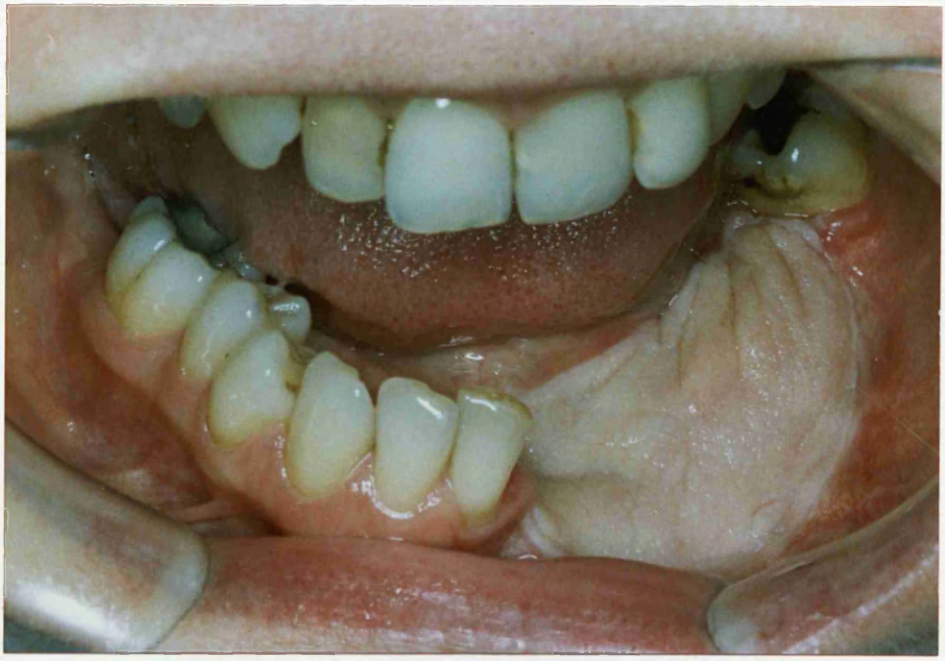


Fig. 43. The light microscopical appearance of the skin of an intraoral flap in the post-operative period, showing marked increase in epithelial thickness, increased glycogen content of the upper spinous layers. Azur II. X 60. (Patient **II** - 3).

Fig. 44. The light microscopical appearance of an unchanged irradiated flap 5 years after surgery showing slight increase in the epithelial thickness, hyperkeratosis and dilated dermal capillaries (patient I - 33). X160.

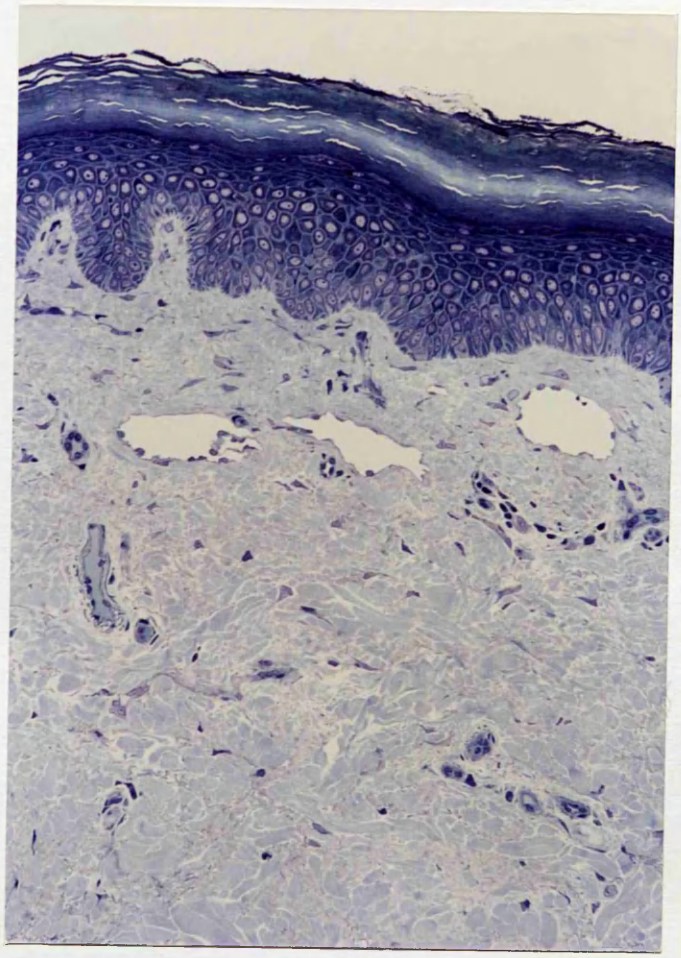


Fig. 45. The light microscopical appearance of non-irradiated flap that acquired a mucosal appearance 24 months after surgery, showing an increase in epithelial thickness (E), parakeratosis (arrows), increased glycogen content of upper spinous cells (G), severe inflammatory reaction in the dermis. Azur II. X 90. (Patient I - 1).

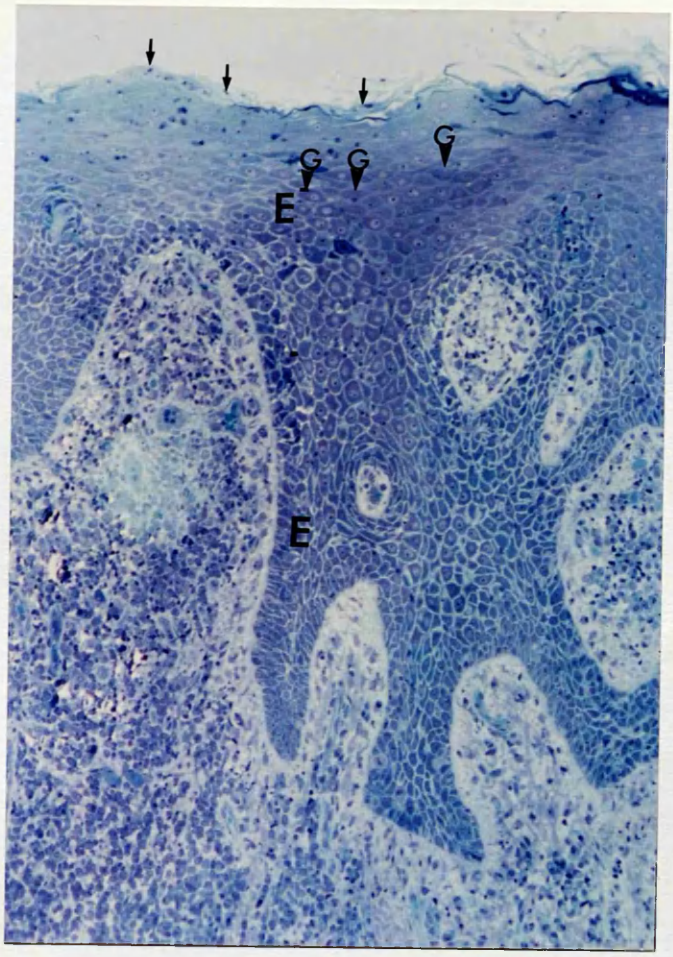

G
G
G
E
E

Fig. 46. The light microscopical appearance of an irradiated flap that acquired a mucosal appearance 4 months after surgery, showing an increase in thickness of the epithelium (E), parakeratosis (arrow heads), increased glycogen content of upper spinous cells (arrows) and inflammatory reaction in the dermis. Azur II. X 85. (Patient II - 1).

