HYPERTROPHIC SCAR TISSUE

Its Microstructure and Mechanical Properties and the Effects of Pressure Therapy

by

ANNE E. TULLY, B.Sc.

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ABSTRACT

The formation of hypertrophic scar tissue during the process of wound healing results in severe disfiguring and disabling consequences for the patient. This thesis presents the detailed micro-structure of hypertrophic scar tissue, as viewed with the S.E.M. and discusses the inter-relationship between the micro-structure and mechanical properties of the tissue. The effect of sustained pressure (in the form of pressure therapy) upon the micro-structure and the mechanical properties is also discussed.

A review of the literature concerning normal wound healing and the formation of hypertrophic scar tissue, together with a review of the different forms of therapy currently in use is presented.

A detailed micro-architectural picture of hypertrophic scar tissue has been established. Abnormalities in collagen fibre size and packing arrangement, together with abnormally large diameter blood vessels and excessive numbers of fibroblasts have been observed.

A motor driven, rotary biopsy punch, for obtaining undistorted, full thickness cores of scar tissue for microscopical analysis was designed and developed.

Pressure therapy has been found to result in an acceleration of the natural remodelling process of the hypertrophic scar tissue. This corresponded with observed changes in fibre micro-architecture and fibroblast concentration, together with a remodelling of the mechanical characteristics towards those of normal skin.

The inter-relationship between micro-structure and mechanical

properties of the scar tissue is discussed. Anisotropy in the mechanical characteristics has been observed to be reflected in the fibre orientation within the scar tissue dermis. The aggravating effect of static and dynamic tensions in the formation of these scars is discussed.

The discussion centres on establishing a better understanding of the aetiology of hypertrophic scar tissue and the way in which pressure therapy works, in order that the treatment of already established scars can be optimised; and in the future, that their formation can be prevented.

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CHAPTER 1

INTRODUCTION

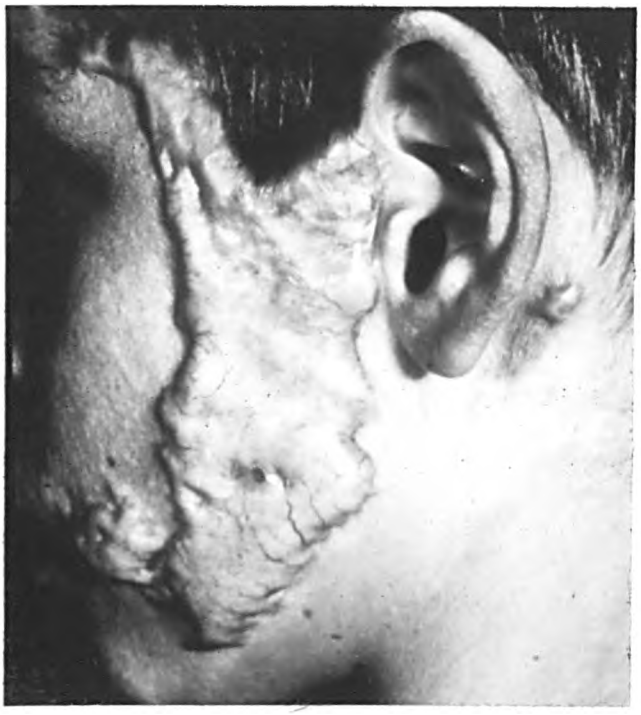


Fig.1.1. Hypertrophic scar of the face, showing the severe disfigurement which can be caused by these scars.

INTRODUCTION

Hypertrophic scars and keloids are conditions found almost exclusively in human beings. In the "normal" events of wound healing, an equilibrium occurs between the synthesising and degradative phases of collagen metabolism; but in some instances this normal equilibrium is lost, and the end result is a greater mass of fibrous tissue and the formation of a hypertrophic or keloid scar.

Clinically these scars are characterised by their raised, red and hard appearance, which can cause severe disfigurement (Fig. 1.1.). If the scarred area lies over or near a joint, severe flexion deformities can also be formed which prohibit normal joint movement.

A hypertrophic scar is defined as a raised scar which remains within the original boundaries of the wound, in contrast to a keloid scar which grows beyond the confines of the original wound.

Although much has been written about the treatment of excessive scar production, very little has been presented concerning the true nature and cause of hypertrophic scar formation. The author has carried out a detailed structural analysis of the micro-architecture of hypertrophic scar tissue, together with an investigation of the inter-relationship between the micro-structure and the mechanical properties of the tissue, in order that a better understanding of the aetiology of these scars can be achieved.

In recent years the use of sustained pressure applied to the surface of a hypertrophic scar to accelerate the natural remodelling processes of the tissue has become one of the most important methods

of treating these scars. Again, although there are numerous case reports in the literature concerning the effectiveness of this form of therapy, there is little quantitative data available on the way in which this therapy works.

This thesis presents the results of monitoring the changes in micro-structure and mechanical properties produced by prolonged pressure therapy, in an attempt to both further the understanding of the way in which the therapy works, and to optimise the forms of pressure therapy used to achieve the maximum benefit for the patient.

CHAPTER 2

LITERATURE REVIEW

2.1. The Functions and Structure of Human Skin

Skin is a remarkable organ - the largest and by far the most versatile organ of the body (Montagna, 1965). With regard to its size, a man of average proportions, approximately 1.83 metres tall will have a skin area approaching two square metres (Montagna, 1965) which will weigh about 4.8 kg (Montagna, 1956).

Mans skin follows a characteristic life cycle. In infancy and early childhood it is dry, soft and apparently hairless. With adolescence comes an enlargement of the hair follicles, which are present in the skin from birth, and a more active growth of the hair. The sweat glands and sebaceous glands go from a nearly dormant state to full activity. Physiologically speaking the skin reaches its peak with puberty, thereafter its condition starts to decline, finally resulting in the wrinkled, flacid skin of old age.

2.1.1. Functions of skin The skin acts both as a container for the body fluids and tissues, and as a protective shield against physical, chemical and biological attack.

The extensive network of blood vessels in the skin performs a variety of functions. As well as providing nourishment for the skin it acts as a thermoregulatory system. When the environmental temperature is high, sweat glands pour water on to the skin's surface. The evaporation of the water cools the circulating blood and hence cools the body. The blood vessels can actively assist this process by relaxing and allowing a maximum flow of blood through the skin. Conversely if body heat needs to be conserved the blood flow is greatly reduced.

The cutaneous circulatory system also assists in the regulation of blood pressure. Depending on the prevailing trends in blood

pressure, the blood may be allowed to course through the capillaries or it may be diverted from the arterioles directly to the venous return system. By altering the blood flow rate in this way the blood pressure may be regulated.

The complicated network of blood vessels in the skin is matched by an equally extensive network of nerves. As well as controlling the glands, blood vessels etc., in the skin, specialised nerve endings sense pressure, thermal and painful stimuli in all areas of the skin, although the sensitivity is more acute in some sites than in others.

Another prominent feature of the skin is its pigmentation. Specialised cells scattered in the epidermis produce a dark pigment, melanin. The prime function of this pigment is to shield the skin's cells by absorbing the ultraviolet rays from the sun. There are approximately the same number of melanocytes in all human skin, the differences in skin colour result from the amount of pigment produced (Montagna, 1965).

Skin is constantly subjected to attacks on its integrity. At a conservative estimate, a person's skin suffers some minor trauma, such as a scratch, about once a week. This high-lights one of the most important properties of the skin - its capacity for self repair. If the trauma is minor the wound will heal leaving virtually no trace. If the damage is more severe the skin will restore its continuity by the formation of scar tissue.

2.1.2. Structure of skin To speak of "The Skin" is an abstraction, and it is necessary for the experimenter to know the exact origin of his tissue specimen, since the gross characteristics of human skin vary greatly on different body sites and the

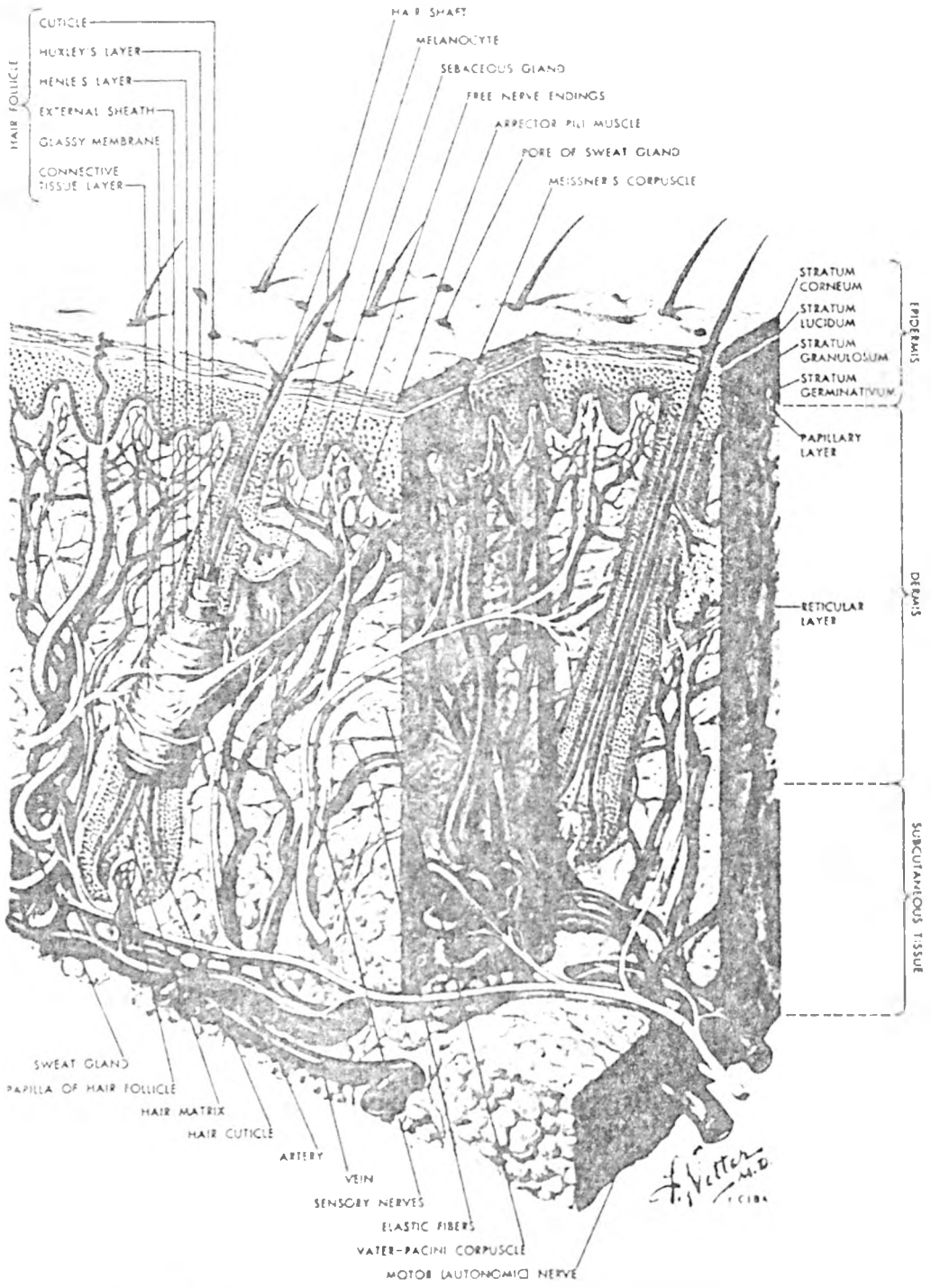


Fig.2.1. Three dimensional cross section of human skin. (From Menaker, 1975.)

histological features vary accordingly (Montagna, 1956). However the structure does follow a generalised pattern. It is composed of two major layers: an exterior covering of specialised epithelium called the epidermis, and beneath this, a dense connective tissue layer called the dermis. The epidermis of mammals consists of two distinct types of cells of different origins. The epidermal cells proper arise from the general surface ectoderm, and those of the pigmentary system come from the neural crest (Montagna, 1962). The dermis and its associated cells are of mesenchymal origin. The deeper limits of the skin cannot be distinctly defined as it merges with a layer consisting predominantly of adipose tissue (Fig. 2.1).

2.1.3. Epidermis The epidermis is a stratified squamus epithelium which covers the entire outer surface of the body. The term "squamus" defines only the superficial cells, which are in the form of scales. The living cells in the lower layers are fusiform, columnar, cuboidal or polyhedral (Fig. 2.2).

The thickness of the epidermis varies between sites on the body. Generally it ranges from 0.06 m.m. to 0.1 m.m., reaching a maximum thickness of 0.6 m.m. to 0.7 m.m. on the palms and soles (Helwig and Mostafi 1971). Although continued wear and stress are factors in the thickening of the epidermis, the actual properties are genetically pre-determined (Bloom and Fawcett 1962).

The epidermis can be divided into five layers. The innermost layer, the stratum basale or stratum germinativum, follows the contours of the underlying dermis. Cell division occurs principally in this layer, the daughter cells being pushed out towards the skin's surface by the continual division products.

The stratum spinosum forms the next layer of the epidermis.

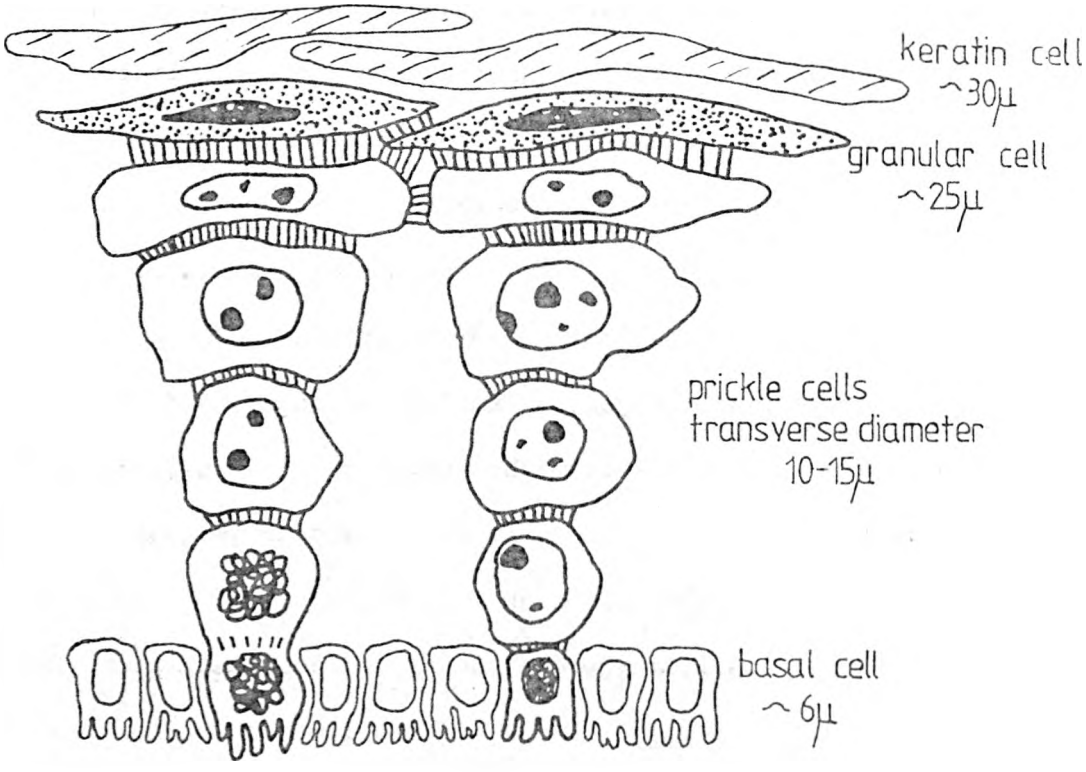


Fig. 2.2. Cells of the epidermis. Cell shape changes as they progress towards the skin surface, going from a columnar form in the basal layer to the flat scales of the keratinised layer. (After Helwig and Mostofi, 1971).

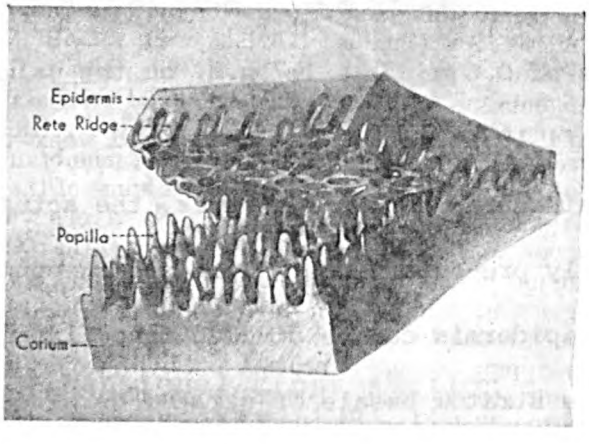


Fig. 2.3. Diagram of dermo-epidermal junction as it would appear when fresh skin is split by acetic acid. (From Pinkus and Mehregan, 1969).

The cells are attached mechanically to one another by cytoplasmic processes called intercellular bridges. Due to cell shrinkage in prepared sections this gives the spinous outline to the individual cells which gave rise to their name. The thickness of this layer varies with body site. As the cells migrate outwards, they become increasingly flattened, whilst showing a progressive accumulation of granules. These granulate cells make up the next layer, the stratum granulosum. The granules are the precursors of keratin, the fibrous protein that constitutes the bulk of the dead surface of the skin (Montagna 1956).

The differentiation of the cells is completed with the loss of nuclei and conspicuous cellular organelles as the next two layers emerge.

The stratum lucidum, in the general body surface, is composed of a thin hyalin layer only one or two cells thick which does not always show up clearly in tissue sections prepared for histology. Whenever the epidermis is thicker than average the stratum lucidum is much more conspicuous.

The outermost layer, the stratum corneum, is made up of fully anucleated cells full of keratin. This thick, horny covering forms a barrier against biological and chemical attack, physical abrasion, and is impermeable to most substances. The cells are plaque-like in form and are continually being shed from the surface.

The pigment producing melanocytes are situated in the epidermis. Exposure to ultraviolet light causes the melanocytes to inject granules of melanin into the surrounding epidermal cells, where the pigment forms a protective awning over each cell nucleus on the side towards the skin's surface.

2.1.4. Dermo-epidermal junction The junction between the epidermis and the underlying dermis in man is rarely smooth. It usually carries a series of rete ridges, the depressions between them being filled with peg-like dermal projections (Fig. 2.3). These projections are the capillary bearing papillae of the superficial dermis.

The architecture of the junction varies with body site and is related to the arrangement of hairs and sweat glands in the skin. In the back, the abdomen, and the breasts the epidermal ridges are inconspicuous, and the papillae, though numerous, are flatter than in other regions (Montagna, 1956). Those regions of the epidermis which are subject to shearing forces (i.e. the soles and palms) are as a rule covered by very scanty hair but are provided with well developed and elaborate ridges and papillae. It has been postulated that skin rich in hair follicles may be anchored to the dermis by these follicles and therefore does not need the presence of an elaborate system of rete ridges and papillae (Montagna, 1956).

The dermo-epidermal interface has been shown by electron microscopy to consist of a complicated interdigitation of basal cell pedicles, dermal ground substance and fibrils (Zelickson 1967). This is organised into a basal lamella, or adepidermal membrane, which closely parallels the cell membrane of the basal cells. The latter are fixed to the lamella by hemidesmosomes and anchoring fibrils. There is evidence that the fibres of the dermis are also attached to the basement membrane by these specialised fibrils (Swanson, 1967).

2.1.5. Dermis The average thickness of the dermis is 1 m.m. to 2 m.m., with a minimum of 0.6 m.m. on the eyelids and a maximum of 3 m.m. or more on the soles, palms and back of the neck. It is

generally thinner on the ventral surface of the body and on the underside of the appendages. The dermis is also thinner in women than in men (Helwig and Mostofi, 1971).

The Dermis of human skin is usually described as having two structurally distinct layers. These layers closely correspond to a biological division into a metabolically active, epidermis associated stroma - the papillary layer; and a metabolically sluggish, tough fibrous layer with mainly a mechanically supportive role - the reticular layer.

In contrast to this classical description, Craik and McNeil (1965) identified three structural layers: the papillary layer, the mid-dermis and the deep-dermis. The mid-dermis is described as having a more compact arrangement of fibres than the deep-dermis.

The following discussion of the components and microarchitecture of human skin, as observed with the Scanning Electron Microscope (SEM) is a précis of the work carried out by Brown (1971). The results presented in Chapter 3 were obtained using similar tissue preparation techniques to those of Brown (1971). Readers are referred to Browns thesis for more detailed information.

Connective tissue in the dermis is composed of the fibrous proteins collagen, elastin and reticulin. Collagen is predominant, 77% of fat-free dry weight. Elastin accounts for 4% and reticulin 0.4% (Montagna, 1956; Weinstein and Boucek, 1960). When operating the SEM in the secondary electron emissive mode for topographical studies, it is not possible to label the tissue components. Thus the following description of dermal architecture will be restricted to fibre arrangement and form. Although taking into account the low percentages of elastin and reticulin in the skin, most of the fibres

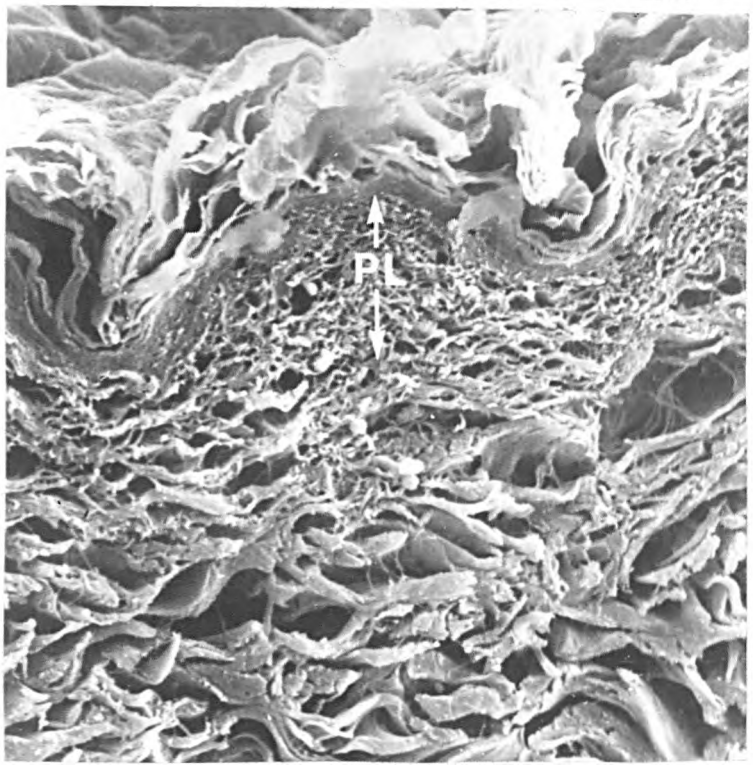


Fig.2.4. ⟵⟶ 25µm. Transverse section of normal skin, showing the epidermis, the papillary layer(PL) and coarse fibres of the mid-dermis. (Brown,1971).