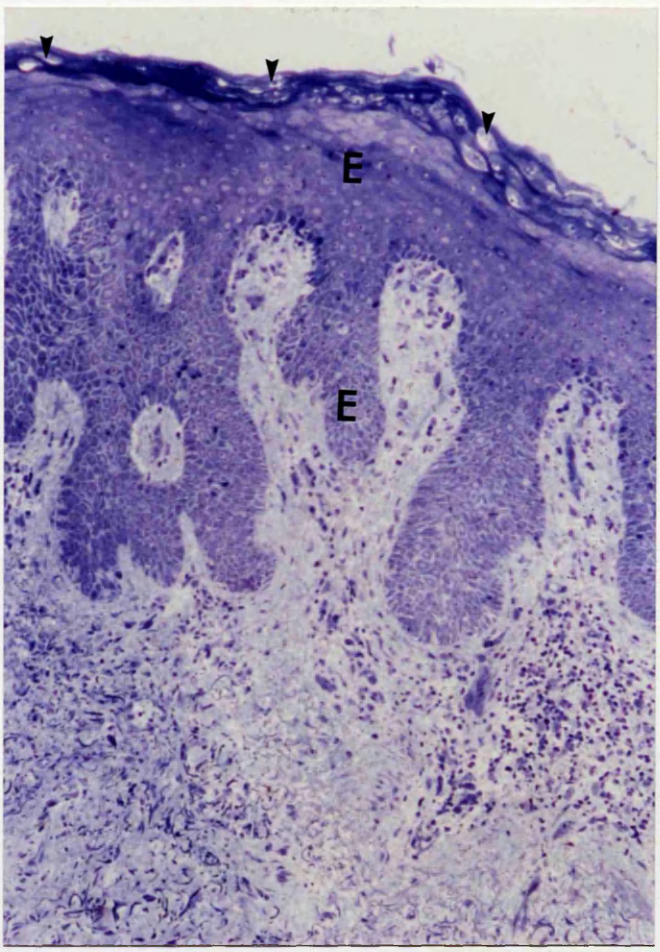

E
E

Fig. 47. The light microscopical appearance of a changed flap 6 months after surgery showing marked increase in epithelial thickness, parakeratosis and vesicles in the upper part of the epidermis. Azur II. X 70. (Patient **II** - 7).

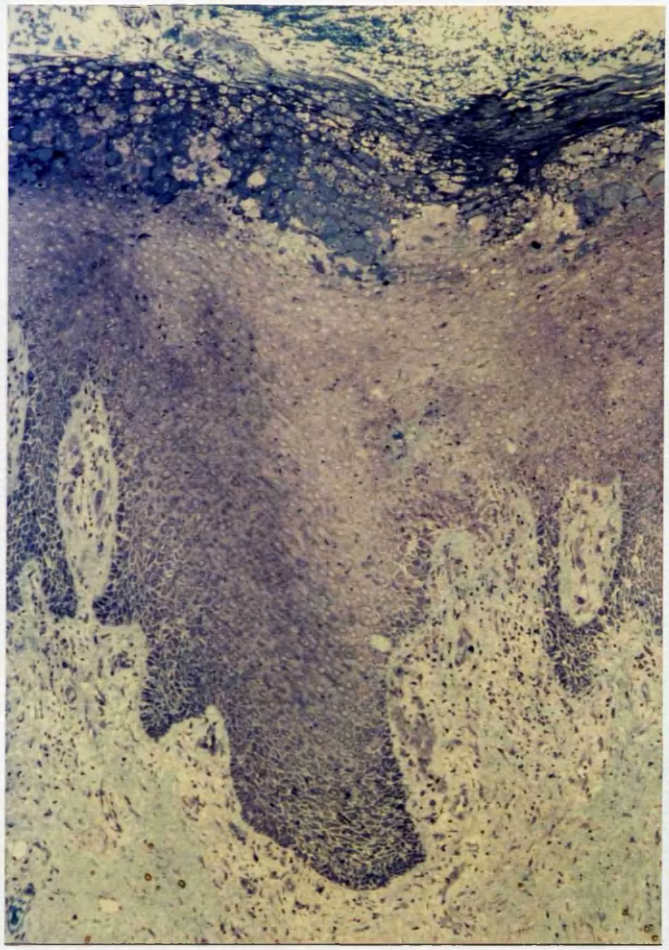
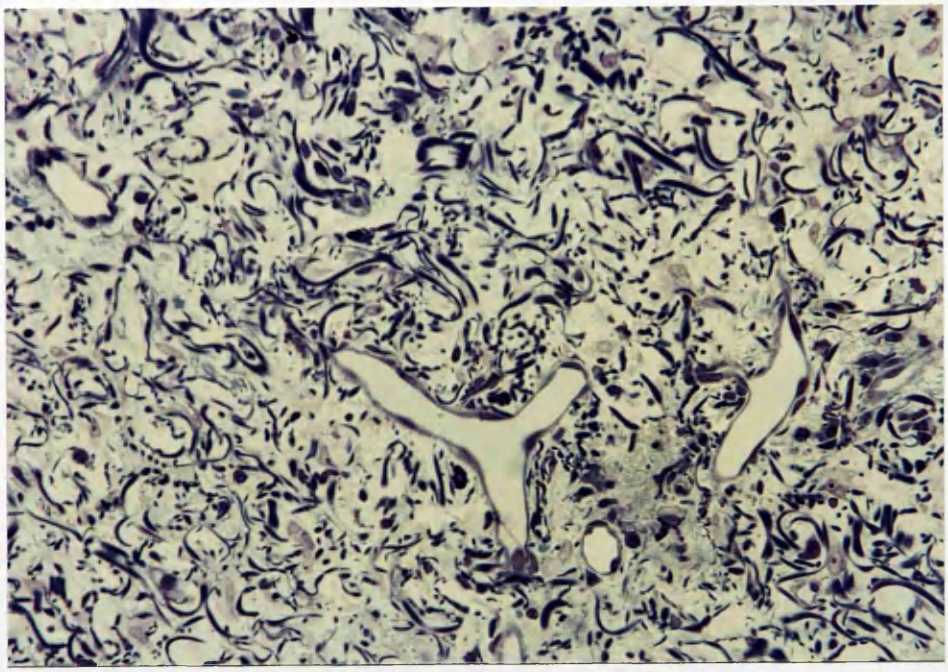


Fig. 48. The light microscopical appearance of the reticular part of the dermis of an irradiated flap showing elastic fibres randomly arranged, curved and thickened. The dermis appears cellular and some dilated lymphatic capillaries are seen. Azur II. X 280. (Patient II - 1).

Fig. 49. The light microscopical appearance of the upper part of the dermis of the flap in Fig. 45, showing inflammatory cells mainly plasma cells (arrows) and lymphocytes (arrow heads). Azur II. X 250.



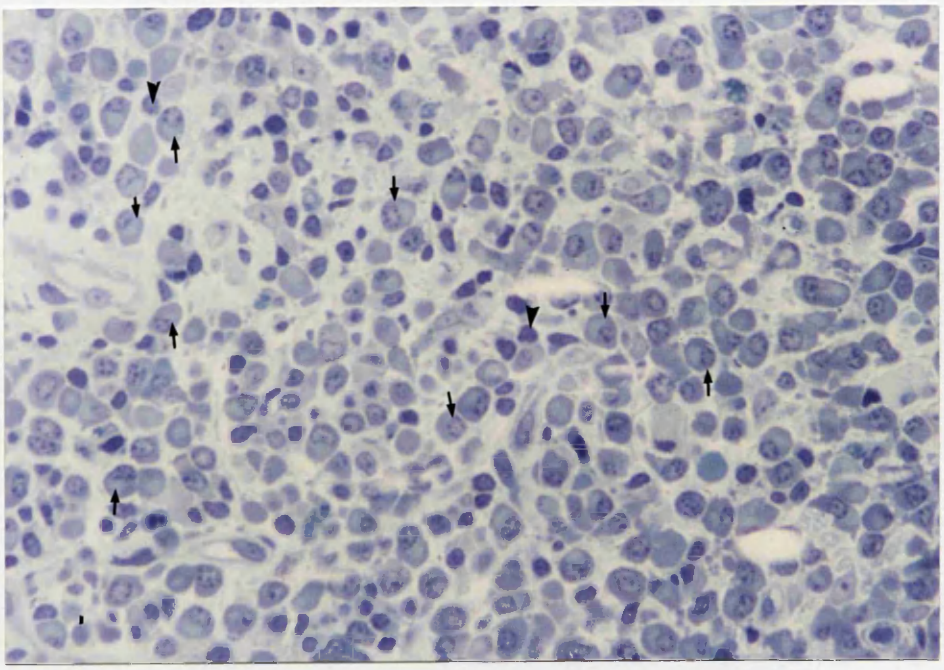
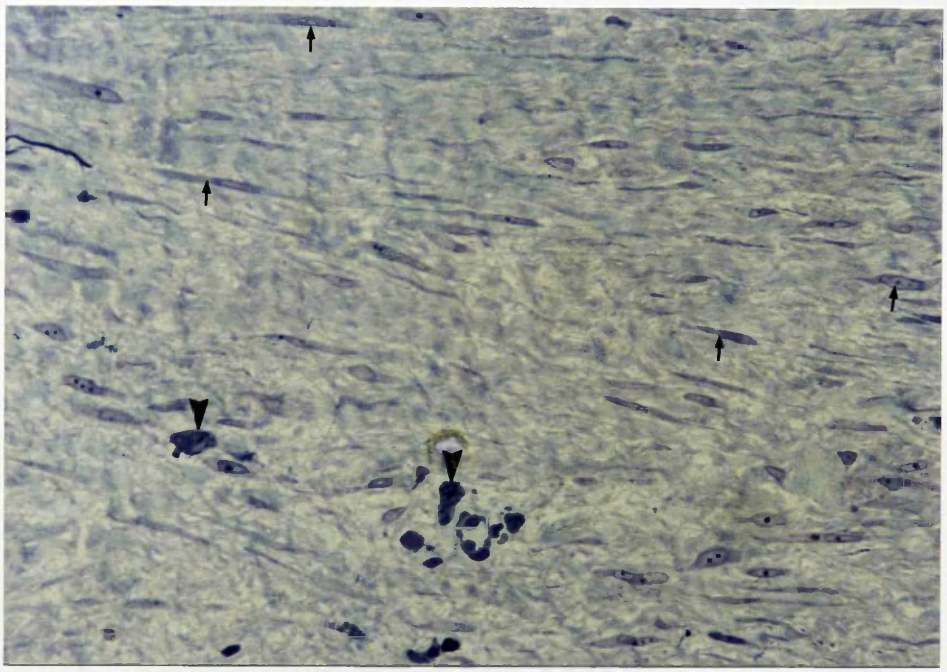