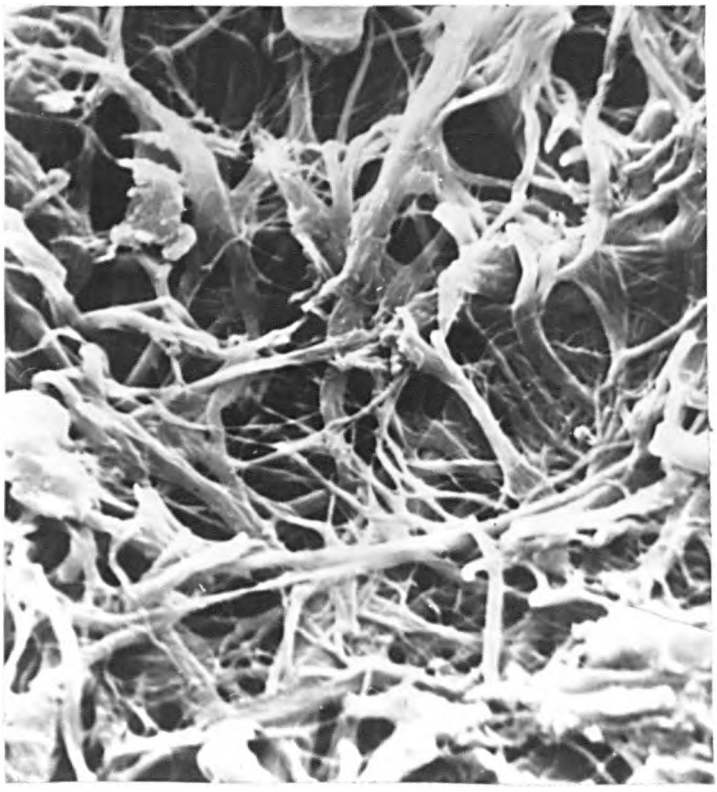


Fig.2.5. ⟵⟶ 5µm. Open network of papillary fibres. (Brown,1971).

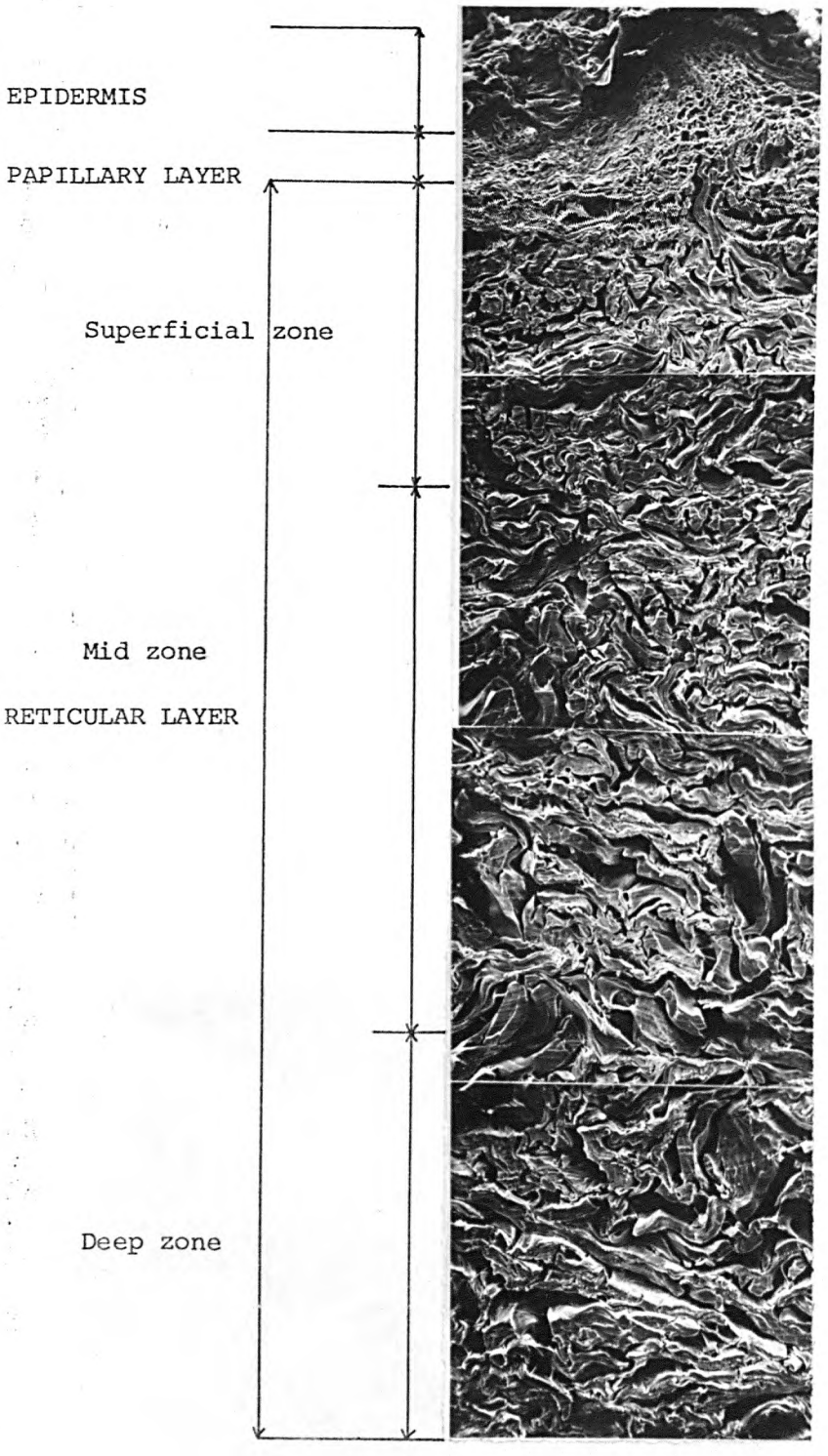


Fig.2.6. The zones of full-thickness human skin.

(After Brown,1971).

Fig.2.7. ⟵⟶ 50μm. Horizontal section through mid-dermis showing densely packed coarse fibres. (Brown,1971).

Fig.2.8. ⟵⟶ 50μm. Horizontal section through deep-dermis showing loose arrangement of coarse fibres. (Brown,1971).

described are likely to be collagenous.

The papillary layer is made up of fine fibres typically 0.3 μm to 3.0 μm. in width. They form a relatively open network in which no regular arrangement is apparent (Figs. 2.4, 2.5).

In the mid-dermis the fibres are more coarse, ranging from 10 μm to 40 μm in width. They are arranged more compactly than the fibres in the deep-dermis, where a looser arrangement prevails. Aggregations of fat cells are found in greater abundance within the networks of the deep-dermis (Figs. 2.6 - 2.9).

Although at low magnifications the fibres of the mid- and deep-dermis appear to be arranged in a haphazard multidirectional system, the fibres rarely run in directions perpendicular to the skins surface. The fibre architecture is arranged in a series of layers, parallel to the skins surface but with many fibres running at a slight angle between adjacent layers. The only exception to the irregular architecture is the concentric arrangement of fibres which often surrounds hair follicles (Fig. 2.10).

In specimens sectioned through the mid-dermis, parallel to the surface the fibres within the microscopic field at a magnification of approximately 200 (field of view 0.5 m.m. square) appear to be partially orientated. But this orientation varies between sites.

The extensibility of the skin is generally anisotropic. Mechanical studies of abdominal skin have shown that the skin is more extensible in the cranio-caudal direction than in the transverse direction (Daly, 1966). Brown (1972) found that although the fibre orientation in abdominal skin varied between different fields of view at a magnification of x 200, in the SEM, there was a "preferred" orientation close to the transverse axis of the specimen. That is,

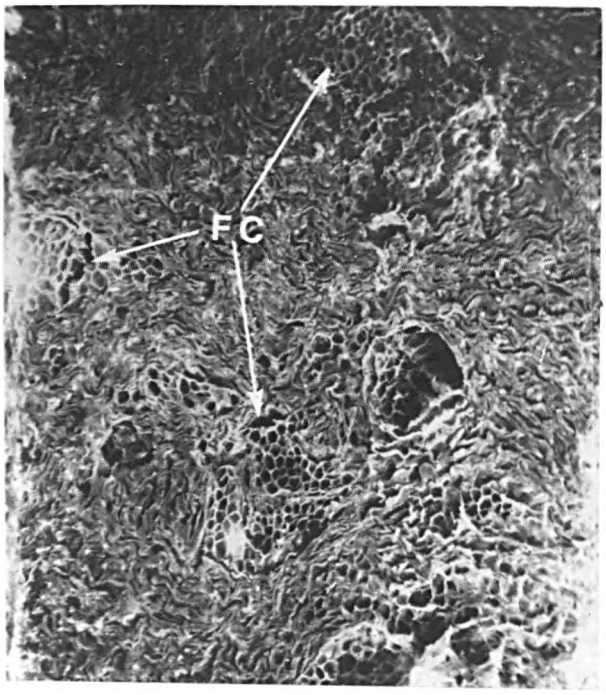


Fig.2.9. Aggregations of fat cells (FC) within network of deep-dermis (Brown,1971).

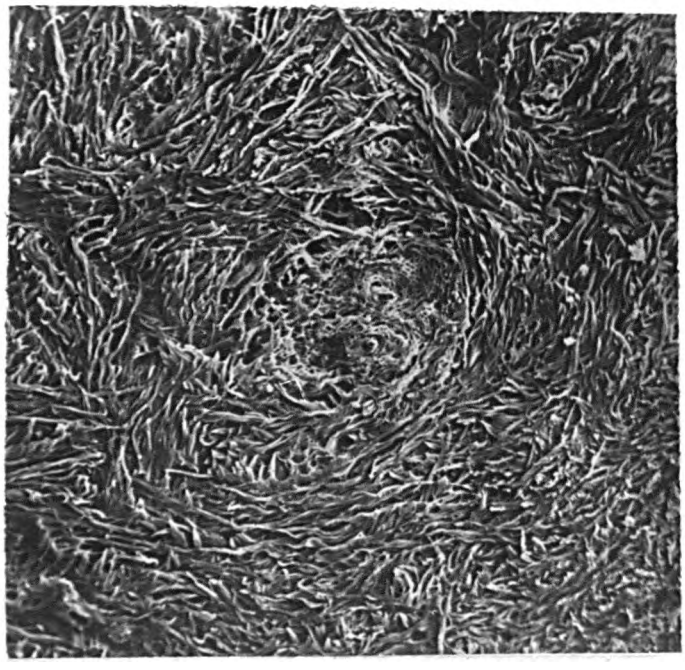


Fig.2.10. ⟵ 200μm. Concentric arrangement of fibres around hair follicles. (Brown,1971).

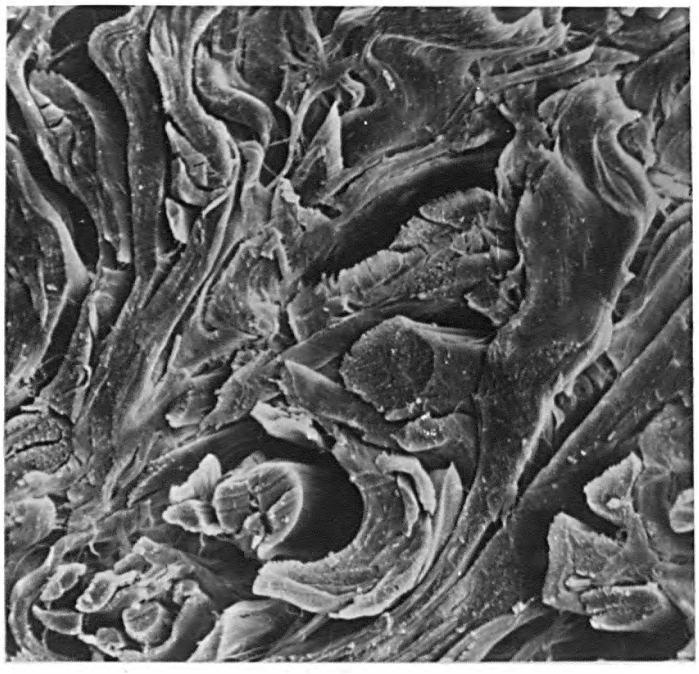


Fig.2.11. ⟵ 50μm. Variation in fibre breadth and cross-section within mid-dermis. (Brown,1971).

there was a preferred orientation of fibres perpendicular to the direction of greatest extensibility, although the network was multidirectional and irregular in overall structure. In general the mechanical properties of the skin are reflected in the fibre architecture as in the specimens of abdominal skin described above.

In the mid- and deep-dermis, aggregations of fibres take various forms ranging from flat ribbon-like bundles to those with almost circular cross section. The breadth and shape of a bundle change along its length as a result of the branching and interconnections between fibres (Fig. 2.11).

Brown (1971) studied skin specimens from 30 adults, in the age range 30 to 85 years. The only age-related change in fibre form within this range appeared to be that the fibres became progressively straighter with increasing age.

The fibrils that make up the fibres are mostly aligned in parallel arrays along the length of the fibre, with a few lying in a haphazard arrangement on the fibre surface. The spaces between adjacent fibres are bridged at intervals by an open network of fibrils. Hair follicles, glands and blood vessels are surrounded by a system of fine fibres which appear to form attachments to the course fibre network comprising the bulk of the dermis.

2.1.6. Elastin fibres The second major fibrous component of connective tissue is elastin. This fibrous protein is not readily replaced when removed and its absence in repair tissue is strongly reflected in the mechanical properties of the scar (Peacock and Van Winkle, 1976).

The elastin fibres in the mid- and deep-dermis are coarse, branching, cylindrical or flat ribbons interspersed amongst the

collagen fibres (Montagna, 1976). The elastin fibres in the papillary layer are much thinner and form a dense belt running just below the base of the dermal papillae (Pinkus, 1970). From this belt fine fibres ascend almost vertically towards the epidermis to end free just below it. In areas of skin with short or no papillae, the belt, which constitutes a highly characteristic base line, is close to the epidermis. In skin bearing papillae it skirts the lower extremities of the epidermal ridges, and long fibrils extend into the papillae (Pinkus, 1970).

Elastin fibres occur in tissues which are subjected to repeated distortional forces. Their role is to restore the original contour after the distorting force has ceased to act. Where the loads applied are high, as in some ligaments, the elastin fibre content is high. Where the loads are smaller, as in skin, the elastic fibres are relatively sparse (only 4% of the dry weight).

2.1.7. Reticulin fibres So called because they branch and form a network, the reticulin fibres make up about 0.4% of the dry weight of skin.

The greatest preponderance of reticulin fibres is in the papillary layer and in its extensions around the cutaneous appendages. The dense bed of reticulin fibres found underneath the epidermis is probably a component of the basement membrane (Montagna, 1956).

In the dermis the reticulin fibres are numerous only around blood vessels. In the extreme deep dermis, these reticulin fibres are well developed around the blood vessels and they also form basket-like capsules around each fat cell (Montagna, 1956).

Evidence suggests that reticulin may be a precursor of collagen and as such does not constitute a separate type of fibre. The

physical and chemical properties of reticulin and collagen are similar, with reticulin demonstrating the same 70 nm. periodicity as collagen. In optical microscopy, reticulin fibres often appear to continue into collagen fibres which are distinguishable by differences in their histological staining properties.

2.1.8. Ground substance The ground substance of the dermis is a semifluid, non fibullar amorphous substance containing salts, water, protein and polysaccharides in solution, that fills the spaces between the fibres and the cells. There is proportionately more ground substance in the papillary layer than in the mid- and deep-dermis (Montagna, 1956).

The ground substance contains heteropolysaccharides that can be divided into two groups: Mucopolysaccharides and Glycoproteins. The acid mucopolysaccharides isolated from the tissue comprise Hyaluronic acid, Chondriotin Sulphates A and C, Dermatan Sulphate, Keratan Sulphate, Heparin and Haparan Sulphate (Craig and Schofield, 1973.). There is no free fluid in the ground substance despite its high water content as the hygroscopic hyaluronic acid binds the water in molecular form.

All substances going to and from the cells in the tissue must pass through the ground substance, so that changes in its state and composition will exert a profound influence on the life of individual cells and tissues.

It has been suggested that the mucopolysaccharides play a role in a number of physiological and pathological processes including calcification, control of electrolytes, water and the extracellular fluids, wound healing, lubrication and the maintenance of a suitable medium for the eye (Craig and Schofield, 1973). Whilst the chemical

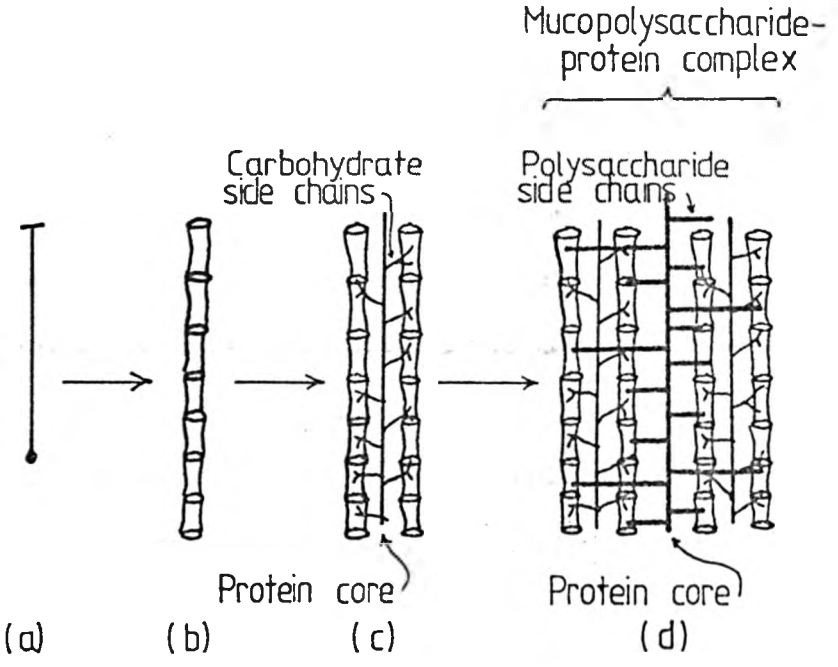


Fig. 2.12. The fibril stabilizing role of mucoproteins and mucopolysaccharide-protein complexes. (After Jackson and Bentley, 1968).

structures of the mucopolysaccharides and collagen carry with them the possibility of significant interaction, the evidence to date suggests that they are not necessary for the formation of collagen fibrils in vivo (Jackson and Bentley, 1968.). It seems likely, however, that mucopolysaccharides, particularly chondroitin sulphate, may be involved in an interaction with collagen that has already aggregated to form fibrils. The mucopolysaccharides appear to be necessary for the further ordering of collagen structure at a higher level of organisation, and are thus important to the overall structural stability of connective tissues (Jackson and Bentley, 1968). Fig. 2.12 shows a model of the possible chondroitin sulphate - protein - collagen interaction. This model shows that the initial collagen aggregates must first be further aggregated by interaction with mucoprotein to attain a level of fibrillar organization at which point the chondriotin sulphate - protein complex comes into play.

2.1.9. Hair and hair follicles Hairs are produced by hair follicles which are tube-like structures extending in a slanting manner from the epidermal surface deep into the dermis (Fig. 2.13). The hair itself is composed of keratinised cells compactly cemented together. Beneath the surface of the skin the hair is encircled by the follicle, which is a sleeve of epithelium continuous with the surface epidermis. The hair is at no time in direct contact with the fibrous components of the dermis.

After an injury to the skin (unless the trauma extends into the deep dermis) the hair follicles provide important islands of epidermal cells capable of taking part in the re-epithelisation of the wound.

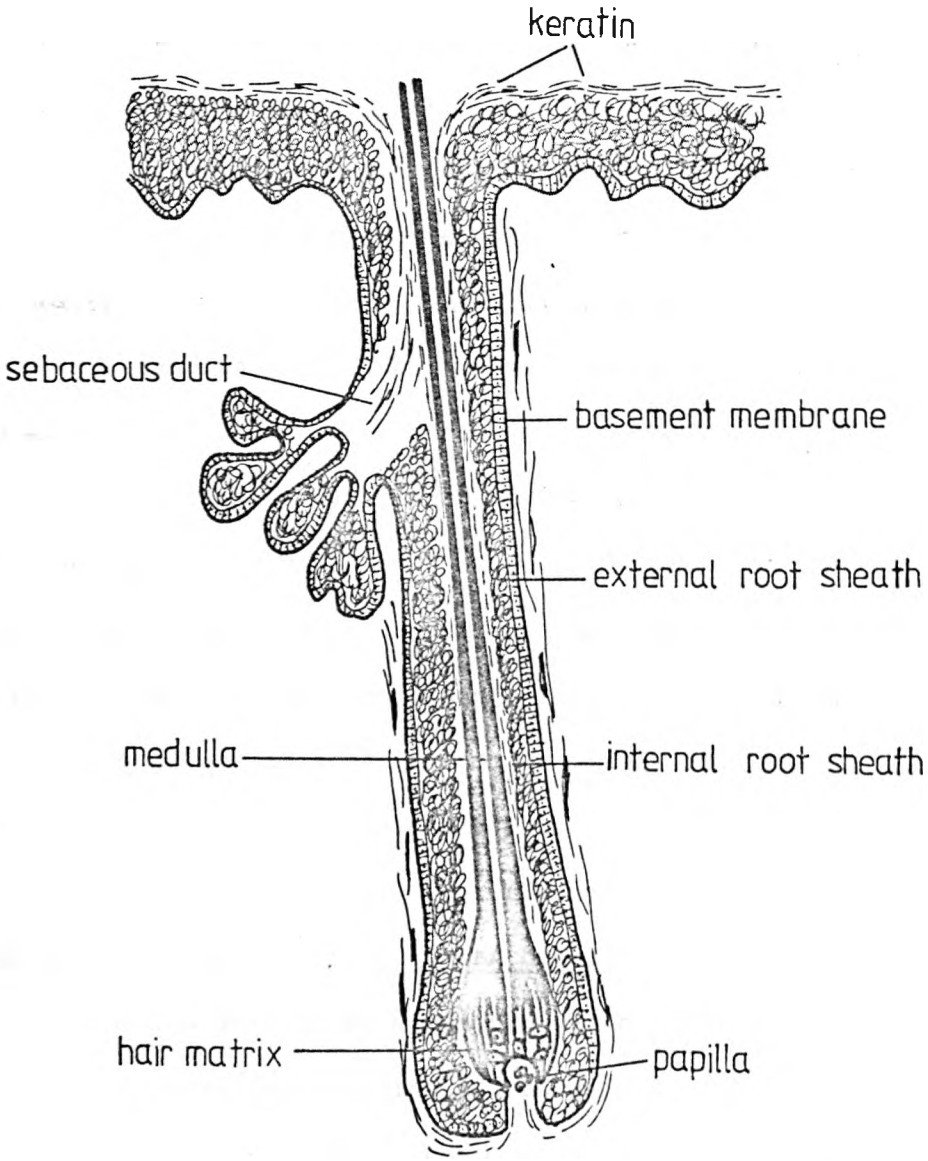


Fig. 2.13. The microanatomy of the hair follicle in cross section. (After Menaker, 1975).

2.2. Wound Healing

The normal process of wound repair differs little from one kind of tissue to another and is generally independent of the type of injury (Ross 1969). A simplified summary of the stages involved in the healing of a linear incision will be presented in sections (2.2.1 - 8). The healing of excised wounds and burns which, although following a similar pattern are subjected to further complications, is discussed in subsequent sections. The reader is referred to Peacock and Van Winkle (1976) for a more detailed description and a comprehensive bibliography.

Wound repair can be divided into three overlapping and inter-related stages, each characterised by the activities of a particular population of cells.

2.2.1. Formation of scab Healing in the dermal layer begins when a clot forms from the blood that flows into the wound immediately after injury. As the blood coagulates, fibrinogen molecules from the blood quickly link up into interconnected strands of fibrin. These strands provide a network throughout the wound defect that somewhat tenuously unite its edges. Meanwhile at the surface, fibrin and other proteins in the blood serum dehydrate and form the protective barrier or scab .

2.2.2. Inflammatory response to injury After formation of the blood clot, blood vessels in the surrounding uninjured tissue start to leak serum into the wound space. The stimulus which initiates this leakage is thought to be a substance released by the damaged tissue, which acts by altering the intercellular structure of the vessel walls so that its cells do not fit together so tightly, thus allowing serum to escape.

The serum contains a number of proteins such as globulin, albumin and anti-bodies. If the wound is infected the globulin and anti-bodies may attack the infecting organisms but normally this fluid merely provides a sustaining environment for the white cells that begin to follow into the wound about six hours later.