Fig. 50. The light microscopical appearance of the deep part of the reticular dermis of an irradiated flap showing giant fibroblasts (arrows) and mast cells (arrow heads). Azur II. X 340. (Patient **II** - 1).

Fig. 51. The light microscopical appearance of the deep part of an irradiated flap showing large amount of extravasated red blood cells and degenerating sweat gland (arrows). Azur II. X 280. (Patient **II** - 1).



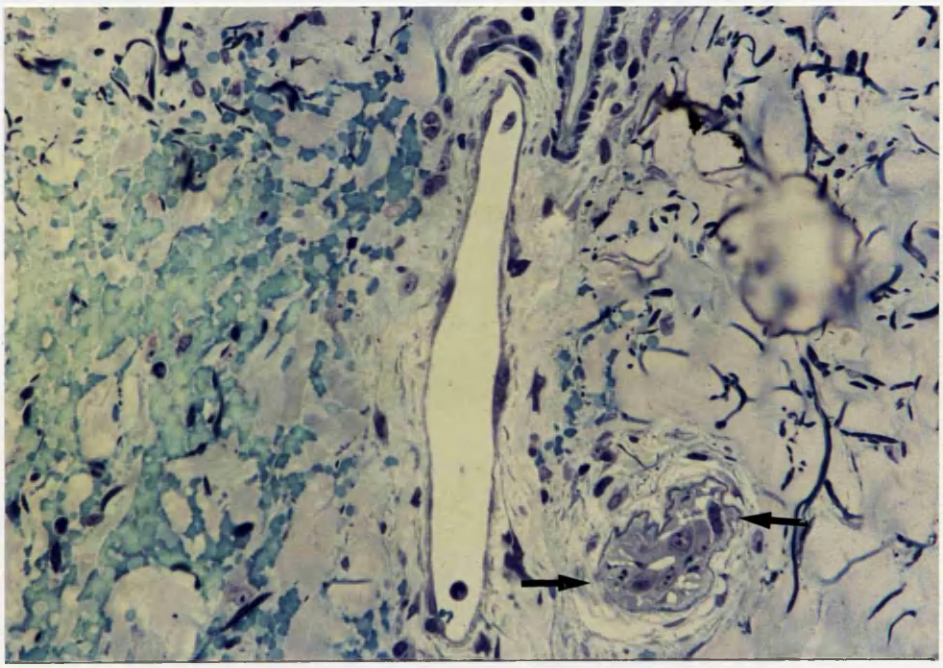


Fig. 52. Light microscopical appearance of a biopsy taken from Patient I - 1, 3 years after surgery when the flap showed partial recovery. The epithelium is still acanthotic, but thin flakes of orthokeratinised cells are seen (arrows). Azur II. X 110.

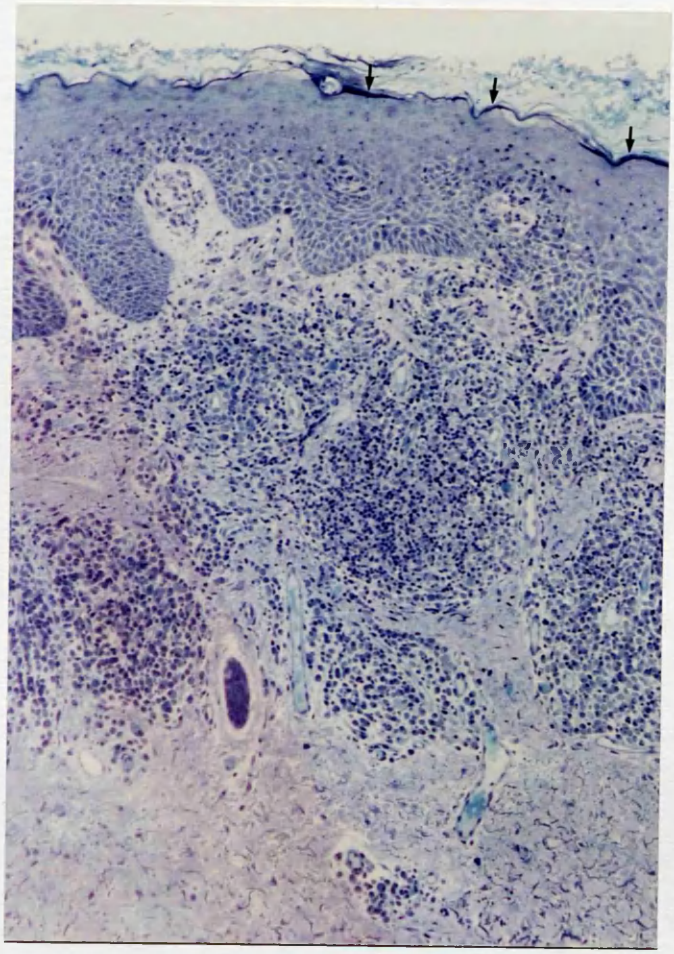
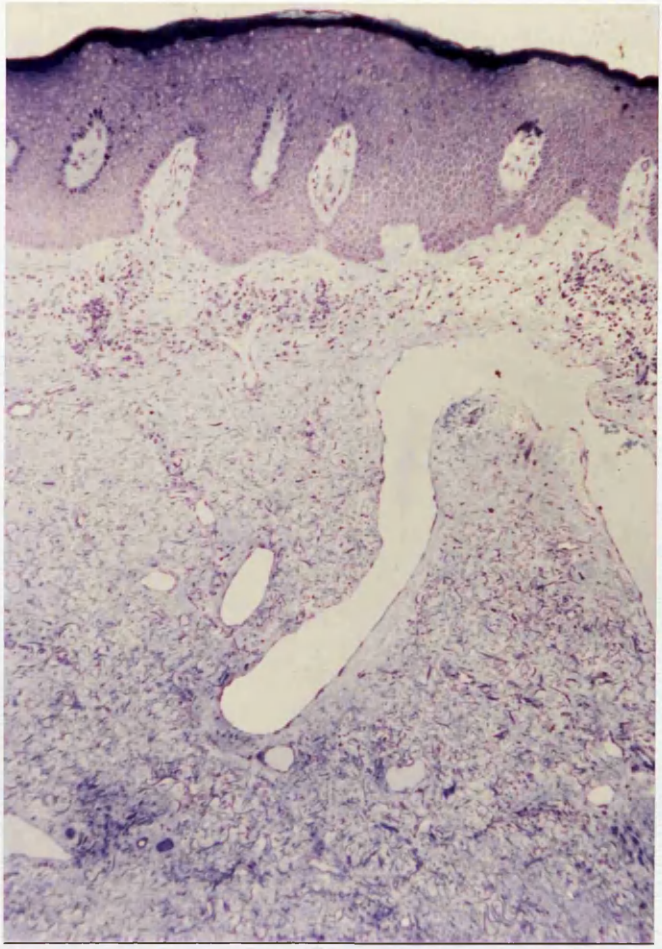


Fig. 53. The light microscopical appearance of an irradiated flap that showed partial recovery 10 months after surgery, the epithelium appears thinner than in previous biopsy and the stratum corneum is orthokeratotic. Dilated capillaries are seen in the dermis. Azur II. X 65. (Patient **II** - 1).