The first of these cells is the neutrophil (a polymorpho-nuclear leucocyte). Once inside the wound this cell can provide two types of defence. Firstly it can ingest organisms by phagocytosis. In many cases the neutrophil kills the bacteria and then digests most of the remains. Secondly if the wound is sterile, with no organisms to be ingested the neutrophil degenerates and dies. At this point the neutrophils outer membrane ruptures, pouring enzyme containing granules into the wound. It is thought that as these enzymes are released from the granules they attack the debris at the sight of injury making it easier for the cells that follow to remove (Ross, 1969).

At some stage during the first twelve hours after injury a second kind of white blood cell, the monocyte, begins to migrate into the wound. On entering the wound the monocyte becomes a macrophage which then proceeds to remove most of the debris by ingesting and partially digesting it. Unlike the neutrophil, the monocyte has a fairly long life span and can synthesise proteins, in particular the enzymes it uses in phagocytosis.

Although the neutrophils and monocytes play a vital role in wound repair, there are times when they can be destructive. Repetetive inflammation, with its overabundance of inflammatory cells and release of their enzymes may cause actual damage to the tissue, as in rheumatoid arthritis.

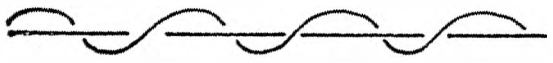
2.2.3. Mast cells The mast cell is ubiquitous in the body, being found mainly in loose connective tissue. There is a controversy as to the multitudinous potential functions, origins or transformations of this cell type, but it is generally accepted that the cell's granules contain sulfated mucopolysaccharides. Substances which play important roles in the different phases of wound repair (Schilling, 1968). The mast cells have also been reported to be associated with the presence of Heparin, Hyaluronic acid and Histamine in the tissues.

2.2.4. Fibroblasts Towards the end of the inflammatory response another kind of cell, the fibroblast, appears and begins to repair the injury by secreting collagen and the protein polysaccharides that go to make up the scar tissue.

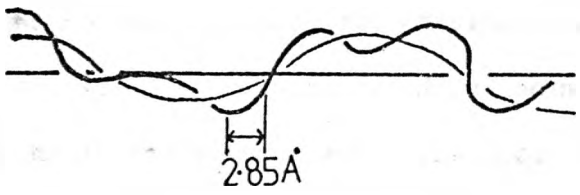
The source of the fibroblast in healing wounds has long been a matter for debate. Ross and Benditt (1961) from their Electron Microscope (EM) morphological studies of fresh wounds, suggested a vascular origin for the cell. The evidence to date indicates that the cells are derived from the surrounding connective tissue, perhaps from the perivascular mesenchymal cells (Grillo, 1964).

In surface view, the fibroblasts have an ameboid shape, with processes of varying lengths stretching out from the body of the cell; in profile they appear spindle shaped. Their shape is also influenced by their environment (Montagna, 1956). In the mid- and deep-dermis they are usually very thin, long and compressed; in the papillary layer they are larger and resemble mesenchymal cells. The large oval nucleus is stippled with very delicate chromatin particles and contains one or more large nucleoli.

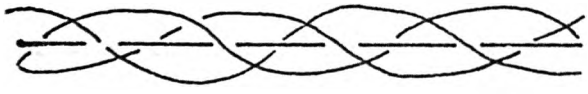
The rough endoplasmic reticulum (rer) of the fibroblasts



(a) single chain polypeptide left hand helix.

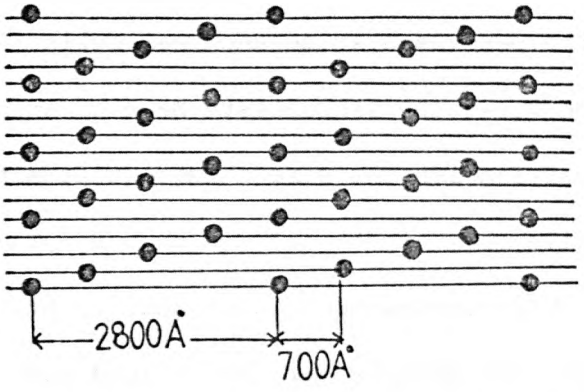


(b) single chain L.H. helix is itself coiled into a right hand helix.



(c) Three chain "coiled coil" structure of collagen molecule.

Formation of collagen molecule from three polypeptide chains



collagen fibril magnification 120,000

Collagen molecules join up in parallel arrays staggered by one quarter of their length. This results in the characteristic 700A° striations found when collagen fibrils are viewed under the electron microscope. (After Evans, 1973).

Fig. 2.14.

synthesises the proteins that form the tissue and secretes them into the extracellular space.

2.2.5. Collagen The formation of collagen fibrils from free amino-acids is shown in Fig. 2.14, which is a reproduction from Gross (1961).

Collagen has a unique amino-acid composition as demonstrated by the fact that the molecule contains exceptionally large amounts of glycine (30%), proline and hydroxyproline (12% each), and substantial amounts of both glutamic and aspartic acids (Ross, 1967). In vertebrates, hydroxyproline and hydroxylysine appear to be unique to collagen, with the exception of the small amount of hydroxyproline present in elastin.

Studies of the amino-acid sequences of the three linear α chains that make up the left handed "super helix", revealed that two of the chains were identical whilst the third differed in its distribution of acidic and basic residues. The two identical chains were termed α1 and the dissimilar chain α2. Thus the collagen in these studies, which was derived mainly from skin or tendon, was made up of α1 and α2 chains in the ratio of 2:1.

In 1969, a second type of collagen was discovered. This was composed of three identical α chains differing from the α1 chains shown previously. The two types of collagen were designated as: Type I with the formula $\alpha 1(I)_2\alpha 2$, present in skin tendon and cartilage, and, Type II with the formula $\alpha 1(II)_3$, present only in cartilage.

In 1971, studies on infant dermis revealed another different α1 chain, designated α1(III). The type III collagen made up of these α1(III) chains had no α2 chain and was found in greatest

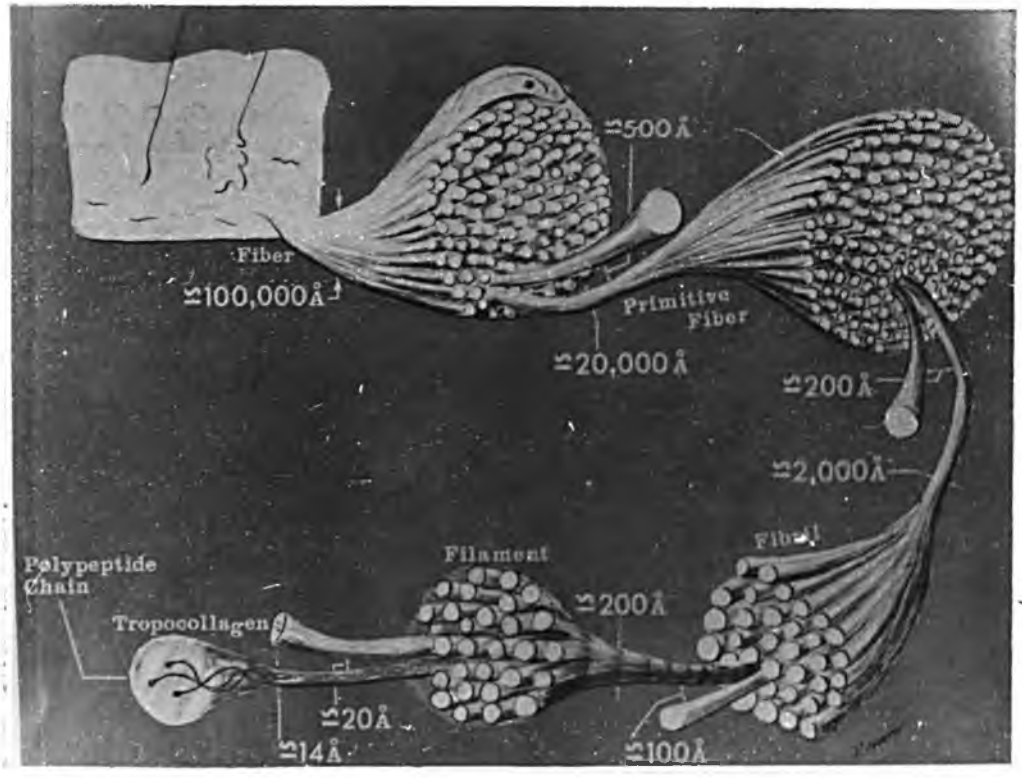


Fig.2.15. Schematic diagram of the assembly of the components of a native collagen fibre. (After Peacock and Van Winkle,1976).

amounts in embryonic tissue.

A fourth type of collagen (type IV) has been identified in Descemet's membrane and the anterior lens capsule of the eye. This is again composed of three identical α1 chains (α1(IV)).

In summary, four types of collagen have been identified, all of which appear to be tissue specific. Synthesis of these collagens is probably directed by different structural genes (Peacock and Van Winkle, 1976).

The basic tropocollagen molecule varies in cross-section and electric charge along its length and these variations are both irregularly spaced and asymmetric. The molecules are approximately 1.5 nm in diameter and 280 nm long., It is thought that when two parallel tropocollagen molecules overlap by one quarter of their length, the three dimensional configuration and charge distribution of adjacent sections are complementary and therefore attract one another. Native collagen fibrils consist of tropocollagen molecules lined up in this quarter stagger fashion giving rise to the characteristic banding of periodicity 64 nm to 70 nm, seen in the electron microscope (Fig. 2.14). This banding occurs at the tropocollagen molecule junctions but is is not clear whether it is the effect of variation of cross section or density, and if it is cross-section, whether this obtains in the native environment or is cuased by differential contraction when dehydrated for electron microscopy.

A schematic diagram of the assembly of the components of a native collagen fibre are shown in Fig. 2.15.

As the aggregation of tropocollagen molecules proceeds the solubility of the collagen changes Fig. 2.16. The most recently

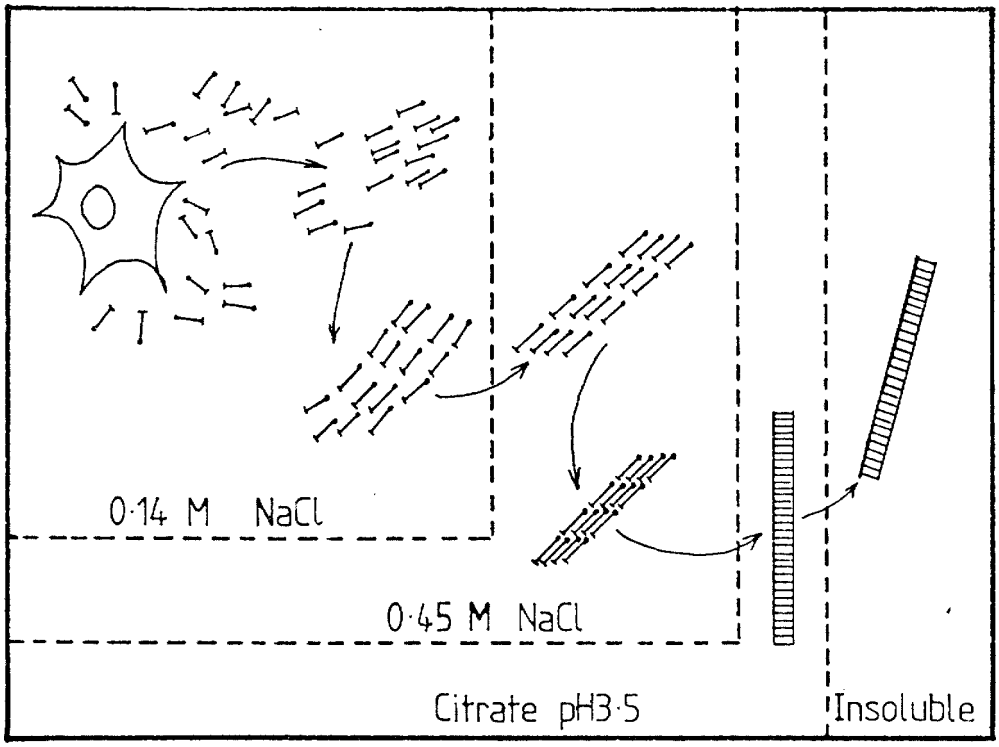


Fig. 2.16. Aggregation of tropocollagen molecules produced by the fibroblast, with associated changes in collagen solubility. (After Cross, 1959).

formed collagen is most easily soluble (in cold physiological saline), the older more tightly aggregated collagen is soluble in hypertonic salt solution and acid citrate. With time the collagen becomes progressively more insoluble, as structurally specific molecules fit together in increasingly stable association. Eventually cross-linking becomes so strong that solubilisation will occur only under the most drastic conditions (Grillo, 1964).

The native collagen fibrils in human adult skin have been shown to vary from 70 nm to 140 nm in diameter (Gross and Schmitt, 1948). In infant skin the fibrils showed diameters as small as 30 nm, although the average diameter was not significantly different from adult skin. The fibrils aggregate into bundles which constitute the collagen fibres. Viewed in the light microscope collagen in the dermis is arranged in fibre bundles ranging in size from 1 μm to 30 μm in diameter.

2.2.6. Collagen remodelling After approximately 14 days (in the case of a linear incision) the synthesis of connective tissue proteins begins to decrease, and a process of remodelling starts. It is this process that gives the scar tissue its tensile strength (Ross 1969).

Forrester and co-workers (1969) carried out an SEM study of the collagen fibre architecture of healing incised skin wounds of different ages. Their results showed that in the 10 day specimen the individual collagen fibrils appeared to be less discrete than in the normal unwounded skin. The fibrils were in a haphazard arrangement and showed little sign of coming together into bundles. Cross-banding was not visible. The wound had very little tensile strength at this time.

Fig.2.17. ——— 500μm. Although parallel and aligned at high magnification, lower magnifications show that the fibres are grouped into bands which run in all directions. (Brown and Gibson,1974).

In the 100 day specimen the collagen was in the form of large irregular masses without the obvious fibril substructure that characterised the normal specimens. (The 100 day specimen was the oldest specimen viewed by Forrester.)

With time the fibrous network undergoes further remodelling into thick bundles and sheets of collagen fibres, which often have a wavy appearance.

At high magnification parallel alignment of the fibres is evident, but at low magnifications the fibre sheets and bundles run in all directions (Brown and Gibson, 1974) (Fig. 2.17).

2.2.7. Mucopolysaccharides There appears to be an inverse relationship between the amount of mucopolysaccharide and the amount of collagen present in a wound. Autoradiographic studies have demonstrated that the same cells that synthesise and secrete collagen are simultaneously synthesising and secreting muco-polysaccharides. Following the proliferation of the fibroblasts in the wound an amorphous matrix with the staining characteristics of acid mucopolysaccharides appears. The amount of mucropolysaccharides reaches a peak on the 5th or 6th day after wounding, declining rapidly thereafter. This decline coincides with the rapid appearance of the collagen fibres (Jackson and Bentley, 1968.).

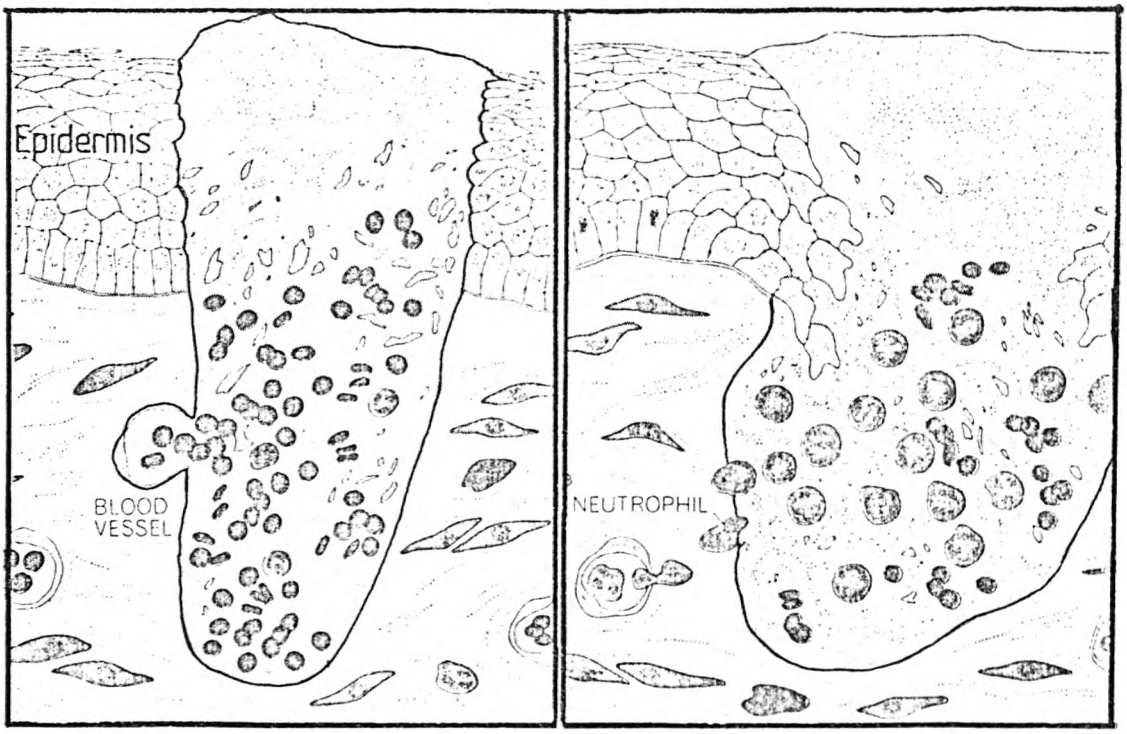
2.2.8. Capillary proliferation As the fibroblasts synthesise the collagen and the protein polysaccharides, large numbers of small blood vessels form throughout the wound. These capillaries originate as budlike structures on nearby vessels, penetrate the wound and grow into loops. The loops then ramify throughout the wound by the division of their cells. As the capillaries from different sites migrate through the wound, they meet and form an interconnected

network of vessels (Schoefl and Majno, 1964).

In the early stages of wound repair this network of capillaries provides comparatively large contents of oxygen for the cells that are actively synthesising the proteins in the wound. Once the continuity of the tissue has been re-established many of the new capillaries regress. Thus the wound changes from a tissue that is rich in blood vessels and actively dividing cells into one that has a much more simple cellular structure.

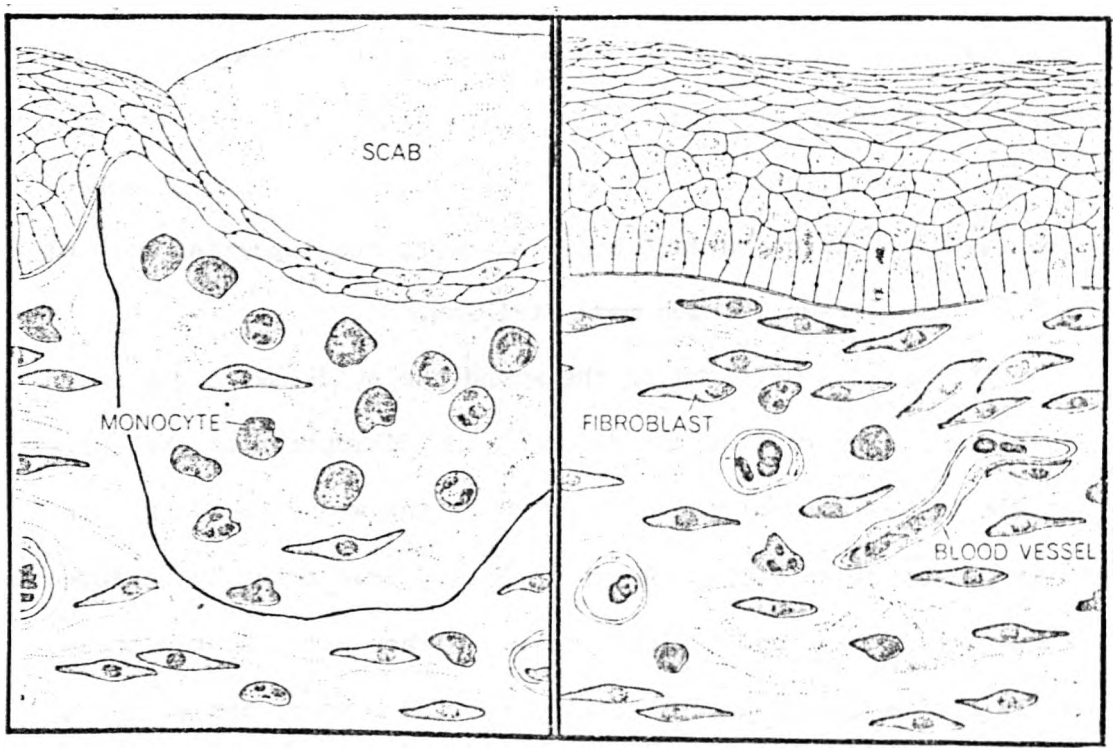
2.2.9. Granulation tissue and epithelisation The presence of capillary proliferation, fibroblasts, collagen fibrils and associated mucopolysaccharides comprise what is termed the granulation tissue. It is over this base that epithelial migration and centripetal contraction of the skin surrounding the wound occurs. Without the presence of healthy granulation tissue wound healing is greatly impaired. Although granulation tissue forms in all wounds, in a linear incision with little gaping of the wound edges the amount is small, and in fact its appearance is often overlooked. In a wound of large surface area, however, the important role of granulation tissue is much more striking.

As scar tissue forms in the wound the epidermal cells begin to close the surface of the defect. The disruption of the epidermal layers causes some of the cells nearest the wound to degenerate. Most of the other cells lose the orderly, orientated appearance they had in their undisturbed state. Within a few hours these cells become amorphous and develop ruffled borders and blisters, much like those seen in the actively moving cells grown in tissue culture. The migration of the epithelial cells seems to be well organised. It is thought that the scaffolding of fibrin derived



At the time of wounding. Cap fills with blood and cellular debris. Strands of fibrin unite wound edges.

One day later. Neutrophils enter the wound. Epidermal cells begin to migrate and reproduce.



Two days later. Epidermal migration completed. Fibroblasts have migrated into defect.

Seven days later. Scab has sloughed from restored epidermis. Fibroblasts actively synthesising collagen.

Fig. 2.18. Wound healing. (After Ross, 1969)

from the clotted blood that forms the first provisional "patch" over the wound also guides the migrating epidermal cells (Fig. 2.18).

As the cells migrate they exhibit a feature quite unusual for epidermis. They appear to actively participate in the ingestion and digestion of the strands of serum and fibrin lying in their path (Ross, 1969).

When the leading edges of the sheets of epidermal cells meet and form a continuous layer under the scab, each cell resumes its normal identity. The cells become more columnar in character and commence mitosis. The daughter cells migrate upwards within the surface of the wound and begin to keratinise.

The question arises of how do the epidermal cells "know" when wound closure has been completed. Abercrombie and Heaysman, (1954) put forward the theory of Contact Inhibition. They observed that fibroblasts grown in culture continued to move and divide until they came into contact with other fibroblasts. They hypothesised that this might also be the case with epidermal cells.

Other investigations suggest that as the cells touch the distribution of electric charge on their surfaces changes, and this change serves as a signal to the cell to stop moving. As yet there is no definite answer to the question.

The epithelisation of excised wounds follows the general pattern of that just described for incised wounds. In an excised wound the epithelial migration commences at the wound edges exactly as it did in the incised wound. In addition if the full thickness of the dermis has not been removed, epithelial cells of the skin appendages, (notably hair follicles) will also commence to migrate. In such an excised wound one is apt to see numerous "islands" of

new epithelium on the surface (Peacock and Van Winkle, 1976).

In large excised wounds all stages of epithelial repair may be seen simultaneously. At the margin of the migrating epithelium, a single layer of epithelial cells is moving across the wound. Further back some cells will be undergoing mitosis, and just beyond them upward cell migration is occurring producing stratification. Where several layers of cells cover the wound, differentiation and keratinisation occur. At the original wound margin a hyperplastic thickening of the epithelium is seen.