Fig. 54. Light microscopical appearance of a flap that acquired a silver-white appearance, clusters of nucleated cells are seen on the surface. The epithelium is thinner than in previous biopsy Fig. 47. Azur II. X 100. (Patient **II** - 7).



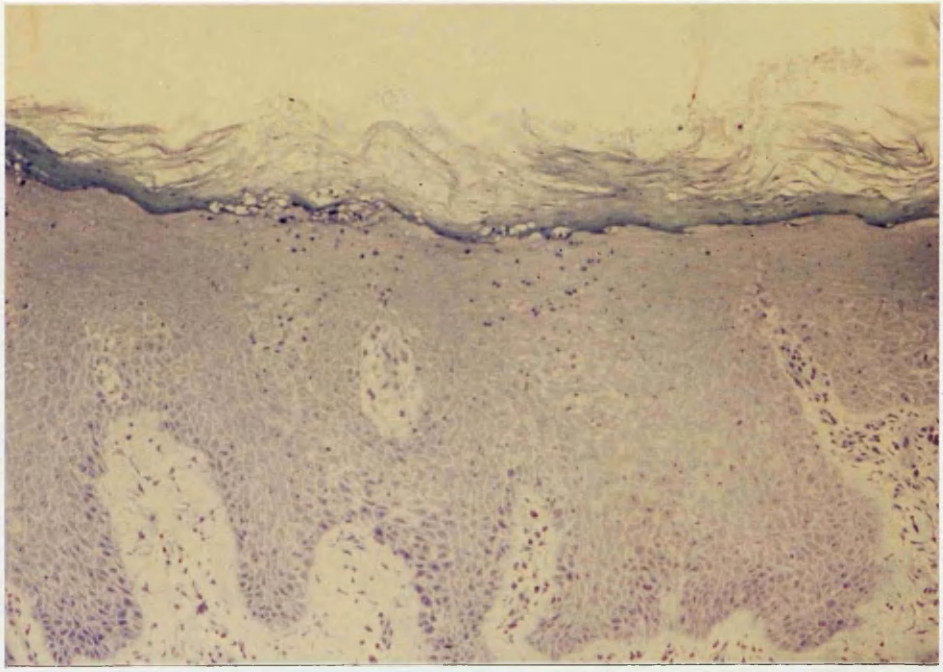


Fig. 55. Transmission electron microscopical montage of epidermis of normal forearm skin.
B = Stratum basale
S = Stratum spinosum
G = Stratum granulosum
C = Stratum corneum
K = Keratohyalin granules
T = Tonofilaments
Arrows = Desmosomes
Arrow heads = Hemidesmosomes
White arrows = Basement membrane.
X 4600.

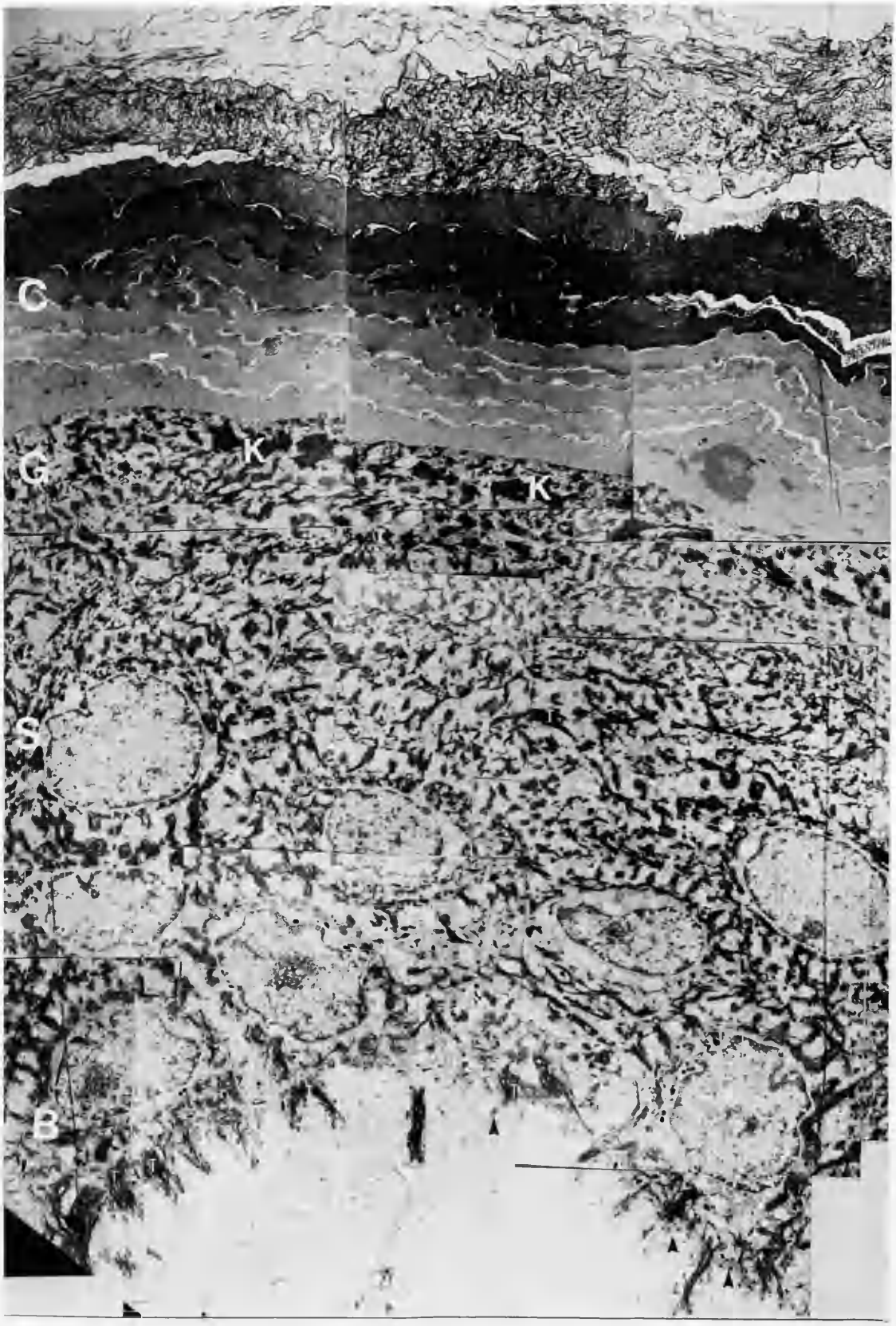

C
G
K
K
S
B

Fig. 56. Transmission electron microscopical montage of the deep part of the normal buccal mucosal epithelium.

F = Tonofilament
Arrows = Desmosomes
Arrow heads = Hemidesmosomes
White arrows = Basement membrane

X 4200.

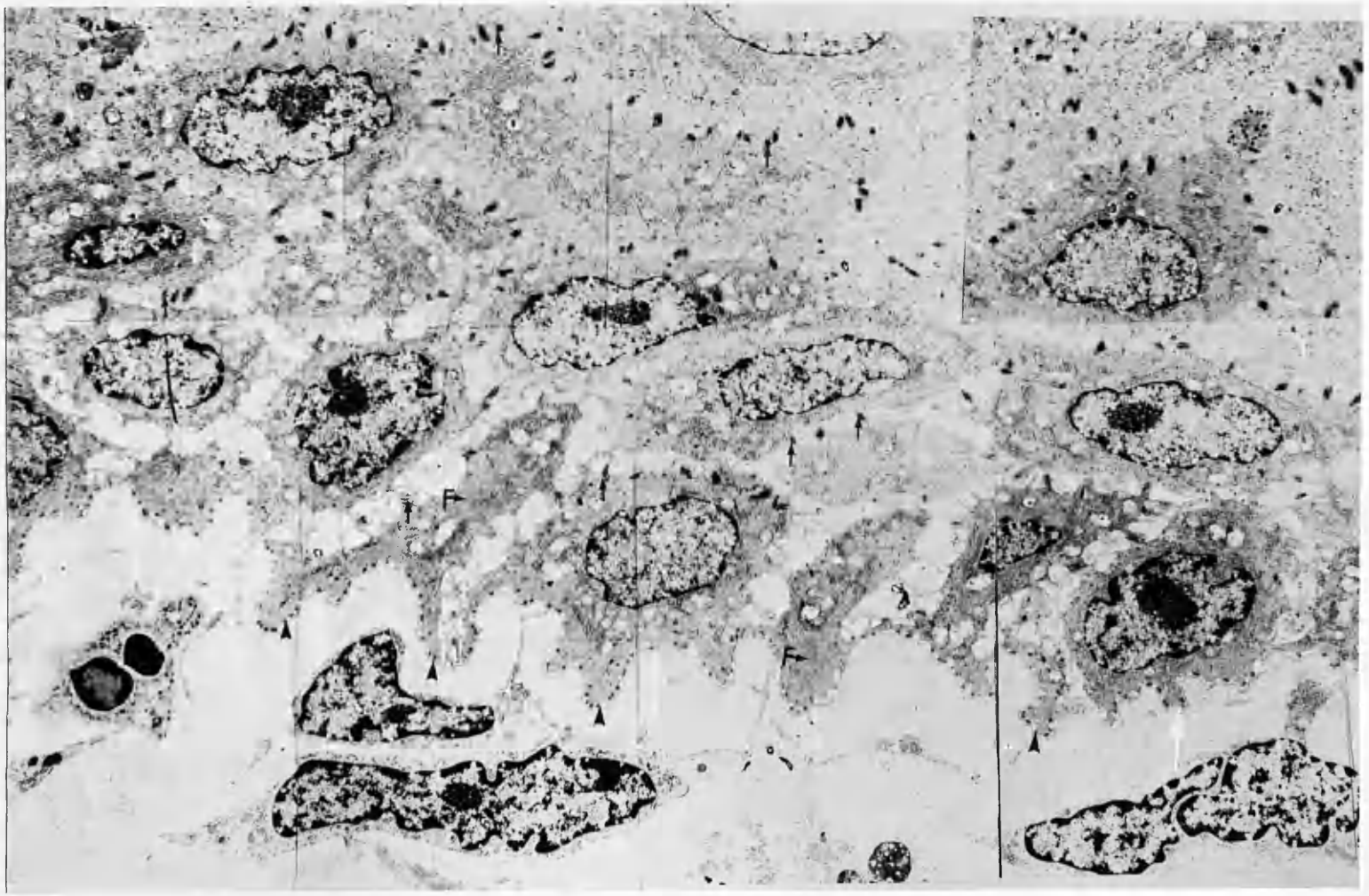

F
F

Fig. 57. Transmission electron microscopical montage of the stratum filamentosum of normal buccal mucosal epithelium.
G = Glycogen granules
Arrows = Microplicae
X 6690.

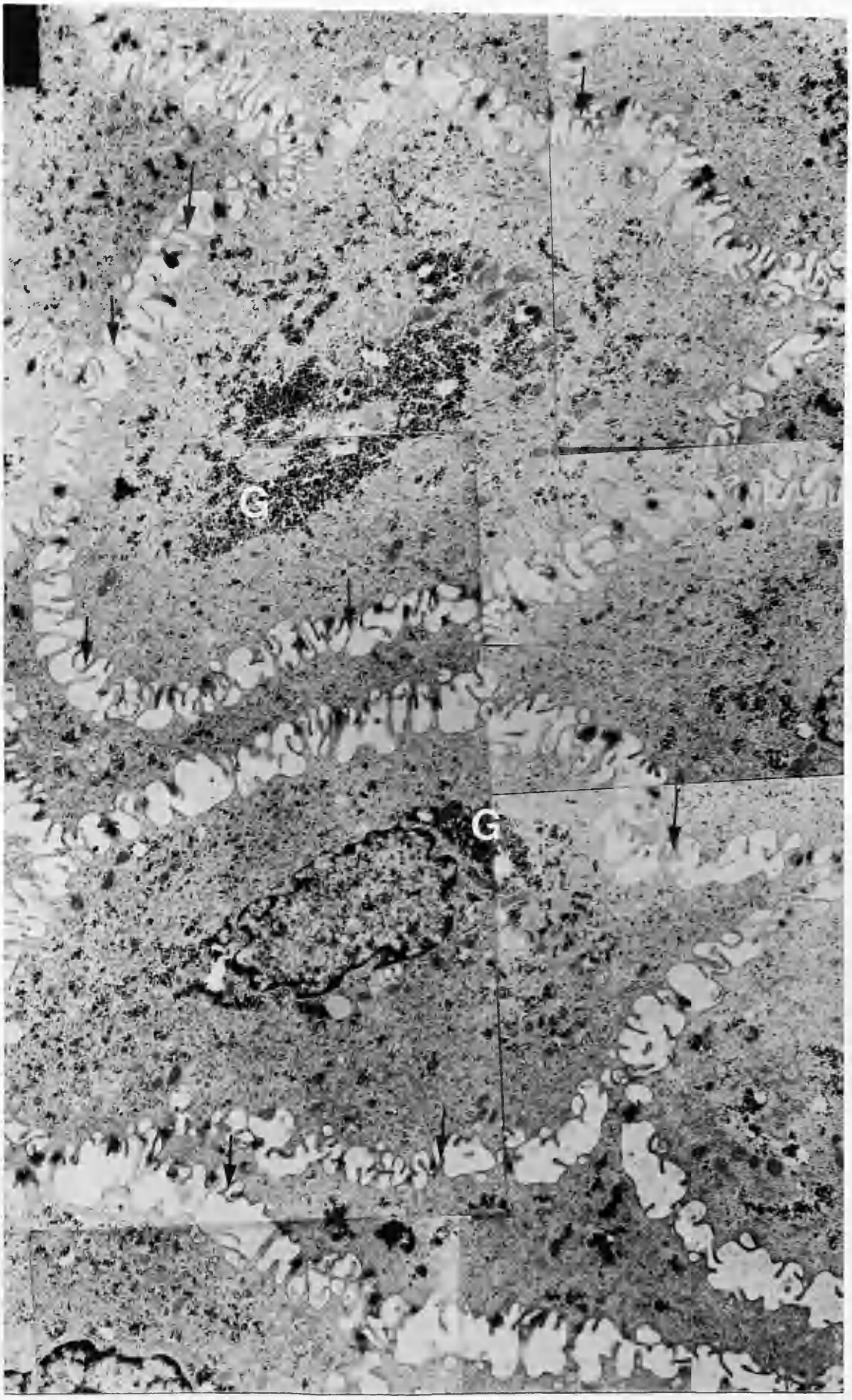

G
G

Fig. 58. Transmission electron microscopical montage of the stratum distendum of the normal buccal mucosal epithelium.

Arrows = Microplicae
Arrow heads = Desmosomes

X 3675.