2.3. Burn Wound Healing

Because of the incidence of burns that heal with the formation of hypertrophic scar tissue a summary of the histological aspects of burn wound healing will be presented here.

The repair of burns is in some ways similar to the healing of excised wounds which have been exposed to drying and have formed a superficial scab. In other ways it is similar to wound healing in the presence of a foreign body. It is complicated by the presence of non-viable tissue and by the graded nature of the injury. Healing is modified by the widespread damage to blood vessels locally and the consequential poor supply of oxygen and nutrients. This also makes the injured tissue unusually prone to bacterial infection.

The wound that results from contact between the skin and a hot body does not have a regular perimeter because heat flows more readily in some compartments of the tissue than in others, and because some cells are very sensitive to heat and others less so (Winter, 1975.).

Furthermore, the injury can be a progressive one. Lawrence,

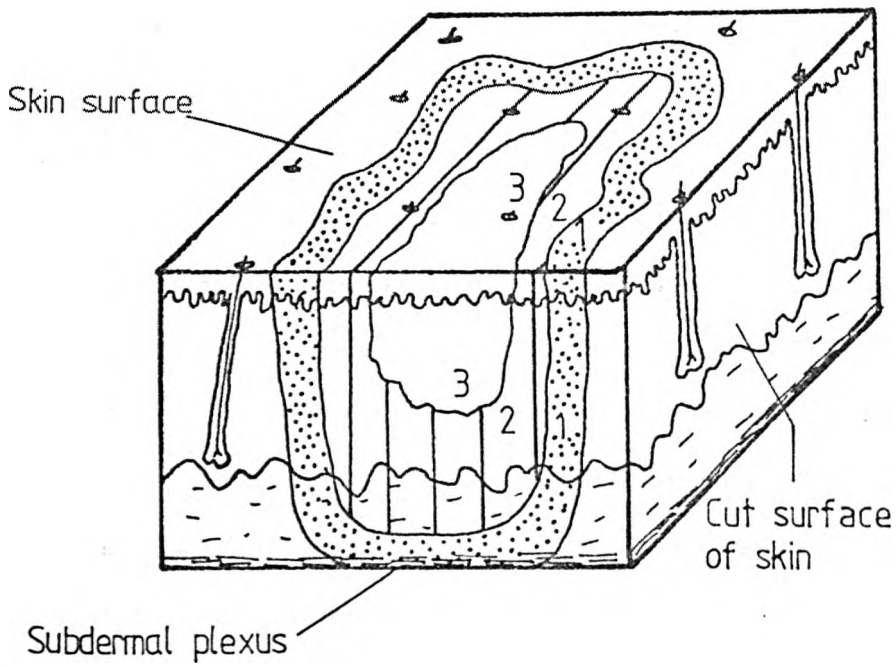


Fig. 2.19. Diagram showing the zones that develop following a thermal burn of the skin. These zones occur in depth as well as on the surface: (1) zone of hyperaemia; (2) zone of stasis; (3) zone of coagulation. (After Lawrence, 1975).

(1975), describes the three well defined zones discernable in a typical thermal burn of the skin (Fig. 2.19). Contact of the skin with sources of heat frequently causes immediate coagulation of tissue protein. This zone of coagulation is surrounded by a region of capillary stasis which is in turn bound by an area of hyperaemia. These zones, which are often visible with the unaided eye on the skins surface, also occur in the depths of the burn.

The capillary stasis develops within a short time of burning and persists for some days. During this period the trapped blood cells and the surrounding tissue become nectrotic and eventually indistinguishable from the coagulated tissue. Thus if the zone of stasis penetrates below the plane of the deepest epithelial structures the burn will be full thickness in depth (Lawrence, 1975). More damage may also be caused by the loss of water vapour through the injured surface, causing dehydration of the exposed dermis.

Winter (1975) in his work on pigs, found that regeneration of epithelium did not begin until the condition of the wound had stabilised. In pigs this took 8 to 9 days. It was impossible to be certain about the duration of this "lag-phase" in humans because of the wide variations in individuals, injuries and anatomical sites observed.

Winter interpreted this delay in healing in the light of the diffuse and progressive nature of the injury, as a failure of any effective stimulus to repair after burning. Studies of surgical wounds have shown that regeneration begins in a zone about 1.5 to 2.0 mm wide bordering the injury (Bullough and Lawrence, 1957). It can be deduced that this is the distance over which wound healing

signals from the damaged tissue are able to diffuse and bring about appropriate reactions leading to repair (Bullough, 1972). In burns the cells in this critical zone are unable to respond because they are dying through lack of oxygen and other essential nutrients. Moreover, in the absence of a circulating tissue fluid the chemical messengers are not dispersed and do not reach such cells as are competent to respond, and the boundaries of the critical zone are not static.

When equilibrium is attained the damaged tissue is at last juxtaposed to living, healthy tissue. The signals emanating from the injured cells and tissue elements then have their appropriate affect and the ordinary processes of epithelial cell movement and fibrogenesis are initiated.

2.4. Wound Contraction

Two major processes contribute to the closing of an open wound: epithelisation and wound contraction. Contraction of a wound is characterised by the centripetal movemement of the whole thickness of the surrounding skin resulting in the size of the open wound diminishing.

2.4.1. Contraction and contracture The differences between the terms Contracture and Contraction must be stressed here. Wound contraction is an active process which tends to close a wound in which an actual loss of tissue has occurred. A contracture refers to an end result which may, in some instances, be caused by the process of contraction, or the shortening and re-orientation of an established mass of scar tissue. For example, the skin surrounding a wound over the flexor surface of a joint will contract in an effort to close the wound. This contraction may be to such an

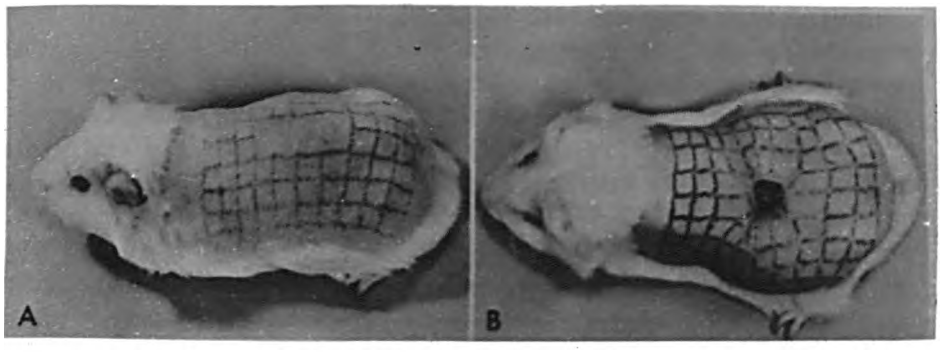


Fig.2.20. Movement of the margins of excised wounds in the backs of guinea pigs. (A) Skin excised, (B) Same animal 104 days post wounding. (After Straile,1959).

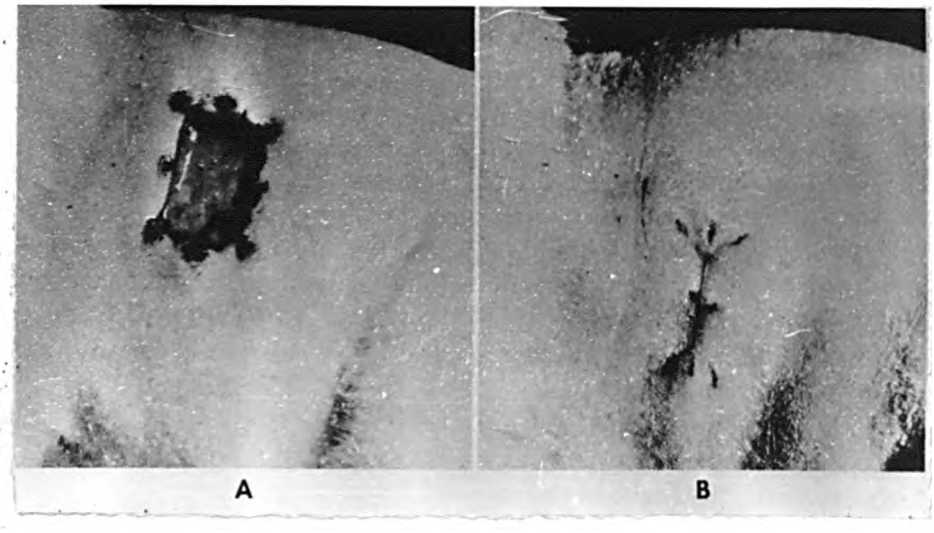


Fig.2.21. Stellate scar which forms after the healing of a square excision. (After Peacock and Van Winkle.1976).

extent that the surrounding skin is too tightly stretched and thus inhibits the joints movement. The scar tissue laid down in the wound may also contract causing further inhibition of movement. The combination of these contractions will result in some permanent flexure of the joint involved, or in other words a contracture deformity.

Contraction does not proceed uniformly from the moment of wounding but appears to be subject to a "lag-phase". However this delay is more apparent than real. A small but significant diminution of the wound size does occur soon after wounding, but contraction is much more rapid after a few days (Abercrombie et al, 1954).

Wound contraction involves the movement of existing tissue at the wound edge, not the formation of new tissue. This results in the tissue surrounding the wound being stretched, thinned and placed under tension (Peacock and Van Winkle, 1976).

Straile (1959) observed the movement of the margins of excised wounds in the backs of guinea pigs. A grid system is printed onto the back of a guinea pig and an oblong of skin excised (Fig. 2.20A).

Fig. 2.20B shows the same animal 104 days after wounding. The wound has contracted, primarily in the anterior-posterior direction. This has resulted in a marked shortening of the lateral borders of the wound and a relaxation in the normal tension of the lateral skin. (It is of interest to note that the cleavage line pattern (section 2.6.1) on the backs of rats and guinea pigs lies in the transverse direction, at right angles to the predominant direction of contraction).

There was an actual reduction in the surface area of skin lateral to the wound, and an expansion of the skin in the anterior-posterior direction.

The centripetal motion of wound contraction is well illustrated by the stellate scar which forms after the healing of a square excision (Fig. 2.21). The centripetal motion causes problems when the wound is circular. The edges of the wound become compressed and this can limit or halt the contraction (Peacock and Van Winkle, 1976).

2.4.2. Source of wound contraction A lot of research has been carried out in an effort to determine the source of wound contraction. The reader is referred to Abercrombie and co-workers (1954) for a more comprehensive review than that which will be presented below.

If, in an excised wound, the granulation tissue within the wound is separated from the uninjured skin surrounding it the isolated island of granulation tissue will contract.

If an island of in-tact skin is left within an excised wound the island will expand as the wound contracts. However this island can be made to contract back to its original size by isolating it from the granulating tissue within the wound.

Both of these experiments pointed to the source of the contraction mechanisms being found within the granulation tissue (James, 1964).

Originally it was thought that the collagen fibres in the wound contracted pulling the wound margins towards the centre. However, this did not explain the closure of wounds made in scorbutic animals. (Collagen production is markedly inhibited in

scorbutic animals (Ross, 1962). Secondly, collagen is not a contractile protein and exhibits shortening only upon denaturisation, which disrupts its crystalline structure.

Attention was therefore focussed on the cells of the granulation tissue, more specifically on the fibroblasts. Gabiani and colleagues, (1972) examined the ultrastructure of fibroblasts in open wounds, as well as in several other models of contracting granulation tissue. They found that certain fibroblasts developed characteristics typical of smooth muscle cells. The name "myofibroblast" was given to this type of cell.

An ultrastructural analysis of the myofibroblasts revealed that they contained an extensive cytoplasmic fibrillar system. They showed immunofluorescent labelling of their cytoplasm with human anti-smooth muscle serum and the nuclei exhibited complicated folds and indentations, indicative of cellular contraction.

The myofibroblasts also showed cell-to-cell and cell-to-stroma attachments. These attachments were postulated to supply the necessary mechanical apparatus for the exertion and transmission of their contractile forces.

The general view today is that the source of wound contraction lies in the cells of the granulation tissue. In particular, in the myofibroblast, a form of fibroblast exhibiting characteristics similar to smooth muscle cells.

2.4.3. Result of wound healing The size of a wound does not affect the rate at which it will contract. The amount of available mobile uninjured skin around the defect will determine whether or not the wound will be completely closed by the process of contraction.

Two events may stop wound contraction. If the edges of the wound meet, contact inhibition of the moving cells sets in and halts further advance. However, if before the edges meet the tension in the surrounding skin equals or exceeds the force of contraction, further centripetal motion will cease, and some reversal of movement may occur.

If wound contraction does result in the apposition of the wound margins, a relatively thin stellate scar will remain as visual evidence of the former wound. Depending upon the amount of mobile skin surrounding the wound, and the structures within the skin, a variable amount of visual distortion may be apparent. The distortion will be particularly noticeable if the extensibility of the surrounding skin is anisotropic (Peacock and Van Winkle, 1976).

The external appearance of a wound that has closed completely by contraction is misleading. Beneath the migrated dermis lies a connective tissue scar equal in extent to that of the original wound. Furthermore, the quality of the expanded skin has been altered. The tissue deficit caused by the stretching and thinning of the skin surrounding the wound is replaced by new connective tissue. This process of "intussusceptive growth" proceeds until the full thickness of the stretched skin is restored.

If equilibrium between the force of contraction and the tension in the skin is reached before the wound edges meet, a defect will remain. Collagen continues to be laid down in the uncovered area and the physical area of the scar tissue will begin to increase in size again. This expansion of the scar is a late phenomenon in the wound healing process and appears to be related to the tension

in the skin (see section 2.6.4).

The final result of wound healing is the restoration of the skins integrity by epithelisation, contraction and scar formation. Unfortunately sometimes the repair process goes further than just restoring continuity and excessive scar tissue is laid down resulting in the formation of hypertrophic or keloid scars.

2.5. Aetiological Factors Influencing the Formation of Excessive Scar Tissue.

It appears that certain individuals have a predisposition to excessive connective tissue production in response to trauma. This predisposition may be general, local and chronological. Affected individuals may be prone to develop hypertrophic or keloid scars in many areas of the skin, or in a few areas only. The same individual may have different degrees of susceptibility at different times of his life.

Many theories in regard to the lesions aetiology have been put forward, the main ones will be discussed in the following sections.

2.5.1. Sex The ratio of the incidence of hypertrophic scars in men to women has been reported as being 1:1 (Alhady, 1964; Koonin, 1964) and slightly weighted towards a higher incidence in women, 1:1.8 (Cosman 1961). However Cosman (1961) states that the apparent higher incidence in women may be due to the fact that the female is more conscious of the uncosmetic appearance of a scar and is therefore more likely to seek the plastic surgeon's help.

2.5.2. Age It is generally agreed that the incidence of hypertrophic and keloid scar formation is greater in young people (Peacock, 1970; Larson, 1974).

Garb (1942) reviewed the incidence of excessive scar formation in 67 patients and obtained the following distribution

First decade	17%
Second decade	34%
Third decade	21%
Fourth decade	18%
Fifth decade	7%
Sixth decade	3%

Approximately 80% of hypertrophic and keloid scars occur in people under the age of 30 (Garb, 1942 (72%); Alhady, 1964 (80%); Ketchum, 1974 (88%)). Roughly 50% of these lesions are in people under 20 years of age (Garb, 1942; 51%; Alhady, 1964, 48%).

Several factors could account for this distribution: that collagen turnover is accelerated in the young due to their "growth spurts"; there is a higher degree of elasticity and tension in young skin than in old skin; young people are more prone to trauma (Ketchum, 1974).

2.5.3. Race A problem arises when reviewing the literature with the differentiation between hypertrophic and keloid scars. It is generally agreed that there is a preponderance of keloids formed in dark skinned races. But in very few cases has the clinical impression been compared with a pathological diagnosis, so that it is uncertain whether hypertrophic scars have been included under the definition of keloids.

Crockett (1964) differentiated between the two types of scars

and concluded that hypertrophic scars were no more common in dark skinned races than in Caucasians. Keloids were, however, more common in Negroes.

Koonin (1964) in his review of the literature formed a hypothesis that keloid formation was linked to an aberration in the Melanocyte Stimulating Hormone (MSH), based upon the following conclusions, each of which will be discussed in turn.

i) The high incidence of keloids in dark-skinned races, whose melanocytes are apparently more reactive to MSH.

ii) The fact that deeply pigmented, swarthy skins, in people of all races, seem more prone to keloid formation than people with fair skins.

iii) The relation of hyperpituitarism to keloid formation, as in acromegalics (people with enlargement of face, hands and feet) and, the higher incidence of keloids in states of physiological hyperactivity of the pituitary eg. puberty and pregnancy, and that these are associated with increased pigmentation.

iv) The main sites of keloid formation are on the parts of the body where the concentration of melanocytes is greatest, such as the face and neck, and that keloids are extremely rare on the palms and soles where the concentration of melanocytes is minimal.

v) The well recorded relation of keloids to thyroid disease and the known increase in pigmentation in certain cases of hyperthyroidism.

vi) Hydrocortisone is an inhibitor of MSH output and local injections of hydrocortisone and its derivatives (ie. triamcinalone, acetonide and decadron) have been used with a fair degree of sucess in treating keloids.

Koonin concludes that it is not known whether the important factor is an increase in MSH secretion, or a hypersensitivity of melanocytes to the effects of MSH.

i) The high incidence of keloid formation in Negroes is generally agreed upon, although the given ratios vary from 2:1 to 14:1. A survey of the literature by Cosman and colleagues (1961) gave an average ratio of 3:1.

ii) Kitlowski (1953) felt that the deeply pigmented, oily skin was more prone to keloid formation because of its higher sulphur content. This was contradicted by the results of Alhady and co-workers (1964). They applied the Chi-square test to population distribution and hospital admission statistics of a city in Malaysia, with a Chinese, Malay and Indian population. Results indicated that the relatively fair-skinned Chinese were more prone to keloid formation than the Malays and Indians.

iii) Bloom and colleagues (1956) and Ketchum (1976) both noted a higher incidence of keloid formation during hyperactivity of the pituitary gland.

iv) Nason (1942) reported that keloids occurred rarely on the face. Cosman and co-workers (1961) found 36% of the lesions studied occurred on the face or neck and only 0.6% on the hand or foot. Crockett (1964) divided the head into regions and found that the beard area had a much higher tendency to keloid formation than the face. The lower limb showed a low tendency to excessive scar tissue formation. When considering the apparent high incidence of keloids on the face and neck reported by some people, the severe cosmetic disability that this causes could have an influence on the patient making him more inclined to seek

medical help. This could bias any conclusions drawn from hospital admission lists.

v) Hyperparathyroidism causes hypercalcemia and Pautrier (1931) found an elevated serum calcium in 9 of 12 patients with keloids. Each of these patients also had an elevated calcium level in the keloid tissue. Garb and co-workers (1942) however, found a decrease in calcium levels in keloid tissue. Cosman and colleagues (1961) could find no association between the hyperthyroid state and the tendency to form keloids.

vi) Hydrocortisone is an MSH inhibitor. Triamcinalone, which is a 9-α-fluorohydrocortisone, produces depigmentation of the skin but also produces a degradation of the collagen in the tissue. (Ketchum 1976). It is more probable that the beneficial effect of Triamcinalone is due to this collagen degradation rather than the inhibition of MSH.

Only the increased incidence of keloids in Negroes and in states of hyperactivity of the pituitary are unrefuted by other authors. The latter has been linked with the hormonal effect rather than with the associated increase in pigmentation.

It was suggested by Glucksman (1951) that the ritual scarring practiced by some Negro tribes had established an inherent tendency to heal with excessive scarring. This is now refuted by most authors.

Why Negroes are more prone to keloid formation is not known. That some physiological difference must accound for this is accepted. The possible connection with melanocyte-stimulating hormone needs to be further investigated.

2.5.4. Heredity In 1956, Bloom reviewed the literature concerned with the possible heredity of hypertrophic scars and keloids. He defined a keloid as an excessive and abnormal overgrowth of the dermis, and further classified the scars into groups depending upon their "degree of proliferative tendency". These groups ranged from the "spontaneous" keloid (where the initiating trauma was so slight that it had been overlooked), through the "traumatic" or "cicatricial" keloid to the hypertrophic scar. The latter showing the least degree of proliferative tendency.

He discovered 31 familial cases of keloids in the literature, to which he added the pedigree of an Italian family with 14 affected members of five generations.

The pedigree indicated that the predisposition to keloids was inherited according to a regular, dominant, autosomal mechanism.

In the Italian family three of the affected members and one other with a questionable keloid had suffered from gastric ulcers. Bloom (1956) hypothesized that the same genetic mechanisms which caused peptic ulcers were implicated in the predisposition to keloid formation.

In Cosman's review of the literature in 1961 only 8 patients out of 240 supplied past case histories of scarring. Whether this was due to a deficiency in eliciting the patient's family history or that the hereditary influence was not strong in the average patient was not made clear. However, some of the severest cases of keloids did have positive family histories.

2.5.5. Hormonal factors Emphasis has been placed on the "glands of internal secretion" as influencing factors in keloid formation by Garb (1942). Out of 80 patients 34% were in their second decade of life, and 12% of the scars followed acne vulgaris lesions. (Acne vulgaris commonly occurs during adolescence).

An increased incidence of keloid formation has been reported during physiological hyperactivity of the pituitary gland, such as during puberty and pregnancy (Edgerton et al, 1951; Cosman, 1961; Ketchum 1974).

Edgerton and co-workers (1951) observed that some keloids resorbed after the menopause, and that keloids were rare in the elderly. The time factor in these resorptions was not recorded. It is quite possible that the scars were just following the natural resorption process that takes place with most scars given enough time. Garb (1942) however suggested that the resorption was due to the diminution or absence of a type of hormonal secretion.

2.5.6. Local factors The most significant contribution to the literature concerned with the "local factors" influencing keloid formation was that of Glucksman (1951). In the examination of 70 cases of scar hypertrophy and keloids he found a foreign body reaction in 45 of the 50 hypertrophic scars. Hair and keratinised material of endogenous origin were present in 31 of these cases. Animal experiments were presented to show the ability of keratin to induce a fibroblast response in connective tissue.

Mowlem (1951) carried Glucksman's (1951) hypothesis further; from a parallel histological analysis of animal and human scar formation he concluded that there were both systemic and local factors involved. He postulated that the skin most likely to

produce hypertrophic scars would have a high density of lanugo and sebaceous glands, and that the possibility of isolating either within a wound would increase in proportion to the thickness of the skin.

However, in both Glucksman's and Mowlem's reports no distinction was made between keloids and hypertrophic scars and no clinical follow-up was given on any resolution of the scars with treatment.

In contrast to Glucksman's (1951) observations, Blackburn and Cosman (1966) found small foreign bodies in serial sections of hypertrophic scars, but none in sections of keloid scars.

It is felt that Glucksman's incidence of foreign body reaction is higher than that usually found. Cosman (1961) found no significant incidence of foreign bodies in the study of 340 lesions.

2.5.7. Initiating trauma Some form of trauma, though it may be so slight that it goes un-noticed, is considered to be the initiating factor of all scars.

The incidence of hypertrophic scar formation after thermal injury is high (Kischer, 1975; Konuralp, 1976; Larson et al, 1976). However, this apparent high incidence could be influenced by the large surface areas involved with burns and the complications involved in their healing.