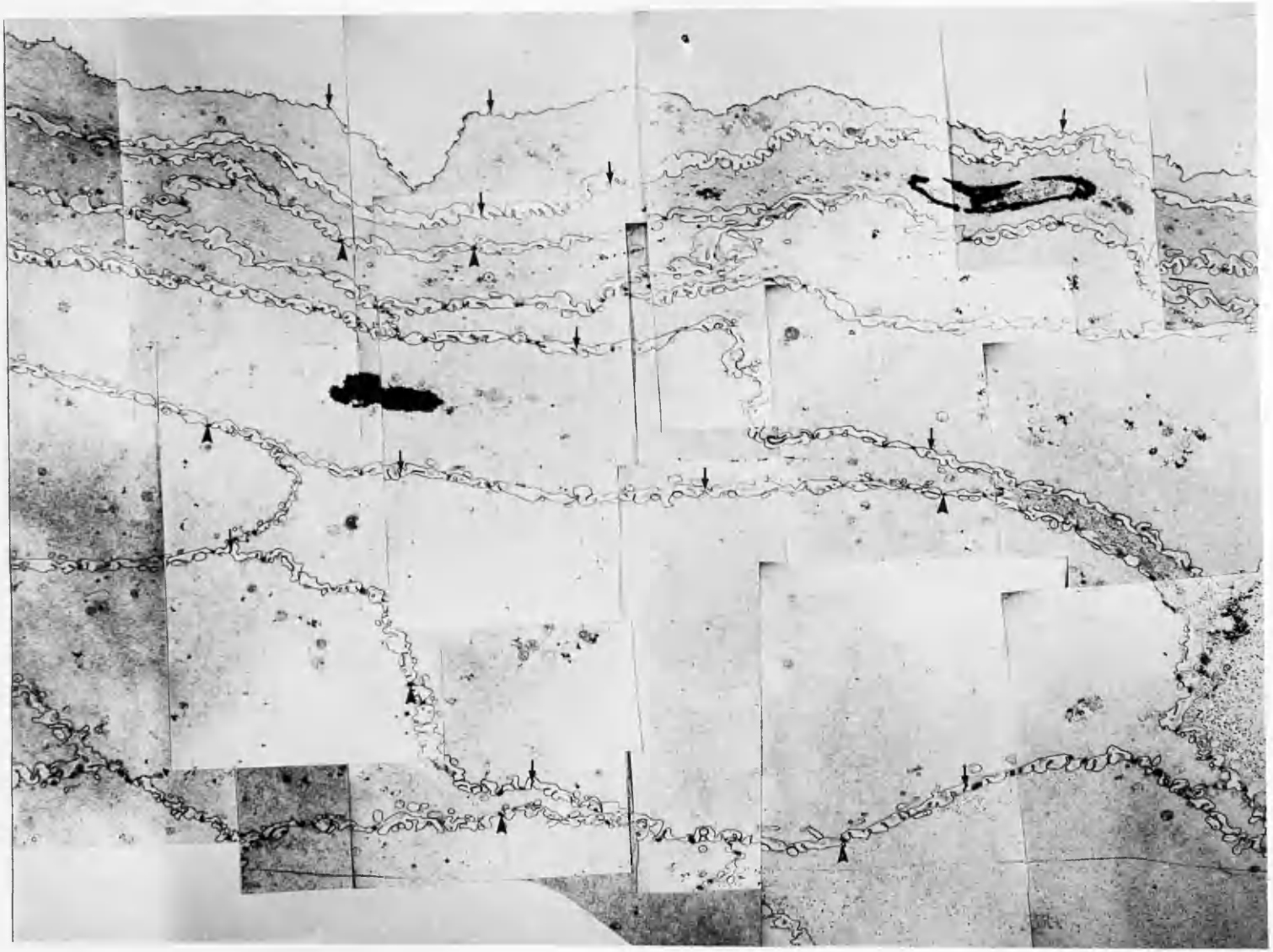


Fig. 59. Transmission electron microscopical montage of the epithelium of an unchanged, irradiated flap 4 months after surgery. Patient **II** - 3.

B = Stratum basale
S = Stratum spinosum
G = Stratum granulosum
C = Stratum corneum
K = Keratohyalin granules
T = Tonofilaments
Arrows = Desmosomes
Arrow heads = Hemidesmosomes

X 4975.


C
K
S
T
T
T
B

Fig. 60. Transmission electron microscopical montage of the deep part of the epithelium of an irradiated changed flap. Patient **II** - 1.

TF = Tonofilaments
NC = Nucleolus
Arrows = Basement membrane

X 4800.

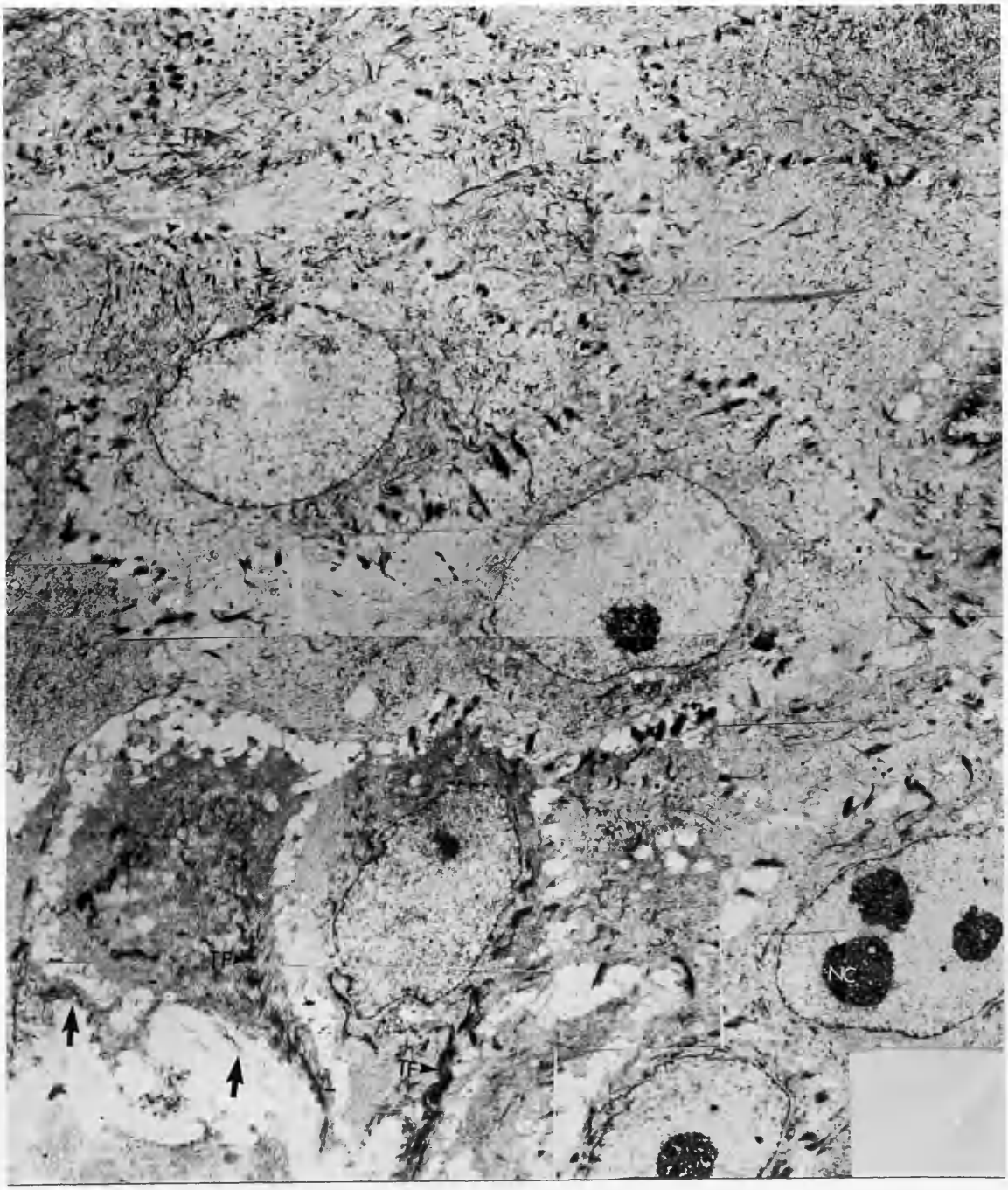

TP
TP
TP
NC

Fig. 61. Transmission electron microscopical montage of the superficial layers of the epithelium of the flap in Fig. 60.

Arrows = Fat droplets
S = Perinuclear space
K = Keratohyalin granules

X 5320.