Smallpox vaccinations and BCG innoculations have also been reported as having a tendency to stimulate the formation of hypertrophic and keloid scars in the injection site (Bloom, 1956; Alhady, 1964). Although no experiments have been reported as to whether a similar effect occurs in injection sites other than

Comparison of Scar Behaviour in Different Regions

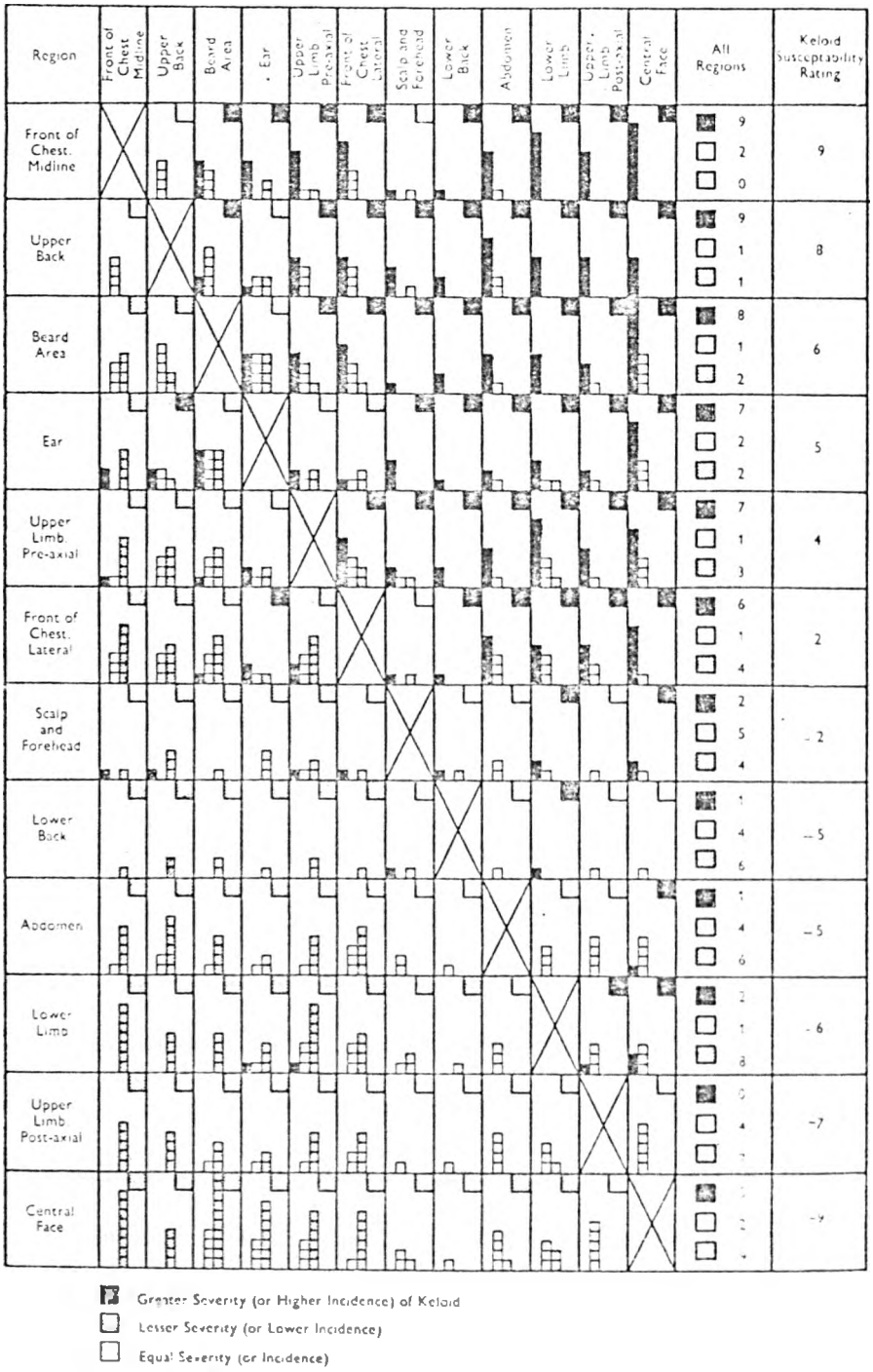


Fig. 2.22. From Crockett, 1964. (see text for explanation of figure.)

the deltoid region of the arm.

2.5.8. Regional susceptibility No part of the body is exempt from scar formation but some areas do seem more inclined to produce hypertrophic or keloid scars than others (Trusler et al 1948).

Fig. 2.22 shows the results obtained by Crockett in 1964 on the regional susceptibility of the body. The scars were divided into hypertrophic and keloid, and the latter subdivided into severe and ordinary. A severe keloid was defined as one which extended beyond the original wound boundary by 2.5 centimetres or more.

46 patients were included in the study, all coloured natives of the Republic of the Sudan. (None of the patients came from tribes which practiced deliberate inducement of scar hypertrophy.)

Fig. 2.23 shows the results obtained when the 12 body regions are compared to one another. Whenever different regions in the same patient were scarred the severity of the scar reaction was compared.

Taking the beard area as an example. When compared with the upper back, 2 scars in the beard area were less severe and 5 of equal severity. This gave a -1 rating to the comparison (scars of equal severity cancelled each other out). After comparing the beard area with all the other regions it was found that the scars were more severe in 8 cases, of equal severity in 1 case and of less severity in 2 cases.

This was used to calculate the Keloid Susceptibility Ratio (KSR) for the beard area.

KSR (beard area) = (8x(+1)) + (1x0) + (2x(-1)); KSR = 6

Regional Incidence of Keloid

Region	Front of Chest Midline	Upper Back	Beard Area	Ear	Upper Line Pre-axial	Front of Chest Lateral	Scalp and Forehead	Lower Back	Abdomen	Lower Line	Upper Line Post-axial	Central Face
Scar Cases	16	11	24	24	21	16	6	2	9	18	10	23
Keloid Cases	15	10	19	18	16	12	2	1	2	6	1	1
Severe Keloid	9	6	11	10	9	4	2	0	0	0	0	0
Non-Keloid Scar	1	1	8	15	15	7	5	2	7	16	9	23

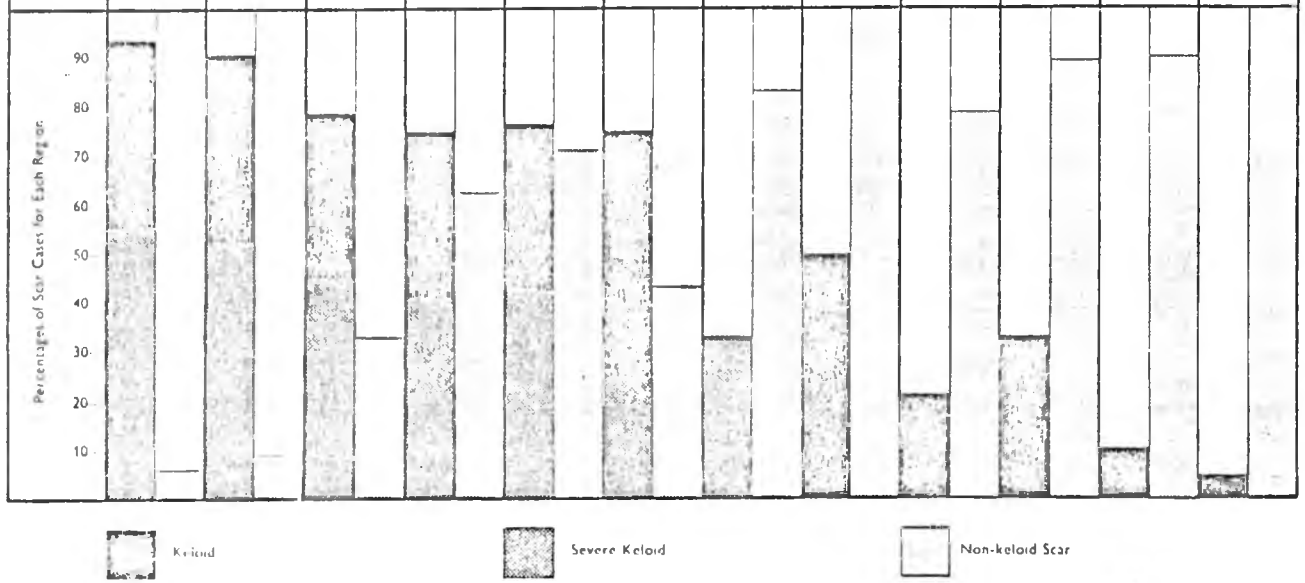


Fig. 2.23. From Crockett, 1964. Diagram showing the results obtained when 12 body regions are compared together with respect to keloid susceptibility.

The KSR's obtained in Fig. 2.23 have been superimposed on a schematic diagram of the body in Fig. 2.24.

Crockett's results should not be taken as absolute values, since there are individual variations in the boundaries of the 12 regions, and only 46 patients were studied. The results do act, however, as a qualitative indication that some body areas are more susceptible to keloid formation than others.

Other authors have reported certain body areas as being more susceptible. In particular the sides and back of the neck, the ears, the sternal area of the chest, the back and the abdomen (Trusler et al, 1948); the deltoid region, the presternal area and the upper back (Ketchum et al, 1974).

Keloids and hypertrophic scars are rarely found on the eyelids, genitalia, palms or soles (Alhady et al, 1964; Peacock et al 1970).

Crockett (1964) concluded by dividing the body region into three orders of susceptibility:

First Order: In a susceptible individual all scars are likely to show keloid change - Pre-sternal region, upper back

Second Order: The detailed nature of the injury can influence the liability to keloid - Beard area, ear, deltoid and pre-axial upper limb, anterior chest wall (excluding the mid-line), scalp and forehead.

Third Order: Keloid change is exceptional and is almost never severe - Lower back, abdomen, lower limb, post-axial upper limb, central face, genitalia.

Why some areas of the body are more prone to hypertrophic scarring is unknown. Various hypotheses have been put forward, the primary one being the degree of extensibility/tension in the

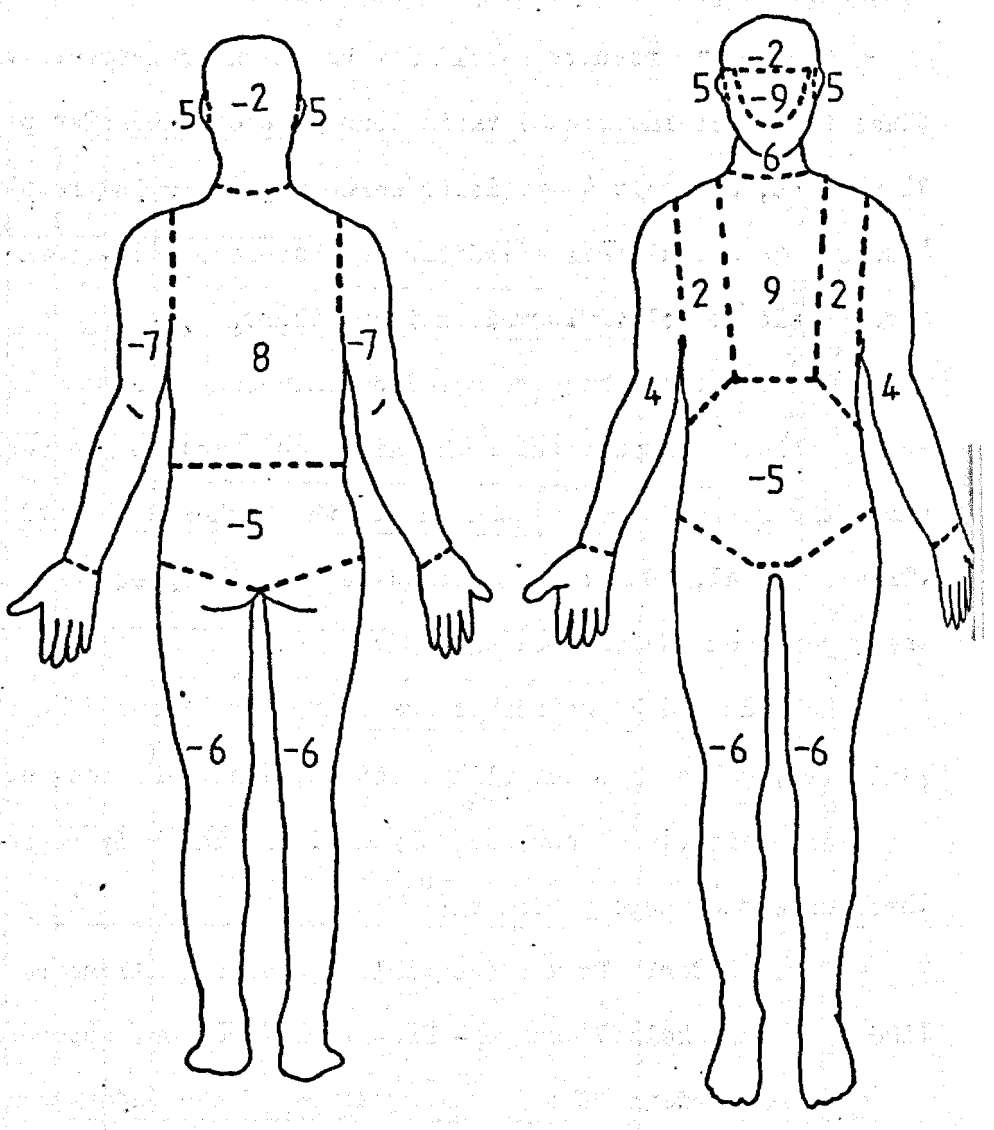


Fig. 2.24. Keloid susceptibility ratios obtained by Crockett (1964) superimposed on a schematic diagram of the body.

skin in the different regions.

2.6 Skin Extensibility/Tension

The skin acts as a container for the rest of the body and as such is under constant tension. Because of the tentlike effect produced by the underlying bony skeleton the tension varies from site to site. Many attempts have been made to map out the tensional forces in the skin, and the patterns obtained have been variously described as Cleavage lines, Langers lines, Crease lines, Wrinkle lines, Lines of Election, Lines of Tension, Lines of Minimal Tension, Lines of Minimal Extensibility and Lines of Extensibility (Flint, 1976). Because the patterns described are due essentially to the tension in the skin they will be discussed in the following section under the generalised heading of Lines of Skin Tension.

2.6.1. Lines of skin tension. The first recorded observation of the tensional properties of skin were those made by Dupuytren in 1834. The corpse of a suicide who had allegedly stabbed himself with an awl showed elliptical wounds instead of the expected round ones. Dupuytren carried out further tests on cadavers and verified that puncturing the skin with a round instrument left elliptical wounds.

Malgaigne (1838) showed that when many punctures were made in the skin, the long axes of the elliptical wounds lay along a pattern of lines.

Langer (1861) using cadavers of all ages exhaustively studied this linear pattern and drew up a detailed map of the lines for the entire body (Fig. 2.25).

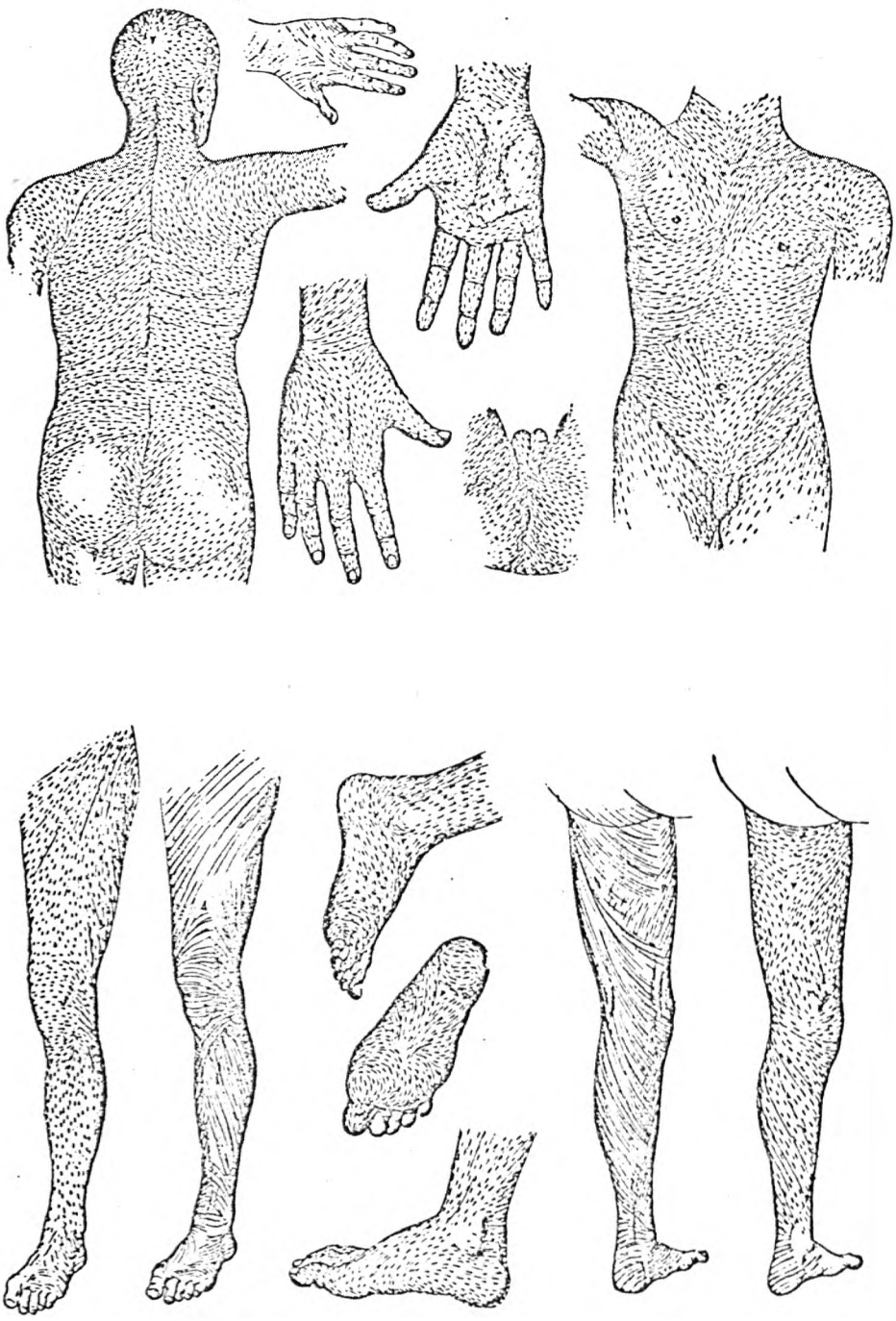


Fig. 5.25a. Langer's lines. A reproduction of the work of Langer (1861).

Kocher (1892) was the first surgeon to recognise the importance of these Cleavage or Langers lines. The assumption was made that the elliptical wound was due to the splitting of the dermis between adjacent groups of collagen fibres. Kocher (1892) advised that surgical incisions be made along Langers lines so that the least tension would be placed across the healing wound.

Other people disagreed with this. Kraissl (1951) argued that the natural crease lines of the skin were the directions of election for incisions and that these did not coincide at all points with Langers lines. He gave the impression that the crease lines were due to the underlying muscle attachment and that they generally ran at right angles to the direction of muscle pull.

Doubt has been thrown on the accuracy of the drawings of Langers lines upon which Kraissl (1951) made his comparison (Flint, 1976). However, even making allowances for these possible errors there are still many areas of the body where Langers lines and Kraissls crease lines do not coincide.

Cox (1941) repeated Langers experiments on cadavers. The cleavage lines that he obtained differed from Langers in certain areas (Fig. 2.26). The main differences were found on the postero-medial aspect of the thigh, sole of the foot, anterior and posterior aspects of the upper extremity and in the region of Pouparts ligament. Langer (1861) stated that the pattern changed with age. Cox (1941) came to the conclusion that the pattern showed variations according to body configuration (degree of muscular development, emaciation, stheric and astheric types etc.), and was only constant for bodies of similar build. The upper extremity line pattern formed an exception to this rule, as it

either due to experimental errors, or other special factors such as muscle, tendon or fascial attachments to the skin, which invalidate the normal criteria of selection. His results also showed that the extensibility of the skin was much greater in the direction perpendicular to the cleavage lines than along them, and that this was reflected in the fibrous microarchitecture of the skin. The histological studies demonstrated that the elastic fibres crossing the cleavage lines were loosely arranged in coils, whereas those along the lines were more extended. The collagen fibres of the mid-dermis also showed a "preferential orientation" along the cleavage lines. This histological pattern was also a reflection of the greater tension in the skin in the directions parallel to Langers lines.

2.6.2. Mechanical properties of skin The mechanical properties of the skin are determined by the physical properties of the extracellular materials and their peculiar micromorphology. Skin has anisotropic, non-linear, time-dependent properties which can be attributed to the intertwined networks of collagen and elastin and to the ground substance matrix (Evans et al, 1971). A typical in-vitro, load strain characteristic is shown in Fig. 2.27.

The non-linearity of the characteristic can be explained in terms of three phases of extension. Initially there is a fairly rapid increase in length for very small increments of force. This is reflected in the microarchitecture by a straightening of the undulating epidermis, and a reorientation of the fibres in the dermis towards the direction of load. The second phase constitutes the "elbow" of the graph. At this stage the cells of the epidermis are becoming elongated, the papillary layer fibres of the dermis are

beginning to compact and the dermal fibres are straightening out. The third phase is characterised by small changes in extension for relatively large increases in force. The fibres of the dermis straighten out and start to compact with increasing load, the deep-dermal fibres lagging slightly behind those of the mid-dermis (Brown, 1973).

Age variations in the load strain characteristic are confined to phase I, showing a shortening of this portion of the curve with age.

The extention of the skin in one direction is accompanied by a similar degree of contraction at right-angles to the stretching force (Fig. 2.27). As the skin is progressively extended there comes a point at which a decrease in volume occurs. This is assumed to be due to a displacement of the interstitial fluid. This fluid must be replaced before the relaxed pattern can be re-established after the removal of the load. In-vivo the fluid is probably displaced into the neighbouring tissues and is therefore readily available on relaxation (Gibson et al, 1965).

The extensibility of the skin is limited to that allowed by the deformation of the fibre network, as little extension is possible once the fibres are aligned. The deformability of the fibre network is determined, in turn, by the fibre configuration in the unstrained condition (Brown, 1973). Thus in cases of hypoextensibility, such as in hypertrophic scar tissue, the low strains achieved with high loads would be expected to be reflected in the fibre orientation of the tissue.

2.6.3. Effect of tension on wound healing When an incision is made in the skin, the static tensions within the tissue will distort the wound. "The area of the resultant defect will always be larger than the original incision or excision, in proportion to the inherent skin tensions" (Thacker et al, 1975). There are also dynamic tensions associated with joint movement and muscle activity which will also have an effect on the wound.

The dynamic forces have an important influence on the static skin tensions. As a joint moves, the magnitude and direction of tensions within the skin change (Fig. 2.28).

Taking the knee as an example, when flexed the extension per unit force is smaller. THe effect of these changing tensions on a circular wound are shown beneath the graph in Fig. 2.28. With the leg extended a circular excision results in an elliptical defect whose long axis is in the longitudinal direction. The flexing of the knee increases the skin tension and results in the narrowing and lengthening of the wound.

Both the static and dynamic tensions have an effect on the wound healing process (Thacker et al, 1975). It has been shown that increased tissue traction across a wound can result in an increase in the tensile strength of the wound tissue (Sussman, 1969; Myers et al, 1969). Forrester and co-workers (1969) emphasised the importance of measuring the energy absorption or toughness of a specimen as well as its breaking strength. In fact Sussman (1969) found no significant difference in breaking strength between wounds closed under different degrees of tension, although they did exhibit significantly different tensile strengths.

Forrester and colleagues (1969), found that tape closed

wounds in rats developed a greater tensile strength than sutured wounds, although the rate of collagen synthesis was the same in both kinds of wounds. The rate of collagen synthesis can be correlated with the gain in tensile strength through the early stages of healing (Madden and Peacock, 1968). However, the major gain in tensile strength occurs during the period of differentiation of the collagen (Dunphy and Jackson, 1962). In fact this difference in tensile strength between the wounds was observed by Forrester and his colleagues to be reflected in the fibre micro-architecture. The tape-closed wound was under increased tissue tension due to the retraction of the cut ends of the panniculus muscle beneath the dermis. The appearance of the 10 day sutured wound was that of a disorganised, random arrangement of collagen fibrils. As healing progressed the fibrils coalesced to form irregular masses of collagen without evidence of fibril structure.