K
K
S
K

Fig. 62. Transmission electron microscopical montage of the deep layers of the epithelium of a changed non-irradiated flap 2 years after surgery, showing widening of the intercellular spaces. Patient I - 1.

Ly = Lymphocyte
Arrows = Basement membrane
X 4200.

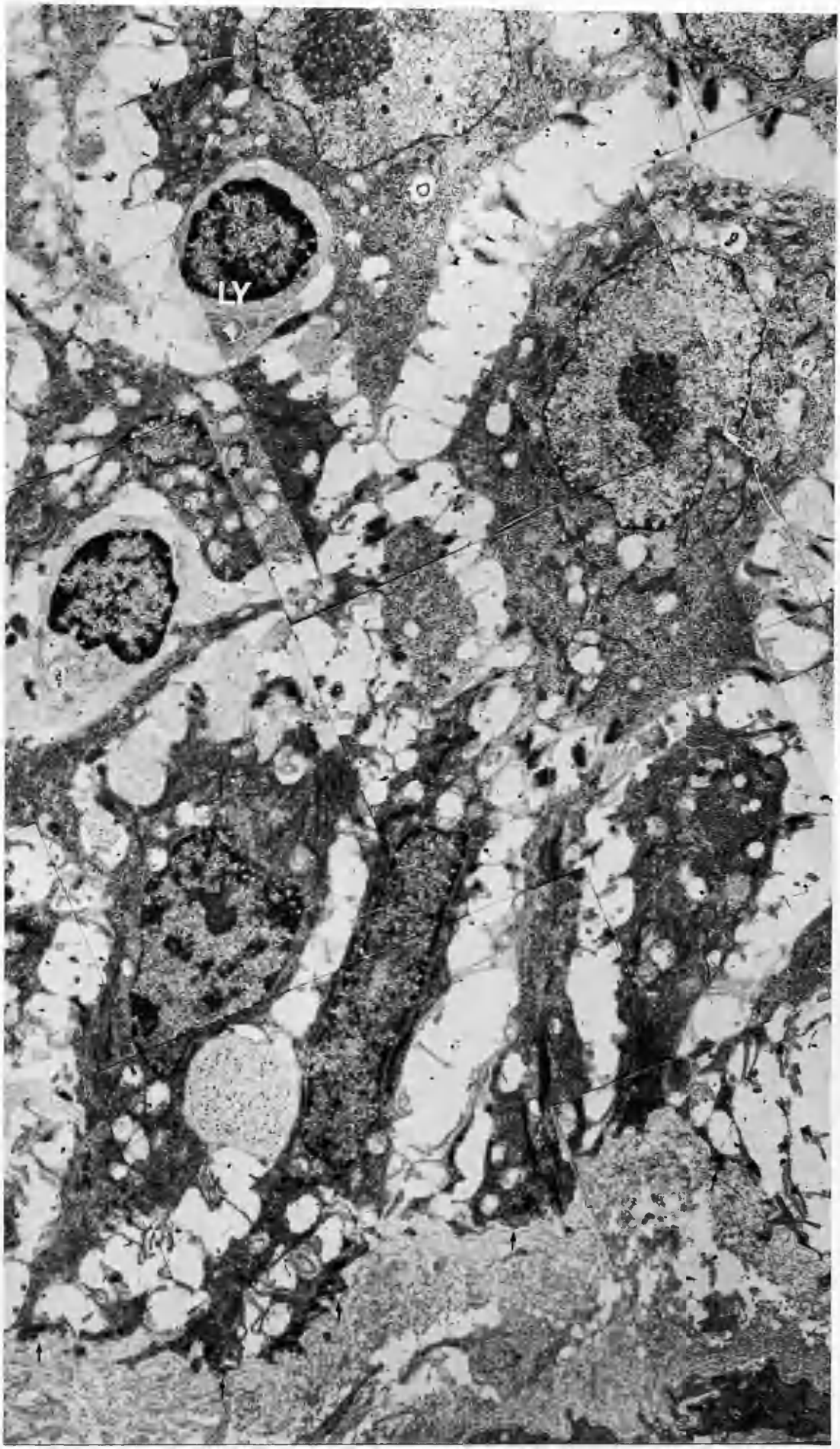

LY

Fig. 63. Transmission electron microscopical montage of the superficial layers of the epithelium of flap in Fig. 62.

Arrows = Fat droplets
G = Glycogen granules

X 4200.

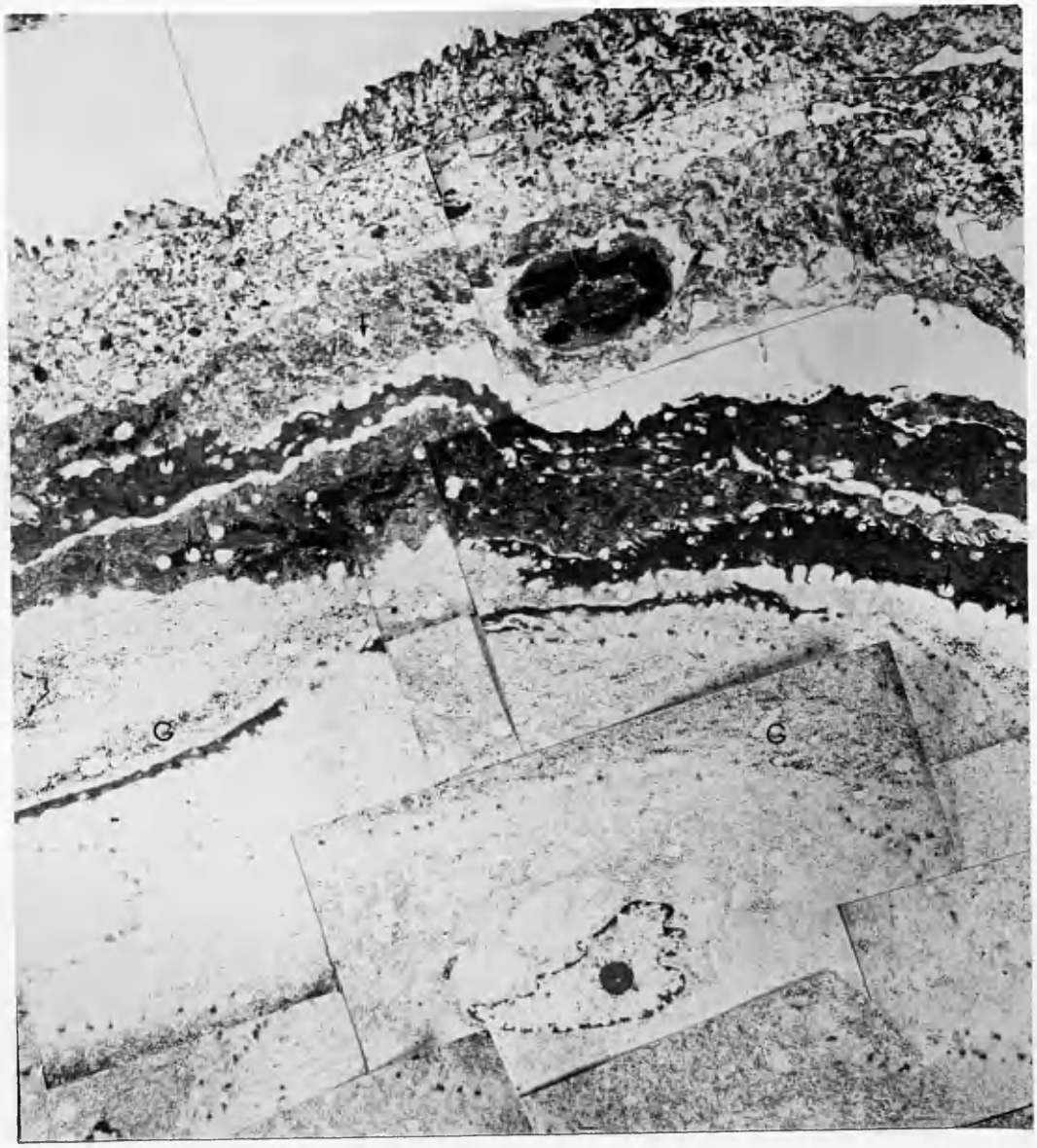

G
G

Fig. 64. Transmission electron microscopical montage of the superficial layers of the flap of patient I - 1, one year later.