In the 10 day tape-closed wound there were already signs of fibril aggregation. Small fibrils lay together in groups and small bundles were orientated across the wound. As healing progressed the fibrils formed irregular masses as in the sutured wound. However, a noted difference was the development of fenestrations in the tape-closed wound. Forrester concluded that the increased tension resulted in an increase in the tensile strength of the wound and that this was mediated by a qualitative change in the collagen laid down in the wound. Although the tape-closed wound had a greater tensile strength the scar was less extensible than the scar in the sutured wound. Thus, the final result was a brittle scar, with no greater ability to absorb energy

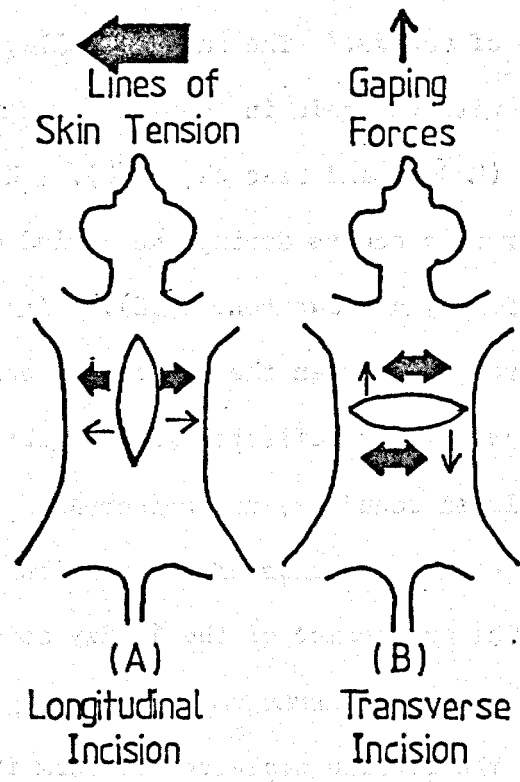


Fig. 2.29. Schematic representation of alignment forces of tension acting upon longitudinal and transverse incisions. (After Sussman, 1969).

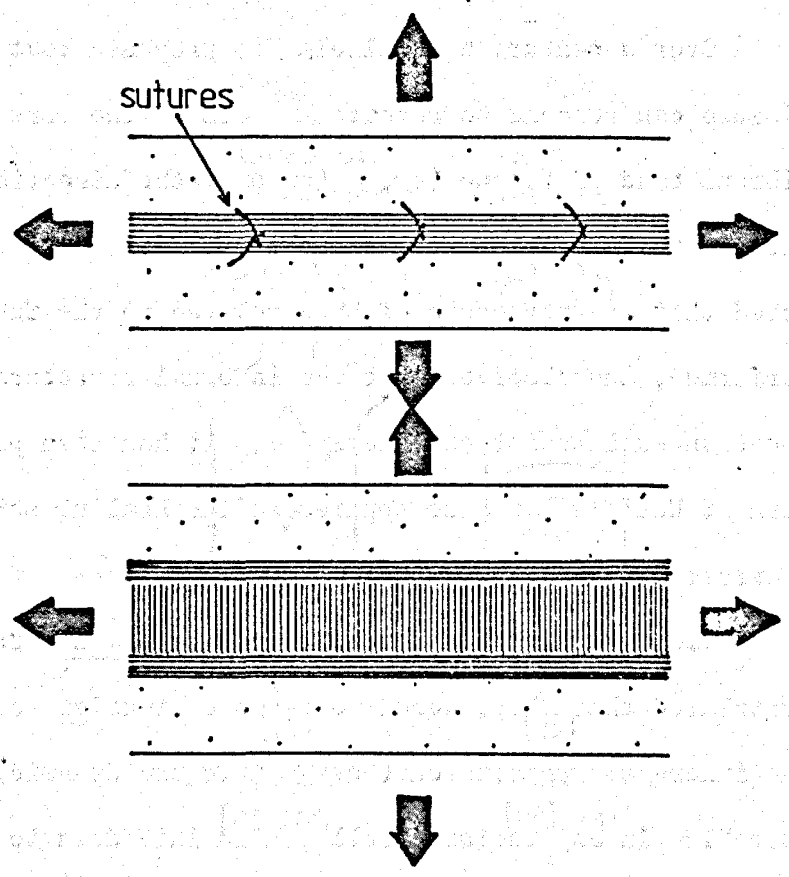
than the supposedly weaker sutured wound scar.

Over a century ago Wilhelm His proposed that connective tissues can respond to repeated stress by the formation of a fibrous band of tissue (eg. a tendon), the direction of whose fibres was parallel to the direction of tension. In 1892 Wolff noted that the structure of bone related to the function it performed, and observed that the internal structure altered to fit the changes in functional demands. It has been postulated that a form of Wolff's Law also applies to the healing skin wound (Forrester et al, 1970).

2.6.4. Wolff's Law and fibre orientation Sussman (1966) postulated that there were two types of tension acting on a wound: The inherent skin tension (both static and dynamic) and a "Gaping force". In any incision collagen is laid down to bridge the defect in the skin. Sussman postulated that these forces acted as stimuli to the collagen formation.

In an incision at right angles to Langers lines both the gaping force and the lines of tension act across the wound. This combination of forces tends to align the new fibrils in such a manner as to smoothly bridge the defect and parallel the pre-existing fibres in the surrounding normal skin (Fig. 2.29). In an incision parallel to the Langers lines the two forces act at right angles to one another. This would result in the collagen being laid down in an haphazard arrangement whilst trying to satisfy both stimulating forces. The scar formed in this way would be of poor structural stability.

Sommerlad and Creasey (1978) observed the fibre orientation in stretched scars from linear incisions in human skin that had



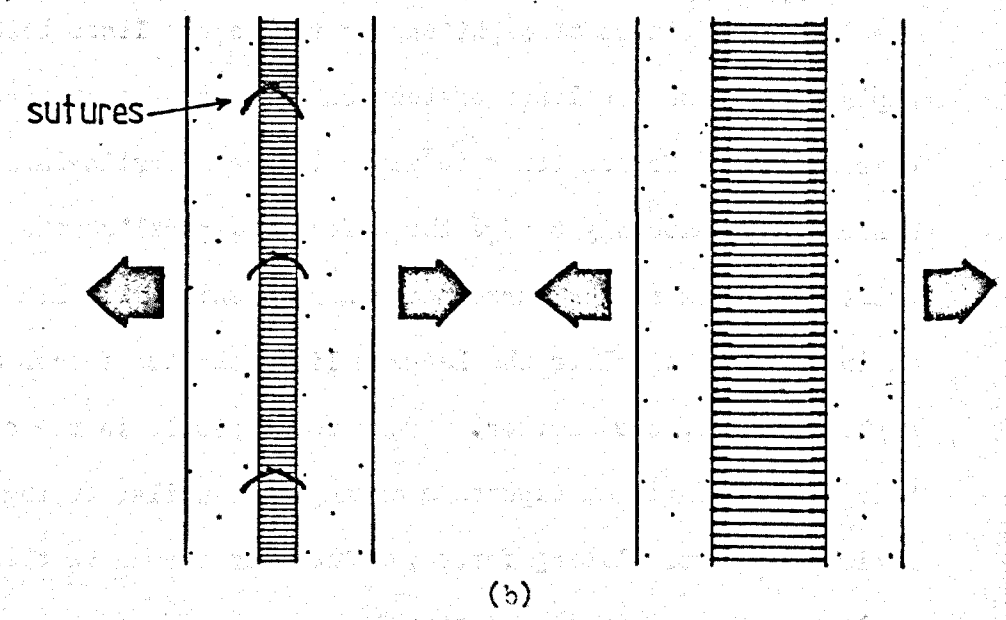


Fig. 2.30. Suggested mechanism of stretching of a scar; (a) where there is tension along, as well as across, the scar; (b) where there is little, if any, tension along the scar but much tension across it. (After Sommerlad and Creasey, 1978)

been sutured. (The effect of suturing would be to remove the gaping force across the wound by providing a mechanical support). In a linear incision parallel to Langers lines the collagen is laid down in the direction of the incision. If the resultant scar tissue is strong enough to resist rupture when the sutures are removed the scar will remain narrow. If however the tensions are sufficient to overcome the bond new collagen fibres will be laid down across the wound to resist the gaping force. This will result in a spreading of the wound and the histological appearance shown diagrammatically in Fig. 2.30a.

If the incision is at right angles to Langers lines the collagen fibres will orientate across the wound. Once the sutures are removed the skin tension will act across the wound. If these tensions are low the scar may resist rupture, if not more collagen will be laid down across the wound (Fig. 2.30b).

Hunter and Finlay (1976) observed the SEM structure of five normal human scars, all running at right angles to Langers lines. After 3 weeks the collagen fibrils were aggregated into small fibres running along the line of the wound, with numerous connections between the fibres. At the edge of the defect the fibrils aggregated to form small fairly discrete fibres, whereas in the centre of the wound the fibres were more "matted". By one year the predominant orientation had changed to a direction across the wound. At the scar edge the fibrils were fairly discrete and orientated directly across the wound, parallel to the epidermis. In the centre the appearance was again more matted, with the fibres passing more obliquely across the wound with many interconnections between the different layers and

interdigitations between the fibres in the same layer. Hunter and Finlay (1976) interpret these results by assuming that the collagen fibres within the wound are undergoing a continuous remodelling process. Whereas Sommerlad and Creasey (1978) interpret their results by concluding that once collagen is laid down in a wound it does not alter its direction in the process of remodelling. Instead, new collagen is laid down in response to the tensional forces. They explain the apparently more mature appearance of the central fibres by stating that the fibroblasts in this region are more active and that these fibres have to resist more stresses than the peripheral fibres which are partially protected by the adjacent normal skin.

The general view is that the maturation of scar tissue involves both new collagen being laid down and existing collagen being remodelled (Arem and Madden, 1976). Since collagen is a non-living material the remodelling that occurs must ultimately depend on the ability of the living cells to sense and transduce the mechanical forces into biomechanical action.

If tissue deformation is the mechanical trigger for biological activity, then the degree of force, the rate, frequency and duration of its application, and the loading velocity may all be important in producing the connective tissue alterations. (Arem and Madden, 1976).

2.6.5. Tension and hypertrophic/keloid scars Tension has been cited as an aggravating factor in hypertrophic and keloid scar formation. In the early literature the authors differentiated between hypertrophic scars and scar contracture bands and stated that the latter were inclined to develop in areas of changing

tension, particularly over joints (Trusler and Bauer, 1948; Mowlem, 1951).

Today no distinction is made between hypertrophic scars and scar contracture bands. Apart from Cosman and co-workers (1961) it is generally agreed that incisions made parallel to the cleavage lines are less prone to hypertrophy than those made at right angles to the lines. Cosman and co-workers (1961) stated that keloids often tended to lie along, rather than across the cleavage lines.

Both the dynamic and static tensions in the wound are aggravating factors. If the area involved is around a joint where powerful muscles exert their pull, hypertrophic scar tissue will start to appear the moment that movement is permitted throughout the normal functional range (Longacre et al, 1968). This thickening of the scar will further interfere with joint motion, causing more tension across the scar and thus a vicious circle is established.

Forrester and co-workers (1969) observed that the fibre pattern in the dermis of a one year old hypertrophic scar had a "ragged" appearance with small fibre fragmentation suggesting that its genesis might have been due to multiple "sub-clinical" ruptures and re-healing resulting in a weak and brittle tissue.

Hunter and Finlay (1976) studied the SEM structure of 4 keloid scars. (They gave no definition of what they classified as a keloid scar and no data on the age of the scar). The collagen in the dermis of the scars was aggregated into large bands of fairly uniformly orientated fibres. A striking feature was that these bands appeared to be orientated in every direction except

directly across the wound. The authors felt that the large amount of fibrous tissue in these scars was due not to excessive forces acting on the wound, but to the inability of the fibroblasts to lay down the collagen in the direction of stress. Thus although the fibroblasts could sense the forces being applied across the wound they were unable to lay the collagen down to resist the tension. The fibroblasts would continue synthesising the collagen until the strength of the tissue was large enough to withstand the forces, and this would mean excessive scar tissue being laid down.

2.7 Hypertrophic and Keloid Scars

In the "normal" events of wound healing, an equilibrium exists between the synthesising and degradative phases of collagen metabolism. At some stage this equation becomes unbalanced and the result is an excessive amount of fibrous tissue (Peacock et al, 1970). It is thought that this imbalance may start in the very early stages of wound healing.

2.7.1. Hypertrophic granulation tissue In the normal process of wound healing the tissue goes from a state of juvenile fibroplasia through to fibrohyalinosis and maturation of the scar. The hypertrophic scar follows the same evolutionary cycle, but the time involved in each stage is longer (Mancini and Quaife, 1961). The essential difference was felt to lie in the growth potential of the fibroplasia, which was considerably increased. This would bring about a greater amount of tissue which would consequently need a longer time to follow through the evolutionary cycle. Attention was drawn to the fourth week of healing as the critical phase; the juvenile fibroplasis instead of going to fibrohyalinosis

persists and continues its growth. (Mancini and Quaife, 1961).

The persistence of a chronic inflammatory reaction in hypertrophic tissue has been reported (Mancini and Quaife, 1961; Schilling, 1968; Linares et al, 1973, 1976).

Differences in the histological morphology of the granulation tissue in wounds which subsequently healed with the formation of hypertrophic scars have been demonstrated (Linares et al, 1973, 1976). The new collagen in the granulation tissue had a tendency to assume a whorl-like or nodular pattern. Blood vessels generally surrounded these collagen formations, but occasionally coursed within them. The degree of this whorl-like orientation differed between patients, with some patients showing almost normal appearing granulation tissue.

2.7.2. Myofibroblasts A large number of the contractile myofibroblasts were found in the granulating and young hypertrophic scar tissue. (Baur et al, 1975). This concentration was higher than that found in normal skin, non-hypertrophic and mature hypertrophic scars. No comparison between myofibroblast concentration in granulation tissue from wounds that healed normally or with hypertrophic scars was given.

Such contractile cells can be found in almost every slow contractile process in human pathology (Ryan et al, 1974). Myofibroblasts have been found in conditions of Dupuytren's contracture (Gabiani et al, 1972). Thus although the myofibroblast appears to play an important role in wound contraction, its appearance in granulation tissue cannot, as was suggested by Baur and colleagues (1975) be taken as an indication of the

Type of skin	NoPts.	PPH (dpm/mg dry wt/hr) Mean ± SD
Keloid	14	4084±524
Hypertrophic scar	7	1338±424
Skin adjacent to abnormal scar	13	376±36
Normal scar	8	301±81
Normal skin	10	179±16

Fig. 2.31. Protocollagen Proline Hydroxylase Activity. (From Cohen et al, 1974).

Age-Patient Years	Age-Tissue Years	Percent Collagen Synthesis Normal	Margin	Keloid
33	-	0·3		
34	-	0·3		
38	-	0·2		
50	-	0·7		
16	-	0·6		
50	-	1·3		
43	-	0·4		
17	1		0·3	2·2
16	2		0·3	6·4
16	1		0·7	9·4
43	1		0·8	0·8
23	6			7·1
62	27			8·3
24	2			7·5
11	2			2·8
32	5			2·0

Fig. 2.32. Collagen Synthesis in Normal Skin and Keloids. (From Cohen et al, 1974).

predisposition of that tissue to hypertrophy.

2.7.3. Mast cells Population counts of mast cells from normal skin, hypertrophic scar, mature scar and granulation tissue indicated that the hypertrophic scar contained approximately four times more mast cells than normal skin and approximately one and a half times more than mature scars. Mast cells were virtually absent in granulation tissue (Kischer and Bailey, 1972). Subsequent studies by Kischer and his colleagues (1978) showed that the mast cells began to appear as the granulation tissue developed interstitial collagen.

The high population of mast cells in the hypertrophic tissue has been postulated to be linked with the chronic inflammation persistent in this tissue. (Kischer et al, 1978).

2.7.4. Collagen metabolism The excessive collagen deposition in hypertrophic and keloid scars may be due to increased collagen synthesis, inadequate collagen degradation or a combination of these factors.

Cohen and co-workers (1974) determined the rate of collagen synthesis in keloid, hypertrophic and normal scar, and in skin adjacent to abnormal scar, and in normal skin (Fig. 2.31). The enzyme Protocollagen Proline Hydroxylase (PPH) specifically hydroxylates proline during collagen synthesis. PPH activity correlates well with direct measurements of the relative rate of collagen synthesis.

The results showed that the mean level of PPH activity in keloids was greater than that in hypertrophic scars, and both levels were greater than those in normal scars and normal skin. There was no significant difference in PPH activity between the

latter two groups. PPH activity in specimens adjacent to abnormal scar tissue was not significantly higher than that found in normal skin and scar.

Methods based on PPH activity could be inaccurate since the enzyme activity can be elevated without actual collagen synthesis (Diegelmann et al, 1974). However, the results shown in Fig. 2.32 confirmed the previous findings. These results were obtained from in-vitro ^{14}C-proline incorporation into tissue biopsies (Diegelmann et al, 1974). The mean relative rate of collagen synthesis in keloid was 5.2%, whereas it was only 0.5% in normal skin. The increase in relative collagen synthesis was due to increased absolute collagen synthesis (collagen/mg protein) as compared to total protein synthesis. Craig and colleagues(1975), using a similar technique found the rate of collagen synthesis in hypertrophic and keloid scars to be about the same in each, and equal to twice that found in normal skin.

Whereas Cohen and co-workers (1974) found that the rate of collagen synthesis did not vary with the age of the lesion (Fig.2.32) Craig and colleagues (1974) found that after two or three years the rate fell to approximately that seen in normal skin.

Craig also reported a difference in collagen concentration in the tissues. In normal scar the concentration remains relatively constant with time, but in both types of abnormal lesion the concentration started off somewhat lower than normal scar and then rose to levels higher than the normal scar after two to three years. Other authors have found no differences in the collagen concentration of the different tissues (Bazin et al, 1974; Soussaline et al, 1976).

Inadequate collagen degradation would also result in an excessive amount of collagen deposition. However, the collagenase activity in all types of scars was found to be higher than in normal skin (which had no significant collagenase activity). The activity was greatest in keloids and approximately the same in hypertrophic and normal scar (Cohen et al, 1974).

It appears that both collagen synthesis and degradation are elevated in the abnormal scars. Two explanations are possible for this apparent paradox: An abnormality in the collagen which makes it resistant to the normal breakdown mechanisms, or an inhibition of the collagenase mechanism. However, Milsom and Craig (1973) demonstrated that neither of these explanations was correct.

Cohen and his colleagues concluded that although the collagenase was present in-vitro, the enzymes activity must be inhibited in some way in the in-vivo situation. A view supported by Linares and Larson (1978).

2.7.5. Collagen type Granulation tissue and young abnormal scars showed significantly higher levels of neutral salt-soluble collagen. The citrate-soluble and insoluble collagen fractions appeared to differ little in the scars and normal skin. (Shetlar et al, 1971; Bazin et al, 1974; Soussaline et al, 1976). However Hayakawa and colleagues (1978) demonstrated an increased level of acid-soluble collagen in granulation tissue and immature hypertrophic scars. He also found that the fraction of insoluble collagen in the tissue varied with time. The concentration was relatively low in the granulation tissue, but this value gradually increased over the next two years until it was

significantly higher than that of normal skin.

An abnormally high ratio of type III to type I collagen was found in the granulation tissue of burn wounds. This ratio gradually fell to normal after approximately two years. (Hayakawa et al, 1978). However Berry and co-researchers (1974) found the collagen to be unlike that of infant dermis (type III) or human cartilage (type II). The amino-acid composition of scar collagen was found to resemble that of collagen from the achilles tendon rather than normal skin although the differences were not striking. They postulated that this difference could be explained on the basis of a change in the ratio of α_1 and α_2 chains in the collagen molecules.

2.7.6. Mucupolysaccharides and glycoproteins An increase in mucopolysaccharide and glycoprotein levels in scar tissue has been reported (Shetlar et al, 1971; Kischer and Shetlar, 1974; Bazin et al, 1974; Soussaline and Nicoletis, 1976). These levels were consistently elevated in hypertrophic scars, but only slightly higher than normal skin in normal scars.

The excess of glycosaminoglycans was found to be due to the chondroitin sulphates, more specifically to an increase in chondroitin-4-sulphate (chondroitin sulphate A). Hyaluronic acid content did not vary significantly between different tissues. (Shetlar et al, 1971).

Linares and Larson (1978) have demonstrated that chondroitin-4-sulphate prevents collagenase from breaking down collagen. This suggests that the presence of great amounts of chondroitin-4-sulphate in hypertrophic scars may contribute to the overabundance of collagen deposition found in these scars.

2.7.7. Water content The water content of normal skin has been reported as approximately 64% (Kischer and Shetlar, 1974). Hypertrophic scar tissue contained a higher level than normal (82%), but normal scar tissue water content closely resembled that of normal skin (76%). (Kischer and Shetlar, 1974; Bazin et al, 1974).

This high water content in hypertrophic tissue did not decrease significantly with time as it does with normal scar tissue. (Bazin et al, 1974).

2.8 Structure and Mechanical Properties of Hypertrophic Scar Tissue

The first description of hypertrophic-or keloid-like lesions appeared in the literature around 1790 (Linares 1977). Retz in his book on skin lesions described the epidermis of these scars as being dark red in colour, and giving the appearance as of being compressed by the accumulation of a dense substance which at times formed nuclei or nodules. These nodules varied in shape from almost spherical to discs of plaques of tissue and were intertwined by fibres of the same substance, but of different thicknesses.

In 1816, Alibert gave a similar description of these lesions. He described them as fleshy, ovular or oblong excrescences which were horizontally situated in one or various parts of the skin. They were pale pink in colour with whitish lines. These lesions deeply adhered to the skin and the edges often demonstrated bifurcated prolongations similar to the legs of a crab. (This gave rise to their being called chancroids or keloids). The scars showed a rich venous network on their surface and blanched when pressed.

2.8.1. Microarchitecture as observed with the light microscope

Early reports in the literature put forward a variety of observed histological differences seen between keloids and hypertrophic scars.

In some keloids dense bundles of collagen fibres in regular and systematic arrangements, running mainly parallel to the epidermis were observed. Other specimens showed an irregular arrangement with curled and branching fibre bundles frequently crossing one another. (Heidingsfeld, 1909). The fibre bundles in hypertrophic scars were more evenly arranged, but again ran mainly parallel to the epidermal surface. Some thin walled, newly formed capillaries were observed in both tissues. Any other vascular differences observed appeared to be purely inflammatory. (Heidingsfeld, 1909).

During the period of fibroplasia, in the keloid tissue, nodules and whorls of collagen appeared firstly in the centre of the defect, and later in the transitional area (Kitlowski, 1953; Mancini et al, 1961). These nodules were separated by long collagen bundles arranged as if they were septa. (Mancini et al, 1961). Few blood vessels were seen associated with the nodules (Kitlowski, 1953). The margins of the keloid scars tended to end abruptly, whereas those of the hypertrophic scars had a wider transitional border region.

The presence of these broad, irregular and branched septal bands of collagen was felt by some to be the single most reliable criterion in differentiating keloid from hypertrophic scar tissue (Blackburn and Cosman, 1966).

Descriptions of the fibre architecture of hypertrophic scars

in the early literature was scant, most authors tended to group the two lesions together under the heading of keloid scars. Since the late 1950's the emphasis has changed to the hypertrophic scars, and keloids are taken to be just a qualitatively different result of the same fundamental process (Peacock et al, 1970). The characteristics originally associated only with keloid scars are now also applied to hypertrophic scars as well. (Linares et al, 1972, 1973, 1974; Kischer, 1973, 1975; Larson et al, 1974 a,b; Longacre et al, 1976).

In the early stages of scar formation, before clinical evaluation, the collagen fibres showed no specific orientation. Although the overall pattern was irregular, some fibrils demonstrated a unidirectional or curvilinear pattern, these were interpreted as the fore-runners of the whorl like arrangements that were observed in the more mature hypertrophic scars by the same authors (Linares et al, 1973; Linares, 1974).