T = Tonofilments
G = Keratohyalin granules
Arrows = Fat droplets

X 4400.

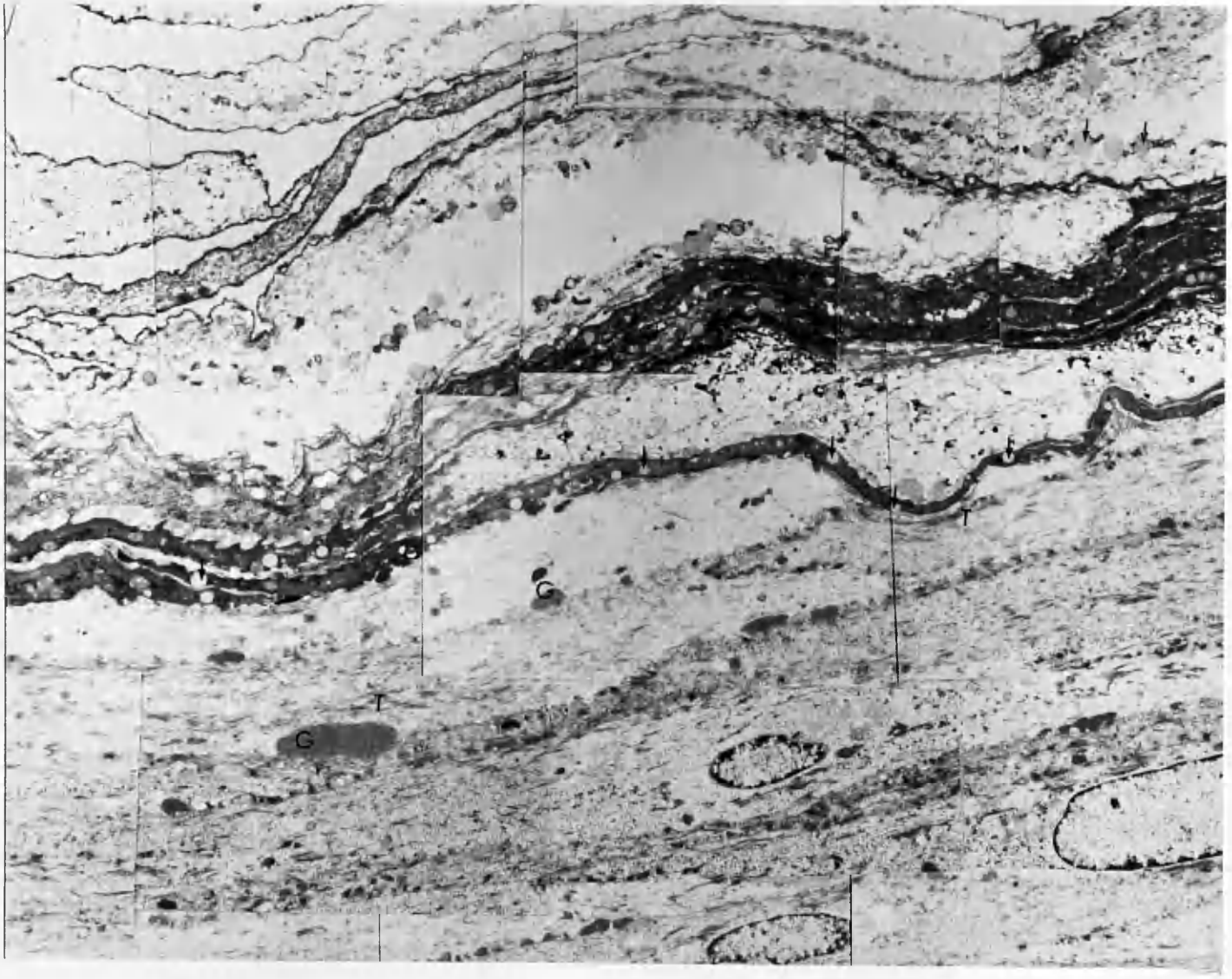
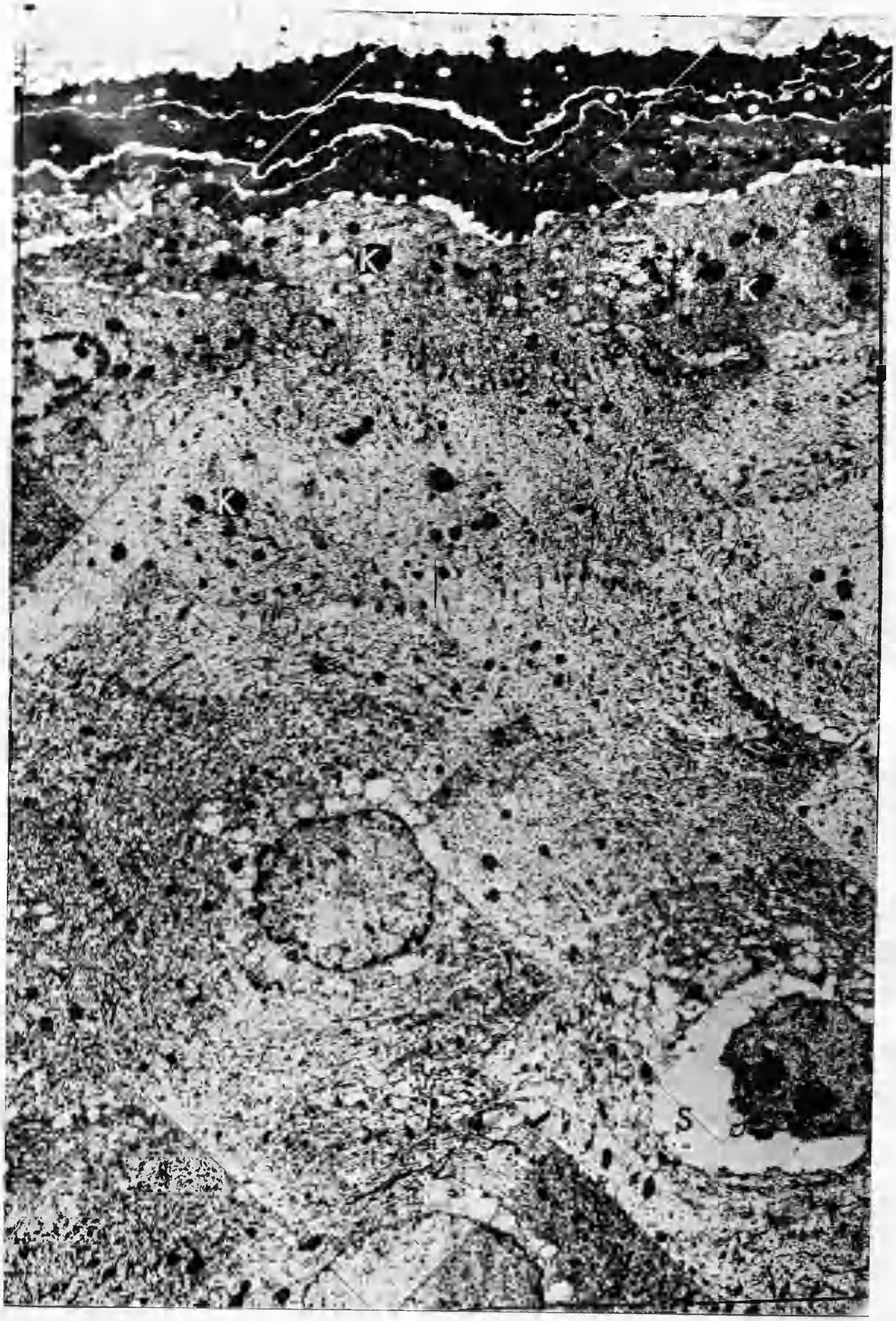

T
G
T
G

Fig. 65. Transmission electron microscopical montage of the superficial layers of the flap of patient **II** - 1, ten months after surgery.

K = Keratohyalin granule
S = Perinuclear space

X 4600.


K
K
K
S