A very rich network of small capillaries was found disposed in an irregular pattern similar to that of the collagen fibres. Approximately half of the scars showed scattered foci of chronic inflammation, but only a few demonstrated foreign body granulomas. In histological sections of the granulation tissue, elastin-like fibres, most of them fragmented with a degenerative appearance and a frequent giant cell reaction were seen at random, particularly in the tissue of patients who later developed hypertrophic scars. (Linares and Larson, 1974). This response could contribute to the persistency of the chronic inflammatory process present in these scars. Linares also postulated that the immunological response elicited by the injured elastin fibres could be also

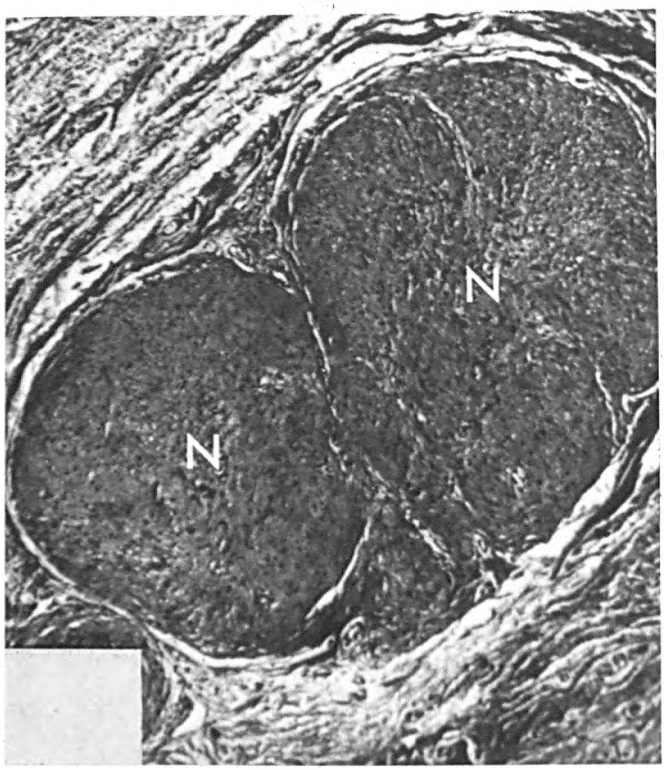


Fig.2.33. ⟵⟶ 500μm. Light Micrograph. Mid-dermis of hypertrophic scar with two large discrete nodules(N), surrounded by septal-like capsules. (After Linares et al,1972).

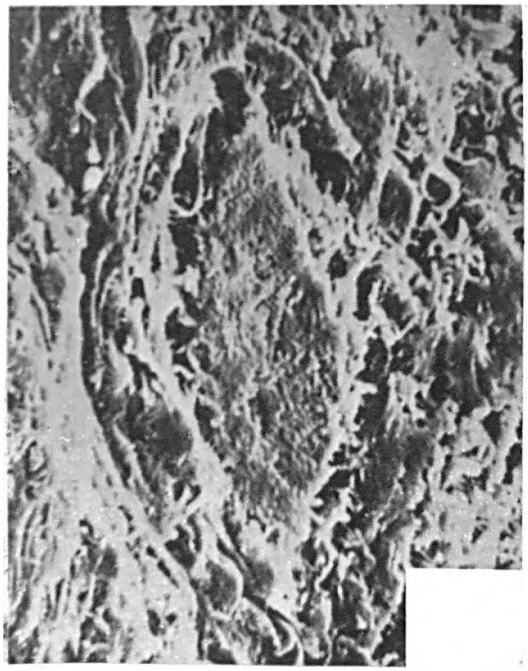


Fig.2.34. ⟵⟶ 10μm. S.E.M.Hypertrophic scar nodule surrounded by whorled pattern of collagen fibres. (After Larson et al, 1974).

interfering with the normal pathway of the biosynthesis of elastin, resulting in a failure of elastogenesis.

In the next stage of development the collagen fibres progressed from the whorl-like arrangement, seen by Linares and his colleagues (1973, 1974) to distinct nodular forms. The fibre bundles increased in thickness and the nodular area became highly compacted. Frequently the nodules became more clearly delineated and appeared to be surrounded by thicker collagen fibres which formed a septal-like capsule (Fig. 2.33). These bands of collagen fibres were observed to run at right angles to the surface of the scar. (Longacre et al, 1976).

A high concentration of acid-mucopolysaccharides had already been observed in the lesions (section 2.7.6). A combined histochemical and histological study of the tissue indicated that the nodules of collagen within the scar tissue contained a high level of chondroitin-4-sulphate. This high concentration decreased as the scar matured (Shetlar et al, 1977). It was concluded that the high level of chondroitin-4-sulphate was related to the proliferation of the connective tissue in the dermal scars and that since the nodules contained the highest concentration, they must be the active sites of proliferation within the scar tissue.

The arrangement of the blood vessels within the hypertrophic dermis is not agreed upon. Whilst Linares and co-workers (1973) described small arteriols and capillaries predominating about the nodules, Longacre and his colleagues (1976) described large calibre, engorged blood vessels running in the large bands of collagen, which were orientated at right angles to the scar surface. Smaller vessels were permeated within the nodule.

A thin band of collagen fibres, with near-normal characteristics was observed below the hypertrophic dermis. This appeared to be acting as a boundary between the scar and the subcutaneous tissue (Linares et al, 1973).

Elongation of both the fibres within the nodules and the nodules themselves was taken as an indication of scar maturation. The pattern proceeded until the fibre orientation was essentially that of a mature "normal" scar. The mature hypertrophic scar showed a regular pattern with a predominant parallel orientation of the collagen fibres with respect to the long axis of the scar and the epidermis (Linares et al, 1972).

The appearance of the epidermis of the scars varied from almost normal in some, to thin and atrophic in others (Linares et al, 1972; Peacock et al, 1970).

2.8.2. Microarchitecture as observed with the S.E.M. The SEM picture of the hypertrophic scar tissue presented by Linares, Larson and their colleagues from the University of Texas (1972, 1973, 1974, 1976, 1978) is very similar to the light microscopy view.

A whorled pattern of the collagen fibres appears to orientate around the central mass or nodule (Fig. 2.34). The centre of the nodule is formed by a complete fusion of the fibres into a homogenous mat of collagen with the fibrils and filaments either just visible or completely indistinguishable (Larson et al, 1974, 1976; Kischer, 1975; Kischer et al, 1974).

Fig.2.35. ⌊____⌋ 1µm. Holey pattern of collagen filaments taken as characteristic of mature scar. (Larson et al, 1974).

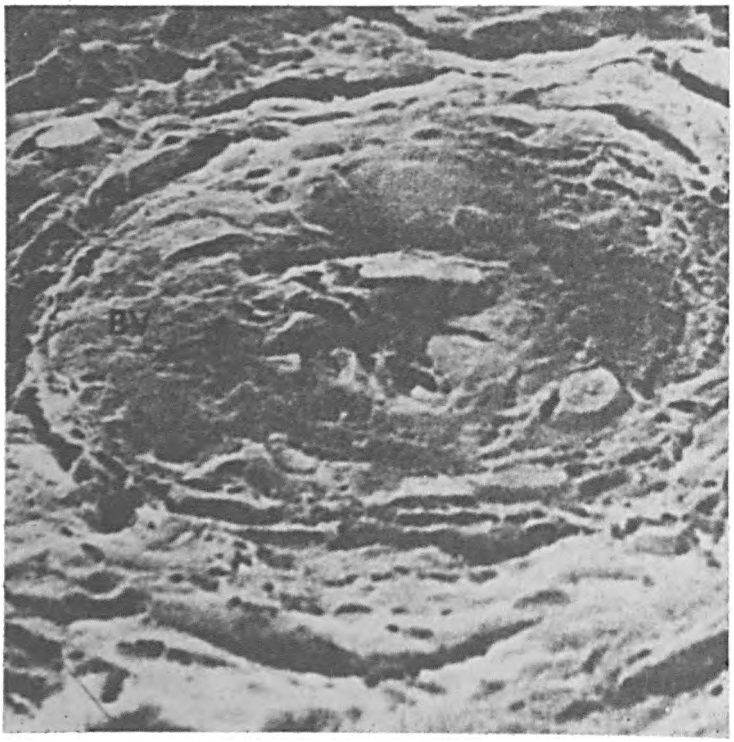


Fig.2.36. ⌊____⌋ 5µm. Whorl like arrangement of collagen fibres orientated around a small blood vessel.
(After Baur et al,1977).

The dermis of the more mature hypertrophic scar demonstrated a loosening of the compact collagen. Individual collagen filaments were once more observed, set within a web-like background of still compacted areas (Fig. 2.35). This left a holey pattern which the authors took to be characteristic for a mature scar (Larson et al, 1974). This picture of mature scar is somewhat different to that presented by other authors (see Fig. 2.17 for comparison).

A micrograph showing a similar arrangement of collagen fibres as in Fig. 2.34 is shown in Fig. 2.36. Here a whorl-like arrangement of collagen fibres is described, but they are orientated around a small blood vessel. (Baur et al, 1977). A similar concentric arrangement of collagen fibres has also been observed surrounding hair follicles in specimens of normal skin (Brown, 1971).

Fig. 2.37 shows transverse sections of the mid-dermis of a 15 month old hypertrophic scar. The collagen is arranged in sheets of fibre bundles criss-crossing in all directions, forming a firmly interlocked structure. Some areas exhibited the parallel aligned wavy fibres seen in mature scar tissue. The criss-crossing bands of fibres enclose roughly circular structures. These are taken to be bands of collagen fibres cut transversely. (Brown and Gibson, 1974; Hunter and Finlay, 1976).

Biophysical techniques (including microradiography, X-ray diffraction and polarising microscopy) have indicated that the collagen in hypertrophic scar dermis was not as well organised as in mature linear scars or normal dermis. Any pattern of fibre orientation that was observed tended to be along the longitudinal axis of the scar. (Holmstrand et al, 1961).

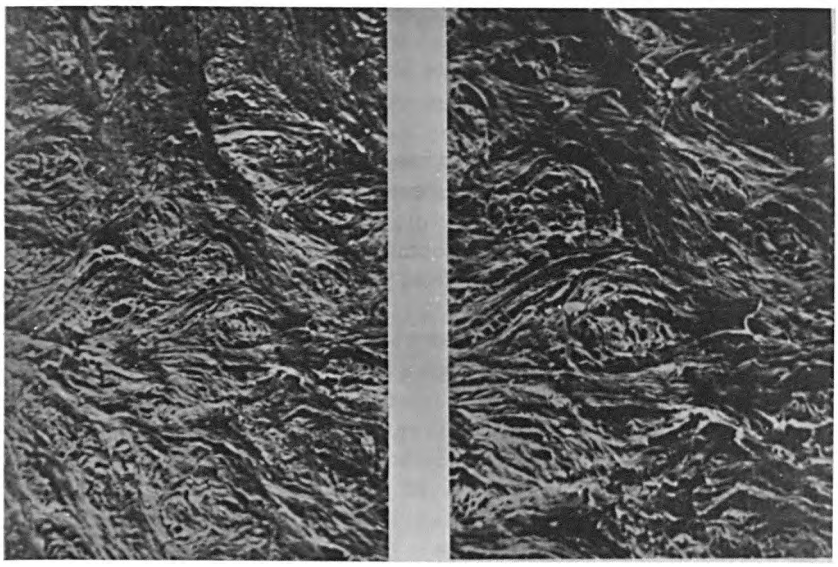


Fig.2.37. ⟵ 50µm. 25µm. ⟶
Mid-dermis of a hypertrophic scar, showing sheets of fibre bundles criss-crossing in all directions.
(Brown and Gibson,1974).

Most of the descriptions of the microarchitecture of hypertrophic scars has been confined to the dermis of the tissue. In fact the mid-dermis appears to be the major area of involvement in the excessive fibrous growth. (Linares et al, 1972; Larson et al, 1974). In the early literature considerable emphasis was placed on the presence or absence of dermal papillae and secondary skin appendages in differentiating between the different types of scar. (Heidingsfeld, 1909). Today, it is generally agreed that these features are variable to the extent that they are of no practical value in the differentiation between the lesion types. (Blackburn and Cosman, 1966; Linares et al, 1972).

2.8.3. Ultrastructure The diameters of collagen fibrils from normal adult dermis usually lie in the range 70nm to 140nm. Some fibrils of fetal dermis are as small as 30nm, but the average diameter does not differ significantly from that of adult skin. The fibrils exhibit a banding periodicity of 64 to 70nm.

A slightly different ultrastructural picture of normal dermis has been presented by Basom and colleagues (1974) based upon work carried out by Haust in 1965. They describe collagen as being made up of subunits, collectively known as unit collagen fibrils. These fibrils ranging from 20nm to 30nm wide with a periodicity of 22nm, grow and soon acquire a 64nm periodicity making them indistinguishable from the unit collagen fibrils of collagen fibres. A third type of fibril, the Microfibril, is described as being the developmental forerunner of the unit collagen fibril. They are slender filamentous structures about 4 to 15nm wide, without periodicity; and it has been suggested that they are the "common denominator" of both the unit collagen fibrils and elastin fibres. (Haust, 1965).

Basom and co-workers (1974) observed the evolutionary cycle of these microfibrils together with the amorphous ground substance represented the promordium of the earliest elastic tissue. The first elastin fibres are small, becoming recognisable about the seventh day. The matrix of each fibre expands, apparently at the expense of the microfibrils.

With increased pressure and attrition from the constantly developing circulatory system larger microfibrils appear. These microfibrils later develop a visible periodicity and become identifiable as unit collagen fibrils. These developing fibrils undergo continuous "transformation" into elastin fibres, a change which Basom states, can occur at any stage of fibrillar development. Thus large elastin fibres are derived from fibrils that have already grouped to form light microscopic collagen fibres. The authors classified the fibril types into five groups: primary fibrils, microfibril, "unit collagen" fibril, collagen fibril and "transforming" fibril. These fibrillar types maintain a constant ratio in normal dermis. When the normal stresses are altered, by immobilisation and/or trauma, the fibrilla matrix responds by altering the ratio of the fibril types and their orientation.

In a later paper (Longacre et al, 1976) Basom described the abnormal ultrastructure of hypertrophic scars as being due to a block in the collagen and elastin synthesis. The dermis of a one year old hypertrophic scar contained a high concentration of transforming fibrils and microfibrils. Aggregates of microfibrils exhibited a periodicity of 150nm to 160nm, creating a fibril never seen in normal dermis. Unit collagen fibrils were seldom

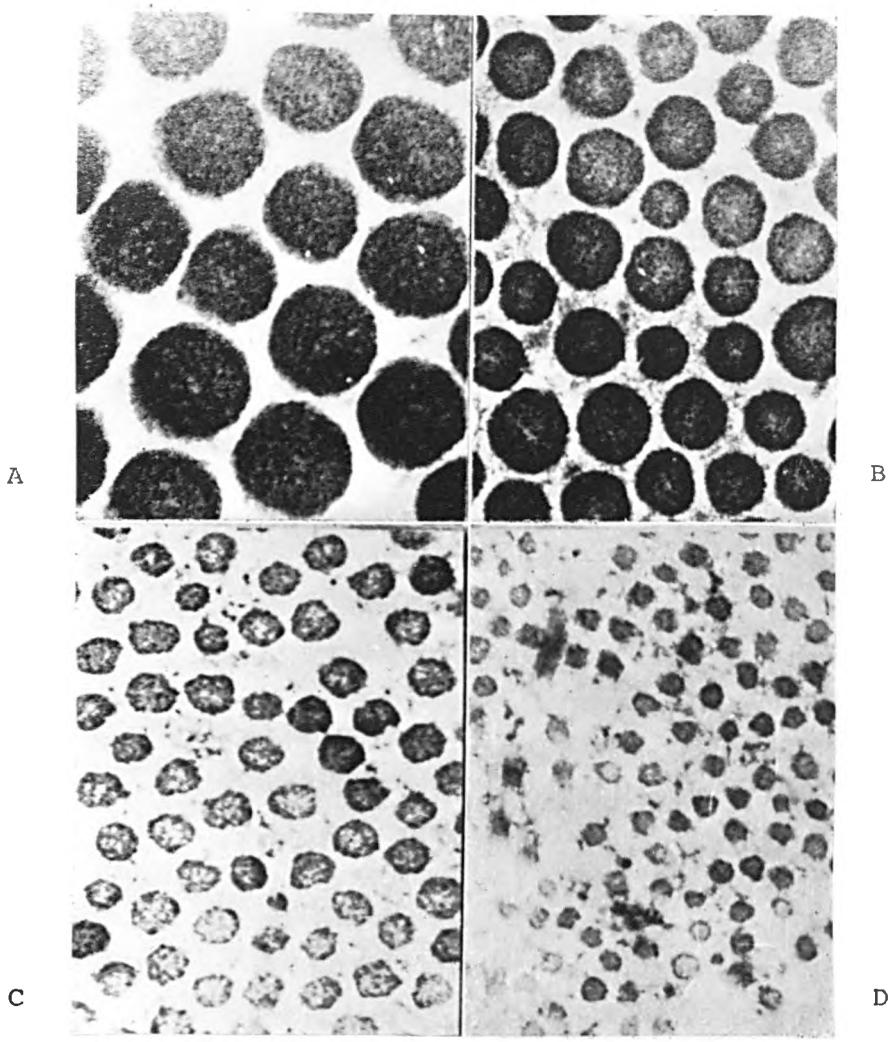


Fig.2.38. ⌊_____⌋ 14nm. T.E.M. collagen filaments.
A) Normal skin, large round filaments.
B) Mature scar, slightly smaller, round to ovoid filaments.
C) Hypertrophic scar, smaller, irregular to ovoid filaments.
D) Granulation tissue, small, angular and irregular filaments.
(After Larson et al, 1974).

seen. The authors felt that the apparent block in synthesis could be due to an inhibition of the enzymes responsible for microfibrillar aggregation. Or, considering the abnormal 150nm periodicity fibril, the synthesis of an abnormal mucoprotein within the microfibrils preventing their aggregation and incorporation into collagen and elastin fibres.

In discussing the transformation of collagen into elastin the authors made no attempt to explain the different chemical composition of the two proteins. Further research based upon their hypotheses needs to be carried out.

Differences in hypertrophic dermal ultrastructure have been reported by other authors (Kischer et al, 1971; Larson et al, 1974; Linares et al, 1972,1973). Collagen filaments in the granulation tissue had diameters in the range 30 to 50nm, with prominent micro-filamentous material between them. In longitudinal sections these filaments exhibited unusual bends and curls with no appearance of a rigid packing pattern. (Linares et al, 1973). Collagen filaments within the nodular regions of the dermis had diameters in the range 40 to 80nm, with the majority about 60nm wide. (Kischer et al, 1971).

The shape of the collagen filaments also varied. In granulation tissue the filament cross-sections were irregular and angular; in hypertrophic dermis-angular to ovoid; mature scar - ovoid to round; and in normal skin all the filaments were round in cross-section. (Fig. 2.38). Micro-filaments of 4nm width appeared to interconnect or wind around the collagen filaments, these were much more prominent in the nodules than in the surrounding tissue. All fibrils exhibited periodicity within the

normal range.

Considerably more cellular processes are present in the hypertrophic dermis. The high concentration of fibroblasts demonstrated markedly dilated rough endoplasmic reticulum, and a paucity of organelles. They continued to show a system of intracytoplasmic microfilaments. (Linares et al, 1972).

The nodules characteristically lacked elastic tissue, both in the extracellular compartments and in the walls of the vessels. The lumen of the vessel walls in the nodules were often partially or completely occluded and the number of perivascular satellites appeared increased over that in normal dermis. (Kischer, 1971).

Two basic forms of interstitial material were observed in the dermis of normal skin, granulation tissue, hypertrophic and mature scar. (Kischer and Shetlar, 1974). A globular form predominated in normal skin, granulation tissue and mature scar, whilst the filamentous form was predominant in the hypertrophic dermis. The latter type appeared to link the collagen filaments in a chain-like fashion, which could explain the near homogenous dermal matrix seen by the same authors when using the SEM.

Globular forms of interstitial material could be demonstrated in stretched samples of hypertrophic tissue. This conversion from filamentous to globular by stretching was felt to reflect possible "recoil capacity" of the material. Conversion to the globular form also occurred normally during the maturation of the scar when softening of the tissue occurs.

2.8.4. Contractures and mechanical properties Numerous case reports have been published on the disfiguring and disabling sequence of scar formation, and much effort has been put into the analysis of the early stages of wound healing. However little experimental data is as yet available on the mechanical properties of the scar tissue and how these properties vary with time.

Clinically hypertrophic scar tissue is hard to the touch and appears inextensible. If the scarred area lies over or near a joint, severe flexion deformities can be formed. These are felt to be due partly to the contraction of the wound edges during the healing process, partly to the excessive fibrous tissue that is laid down in the open granulating space, and partly to the muscle pull around the joints.

Patients with substantial areas of skin loss will assume the position which gives them the most comfort. This usually involves a flexing of the joints, and a patient with extensive skin loss will assume the fetal position. Unfortunately this position of comfort is also the position of contracture. (Larson et al, 1974).

The main areas of contracture are listed below:

Neck - the chin is drawn down to the chest, the mandible fixed and the lower lip everted.

Axilla - Contracture webs of fibrous tissue over the axilla, limiting abduction of the arm.

Elbows and Knees - Flexion contractures preventing extension of the limb.

Hips - The hip is fixed in a position of adduction and flexion, with a predisposition to subluxation and dislocation.

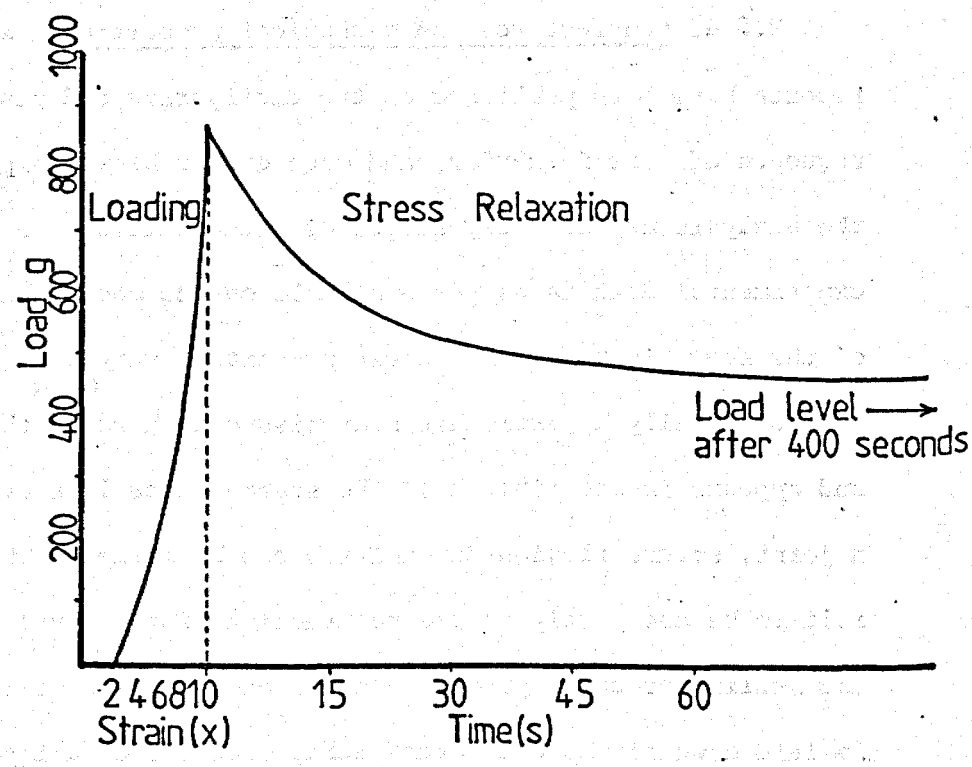


Fig. 2.39. In-vitro stress strain curve obtained for a 15 month old burn scar, demonstrating the inextensibility of the scar at low loads and the visco-elastic stress relaxation at high loads. (After Brown and Gibson, 1974).

Ankles and Feet - Shortening of the heel chord, preventing placement of foot flat on the floor.

Wrist and Hand - The wrist is flexed, the metacarpophalangeal joints extended and the proximal interphalangeal joints flexed. The thumb is adducted and extended.

Fig. 2.39 (Brown and Gibson, 1974) shows an in-vitro stress strain curve obtained for a 15 month old burn scar of the dorsum of the hand. It demonstrates the inextensibility of the scar at low loads and the visco-elastic stress relaxation at high loads. It has been suggested that the ground substance of the tissue is responsible for the stress relaxation of a tissue (Brown, 1971). This would act by delaying the deformation of the fibrous networks to loading changes.

Chu and Brody (1975) obtained a similar stress strain characteristic in-vivo. Mechanical tests were performed on a 7 month old scar of the forearm. The scar was very stiff, but the possible extensibility varied with the position of the arm. With the elbow flexed the scar showed a higher degree of extensibility than when the elbow was extended.

2.9 Methods of Treatment

The types of preventive or corrective therapy reported in the literature are too numerous to list. The procedures can however be classified into four major groups.

2.9.1. Surgical procedures If a wound is too large to be allowed to heal by contraction and epithelisation skin from another source must be grafted onto the defect. Essentially three methods are available: free grafting, pedicle flaps and free flaps. (Peacock and Van Winkle, 1976). Free grafts are

completely detached from all connections with the donor site and thus have to reconstitute new connections with the host sites vascular systems. Pedicle flaps are never completely detached from all of their vascular connections, but are transferred in stages. In the past few years the use of free flaps has increased. These take the same form as pedicle flaps but now the tissue is completely freed from its donor site vascular connections and surgically connected to suitable host site blood vessels by microvascular anastomoses. The free skin graft can be of various thicknesses, usually divided into split skin grafts (SSG) or a thick skin graft (TSG).

In a SSG only a thin layer of skin is transplanted and the donor site will be left with enough epithelial remnants to heal spontaneously, with minimal scarring. If a flap or TSG which is sufficiently thick to interrupt the integrity of the deep-dermis is used the donor site may heal with severe scarring (Larson et al, 1974). To prevent this the donor site is treated as any other partial or full thickness wound. The technique of applying a graft to the host site essentially provides it with replacement epithelium, in the case of a SSG, or replacement skin in the case of a pedicle or thick skin graft. If the amount of patients donor skin is not sufficient to cover the defect, skin from another individual (a homograft) or from another species (a xenograft) can be used. The latter two types of graft are only temporary, and are usually used as biological dressings whilst the patients donor sites are healing. (A SSG donor site can be re-used after it has healed, although care must be taken to prevent the risk of its healing with scar formation from increasing).

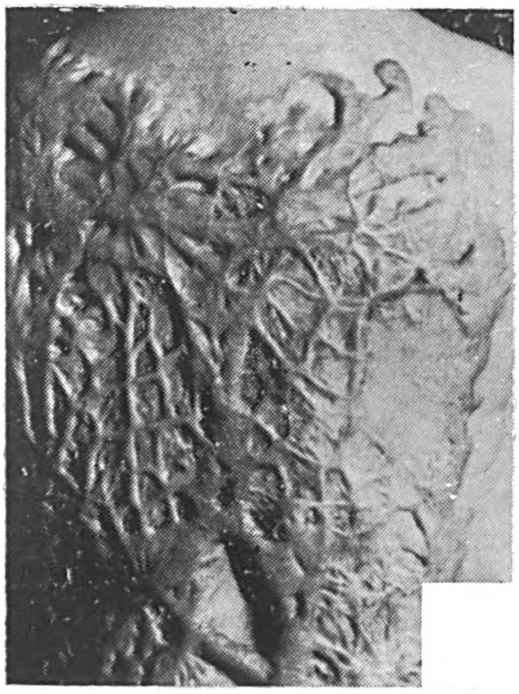


Fig.2.40. Uncosmetic appearance resulting from the re-surfacing of a wound with postage stamp grafts. (After Larson et al 1974).

Skin autografts do decrease the tendency for a wound to heal with a hypertrophic scar (Larson et al, 1974; Pitanguy, 1976; Janzekovic, 1975). It appears that granulation tissue proliferation is strongly related to its coverage by epithelium. Whilst the granulation tissue remains uncovered, it continues to grow, sometimes obtaining levels above those of the surrounding skin, becoming exuberant and hypertrophic (Pitanguy, 1976). This is further supported by the formation of excessive scar tissue at graft borders where epithelisation has been required. (Larson et al, 1974). This process limits the effectiveness of other types of autograft that have been modified in order to increase the area of wound that can be covered. In these grafts the "effective" area that can be covered is increased by either cutting the graft into small pieces (sometimes called postage stamp grafts) or making a series of incisions in the graft which allow it to be expanded, like pulling out a length of mesh fencing. However both of these methods (postage stamp and mesh grafting) just convert the large wound into a number of smaller wounds, each of which will then heal by contraction and epithelisation. Frequently these uncovered areas form hypertrophic scars, giving an unpleasant appearance to the tissue (Fig. 2.40).

The application of a skin graft does not stop a wound from contracting (Sawhney and Manga, 1970). Depending upon the thickness of the graft, however, it will limit the process. Because the application of a graft does not instantaneously halt the contraction of the wound edges the graft itself is prone to contract. This can result in either giving a wrinkled appearance to the graft, or the graft area will actually decrease.

(50% reductions in area have been reported (Peacock and Van Winkle, 1976)). This decrease in area suggests that an active remodelling process is taking place.

The thickness of the graft appears to be related to the degree of contraction. A thick graft will tend to contract less than a thin one (Sawhney and Monga, 1970).

Other factors, including the orientation of the graft on the host site with respect to the cleavage line pattern and the degree of skin laxity in the host site affect the contraction. Sawhney and Monga (1970) and Cronin (1961) found that the amount of graft contraction increased in proportion to the skin "laxity" at the host site. So that grafts placed on the neck contracted by up to 80%, whereas those on the scalp only contracted by a maximum 27%. The maximum contraction occurs during the first few weeks after application (Ragnell, 1952; Sawhney and Monga, 1970). The skin grafts contracted by the least amount in the direction of the cleavage lines (see section 2.6.1) around the host site. This suggested that the inherent tensions within the skin play an important role in graft contraction. Longacre, (1974) recommended the "matching" of cleavage line orientation between the grafts and the skin surrounding the host site to help minimise this contraction. A mechanical support, such as a splint, can also be applied over the graft to physically oppose the graft contraction (see section 2.9.5).

Once a disfiguring or debilitating scar has formed various surgical procedures can be used to try and improve the appearance and function of the area. The scars can be partially or completely excised, the resultant defect being covered with a skin

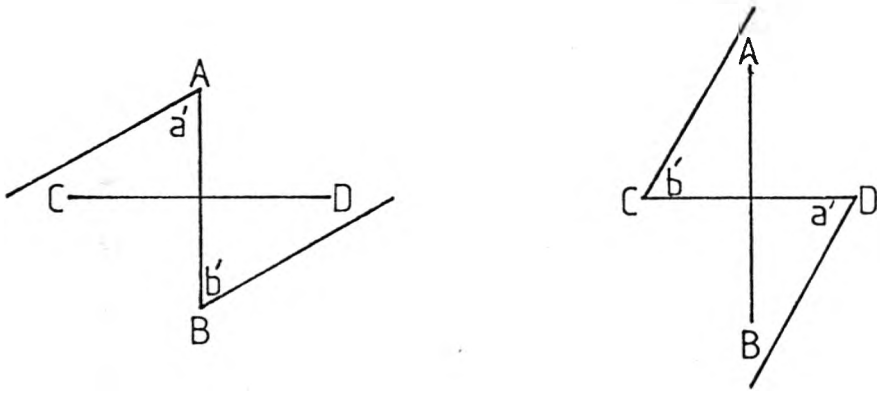
graft.

The success rate of any surgical procedure depends on whether the scar is a hypertrophic or keloid lesion. The complete excision and resurfacing with a skin graft of a hypertrophic scar has a good success rate (83%), whereas this is much lower if the scar is of the keloid type (53%). (Cosman et al, 1961). A higher success rate with keloids has been achieved using partial excision. In this procedure the outer rim of the scar is left in tact. It is felt that this helps to splint the wound, preventing the retraction of the wound edges or the transmission of the external deforming forces to the central area. (Pitanguy, 1974; Peacock and Van Winkle, 1976).

A variation on the partial excision technique is that of "shaving" the scar surface until it lies flush with the surrounding skin. The defect is again resurfaced with a skin graft (Hynes, 1957; Moustaffa and Abdel-Fattah, 1976).

Contracted scar tissue, or contracture webs of scar tissue present a slightly different problem to the surgeon. Here the main problems are the functional limitations caused by the scar tissue. The contracture has to be released either by the addition of more skin to the area, or by rearranging the scar to effectively lengthen it.

If a contracture band is divided at right angles to its long axis the resultant defect is an elliptical gaping wound. The author observed one such operation in which the wound gaped by approximately 8 centimeters immediately after the incision. After division of the tissue the defect is resurfaced with a skin graft. Conway (1939) advocated the use of a full thickness skin graft to



(i) Initial lines of incision (ii) After flap transposition

Fig. 2.41. The Z-plasty. (From Furnase and Fischer, 1971)

prevent the recurrence of the contracture, since SSG appeared to be more prone to contraction. Longacre (1974) found the recurrence high even when thick skin grafts were used. Conway (1939) noted that with the successful release of tension however, the scar tissue appeared to thin and soften.

2.9.2. The Z-plasty The possible complications that can arise when a skin graft is used are avoided to a large extent by using a Z-plasty. In this operation the tension in the scar is released by "repositioning" the tissue. This is achieved by the transposition of two triangular flaps of the tissue (Fig. 2.41). The original markings made to define the flaps also define a parallelogram on the skins surface. Transposing the flaps has the effect of interchanging the diagonals of this parallelogram. The longer diagonal is tranposed to lie in the direction of the contracture, so that the scar is effectively lengthened in this direction. The Z-plasty is usually diagrammed as a symetrical 60°-60° figure, but this orthodox design is often altered to tailor a particular defect. (Furnase and Fischer, 1971).

The release of tension by a Z-plasty on a hard, thick, raised erythmatous hypertrophic scar at any age of its development is associated with a softening, thinning, flattening and blanching of the tissue. Changes in the biochemical, ultrastructural and histological properties are also observed. (Davis and Kitlowski, 1939).

Berry and co-workers (1974) found that collagen degradation products in the urine of patients increased in amount following the release of hypertrophic scar contractures by Z-plasty. This increased excretion of degradation products coincided with a

softening and thinning of the scar.

The excessive amounts of microfibrils and transforming fibrils (reported to have been observed in hypertrophic scar dermis by Townsend, Longacre and their colleagues) start to disappear after Z-plasty (Townsend et al, 1974). They are replaced by normal appearing unit collagen fibrils and elastic fibrils. The vascularity of the dense nodules of the scar tissue was increased after Z-plasty, suggesting that new blood vessels had grown into the lesion. (Longacre et al, 1976).

The results achieved with Z-plasty have been quite good. As well as the release in the contracture obtained with the repositioning of the tissue the scar also starts to soften and thin.

The role of surgery in the treatment of keloids and hypertrophic scars is often a supportive one. Primarily its role is in the reorientation of scars to minimise the tension across them, and the reduction of the mass of the scar tissue so that with other forms of therapy an acceptable scar can be achieved.

2.9.3. Irradiation The principal effect of short wave irradiation upon cell action is to injure, kill or depress cell function through mutation (Peacock et al, 1970).

X-ray treatment of keloids was first described by DeBeurman and Gougerot (1906). The usual procedure is for the lesion to be excised and the defect closed, taking great care to leave as little tension as possible across the wound. (Craig and Pearson, 1965). The irradiation therapy consists of giving controlled doses of radiation pre- and/or post- operatively. Successful prevention of recurrence have been reported using various combinations of pre- and post-operative irradiation.

Levitt (1951) used just pre-operative, or both pre- and post-operative courses of therapy. Cosman (1961), Konuralp (1976) and Craig and Pearson (1965) all advocated early post-operative therapy.

One of the problems of using radiotherapy is that of restricting the radiation dose to a particular area. Soussaline and Nicoletis (1974) have developed a technique for applying interstitial irradiation using a piece of iridium 192, placed inside a lumbar puncture needle and inserted beneath a linear hypertrophic scar.

Any successes achieved with radiotherapy can be explained on the basis of altering the collagen metabolism through damage to the highly specific stem cells necessary to this process. (Peacock et al, 1970).

Although it is theoretically possible to administer a dose of radiation which does not increase the hazard of subsequent neoplasia, finding such a patient variable dose with today's technology is largely a matter of trial and error. Because of this, radiotherapy is not routinely used as a therapeutic measure in the correction of excessive scar tissue (Peacock et al, 1970).

2.9.4. Chemotherapy For centuries, almost every herb known to man has been applied to scars in an effort to soften and/or flatten them. In 1950 Baker and Whitaker demonstrated that direct application of steroids to the skin over a prolonged period of time produced a thinning of the dermis, limited to the area of treatment. They also noted that steroid therapy applied to a healing wound stopped the growth of granulation tissue completely.

Fibroblasts did not proliferate, and there were few outgrowths of endothelial buds from the blood vessels.

Conway and Stark (1951) observed the affect of applying Adrenocorticotrophic hormone (ACTH), both systemically and locally to keloid scars. Scars were either excised and the patient given ACTH post-operatively, or ACTH was injected directly into the wound. Both types of treatment alleviated the symptoms of the lesion but did not appear to affect the physical size of the scar.

The successful use of hydrocortisone, or one of its derivatives in preventing the recurrence of hypertrophic scars has been reported (Asboe-Hansen, 1956; Murray, 1963). Although some successes have been reported when treating keloid scars (Conway, 1960), the therapy was generally ineffective, particularly when treating Negroes. (Murray, 1963).

The intralesional injection or topical application of triamcinalone can give relief of paraesthesia and regression in both hypertrophic and keloid scars. (Ketchum 1966; Griffith, 1966; Ketchum et al, 1971; Macmillan, 1976; Garcia-Velasco, 1973). Various protocols were used. Small scars can be treated with either topical or intralesional applications. Larger scars are usually excised and the defect resurfaced with a SSG. The steroid being injected into the wound edges at the time of surgery.

It was suggested that the injection of the steroid, in some way altered the collagen metabolism within the lesion, resulting in either preventing its recurrence or in causing its regression once formed. It was demonstrated that triamcinalone did not affect collagen synthesis (Cohen, 1974). Thus it has been

postulated that the steroid in some way enhances the collagen degradation (Ketchum, 1967; Cohen, 1974, 1977).

Because of the effect of external pressure applied to a hypertrophic scar causing its regression it was also hypothesised that the high pressure under which the steroids were injected into the lesion could result in the beneficial affect observed. (Peacock, 1970). However Im and co-workers (1976) injected saline into hypertrophic scars to investigate this hypothesis and found no evidence, either visual or enzymatic, of scar resolution other than that associated with the normal maturation process.

As with the use of any potent drug, there can be serious complications if used wrongly. Such untoward effects as atrophy, depigmentation, telangiectasia and symptoms of Cushings syndrome have been reported. However, 95% of these arose from the indiscrete use of the drug. (Ketchum et al, 1974).

In its present form steroid therapy is one of the two most successful ways of treating hypertrophic and keloid scars. With the development of new drugs and a better understanding of the ways in which they act, steroid therapy will probably become one of the standard methods for treating these lesions.

2.9.5. Pressure therapy and splinting The first reported use of pressure therapy in the prevention/correction of hypertrophic scars was in the 1800's. Warren (1893) cites Rayer (1835) and Blandin (1842) as being two of the pioneers of pressure therapy. Panasis (1863) (again cited by Warren (1893)) described the case of an actress who had smallpox scars on her face, and for a period of 6 months wore a mask both day and night. At the end of the treatment the skin presented a polished surface.

He stated that the compression often removed the redness, as well as the elevation of the scar. It was his theory that the earlier the treatment was employed the better the results, and that treatment should be continued for some time after the scars had flattened. Since that time there have been repeated case reports published on the effectiveness of applying pressure to scars.

Blair (1924) was one of the first people to use pressure therapy in the treatment of the wounds themselves. He listed the major advantages of elimination of dead space; control of oozing, limitation of venous and lymph stasis; elimination of the plastic material that pours into a wound and the splinting and support given to the tissue.

Owens (1947) advocated the use of pressure dressings in the treatment of burns to diminish the plasma loss and to keep the granulating surface firm, flat and free of oedema.

Pressure therapy has been used to prevent recurrence of contracture deformities of the neck after corrective surgery and grafting with good results (Cronin, 1951; Gottlieb, 1963). The pressure therapy was also noted to prevent excessive scar formation in wounds and to flatten already existing scars. (Gottlieb, 1963; Fujimori, 1968).

Since 1968, the Shriners Burns Institute, linked with the University of Texas has routinely used continuous pressure, traction, or both to prevent or diminish scar contractures and hypertrophic scar formation with excellent results (Larson et al, 1971, 1974). Since the major part of the literature on the effects of pressure therapy have been published by this group, their methods and results will be discussed in detail.

The severity of a contracture can be classified under four headings (Huang et al, 1978):-

None - When the joint possessed no limitation of motion

Mild - The scar around the joint causes less than 25% limitation of normal range of motion.

Moderate - Restriction between 25% - 50% on range of motion.

Severe - Range of motion less than 25% of normal.

Huang and colleagues (1978) reviewed the case histories of 625 patients who had sustained burns over their joints. Of the 219 patients who had had no form of pressure therapy a high percentage developed contractures. The severity of the contracture seemed to vary with area. More than one third of the axillary burns developing "severe" contractures, whilst only 10%-15% of the burns over the other joints healed with "severe" contractures.

Of the remaining patients who received prolonged pressure therapy few developed any contracture deformities.

Larson and his colleagues (1971, 1974) recommended starting pressure therapy as soon as possible after injury. Contraction of a wound margin starts within days so that early measures must be taken to prevent subsequent development of contractures. In the pre-grafting phase this should involve the correct positioning of the patient using splints or traction to prevent him from assuming the position of comfort, which is also the position of contracture (Larson et al, 1974).

Even after the wound has been grafted the correct positioning of the patient must be maintained to prevent the secondary contraction of the grafts. This is done using splints and pressure wraps.

The importance of maintaining range of motion and muscle tone is stressed by the Shriners group. The duration of splint wearing is individually programmed for each patient to prevent contracture without permitting stiff or immobile joints. The pressure wraps are worn continuously until the scar is no longer "active" since if the pressure is removed too soon the hypertrophy can still occur. (Larson et al, 1974).

Although the Shriners group state that the magnitude of the pressure must be over 25mm Hg (ie. above capillary pressure), Naismith (1979) has found that each patient has a different threshold of effective pressure magnitude.

The pressures can be applied using ordinary elastic bandages, or at the other extreme, custom made elastic pressure garments (Jobst pressure garments). The prime factor determining the material used is that the pressure applied must not, ideally decrease with time or body position.

The length of time that the pressure must be applied again varies with the individual. As long as the scar is in its active phase, it will respond to treatment. (Larson et al, 1974). The pressure must be continuously applied throughout this active phase (6 months to 1 year) to prevent recurrence.

The application of a pressure wrap results in an immediate blanching of the tissue and a reduction in its thickness. After several days the consistency of the originally raised and rigid mass changes with shrinkage and scar softening commonly observed. (Baur et al, 1976).

Traction has also been used to correct contracture deformities. A 65^{o} to 75^{o} flexion deformity of the arm was corrected by applying

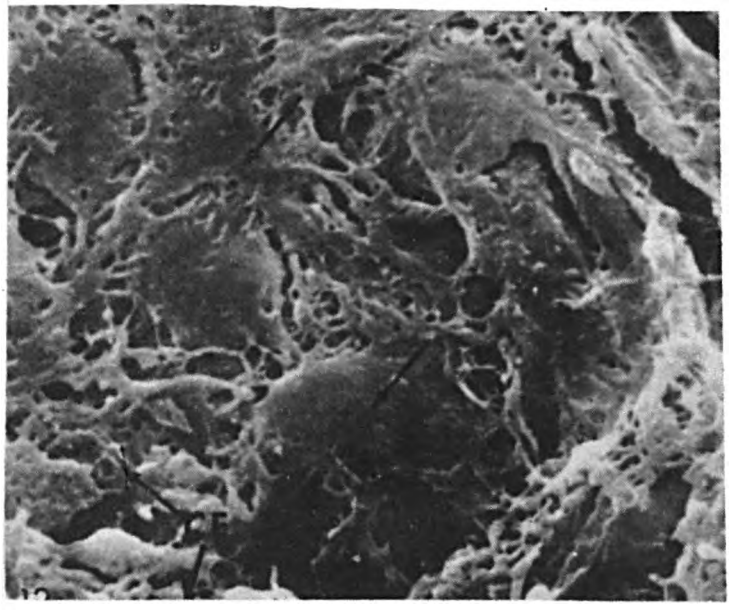


Fig.2.42. ——— 1μm. Meshy network of collagen fibres representing remodelling of hypertrophic scar by pressure therapy. (After Larson et al, 1976).

traction to the joint for 20 minute periods, maintaining the improved position between therapy sessions with splints. After 2 weeks of therapy the flexion contracture of the arm was less than 10^{o} and further therapy resulted in a complete correction, with a softening of the scar tissue.

The overall effect of pressure and traction appears to be an acceleration of the natural remodelling process. (Baur et al, 1976).

Exactly how pressure therapy works is not known, although various hypotheses have been put forward.

It has been suggested that the application of pressure reduces the amount of water in the scar resulting in a loss of tissue bulk. However, the degree of pressure needed to do this and maintain the dehydration was felt to be greater than that supplied by the pressure wraps. (Peacock and Van Winkle, 1976).

Scanning electron microscopy of the scar tissue undergoing pressure therapy demonstrated a remodelling process taking place. (Baur et al, 1976). The heavily solidified nodules were apparently being resorbed or remodelled into the "sinusoidal fibre patterns" seen in normal skin. The central nodular portion remained a solified mass with a uniform texture. The remodelling area appeared as a meshy network (Fig. 2.42). This fenestrated pattern was felt to be due to the loss of the mucopolysaccharide ground substance which had originally fused the collagen filaments into the nodular mass. With the decrease in mucopolysaccharide content individual collagen filaments became discernable. (Kischer et al, 1975).

After prolonged pressure therapy (greater than 6 months) the picture observed was similar to that seen in very old and naturally

remodelled scar tissue. The large collagen fibres had nearly assumed the size, structure and orientation seen in normal skin. No evidence of the nodules was visible. (Baur et al, 1976; Linares et al, 1976).

Pressure therapy also had an effect on the ultrastructure of the tissue. The diameter of the collagen filaments increased and the amount of interstitial filaments decreased. (Kischer et al, 1975).

It was proposed by Kischer (1975) that the effect of the pressure therapy was to increase the tissue hypoxia to such a level that a portion of the fibroblast population could no longer survive. This would result in a "shut down" of excessive collagen synthesis. If this hypothesis was correct then atypical cellular configurations, non-viable cell remains, debris and/or ghosts should have been visible in tissue sections. This cellular debris was not visible. (Baur et al, 1975).

Pressure therapy reduces the blood flow into the scar, Baur and co-workers (1976) suggested that the concomitant reduction in blood volume reduced the amount of collagenase inhibitor in the tissue. (Milsom and Craig,(1973), Cohen and colleagues (1974) and Baur and colleagues hypothesised that the increased collagen deposition was due to insufficient degradation of the protein. And that this was due to an inhibition of the collagenase mediated remodelling by an increase in the level of α-globulins in the blood). With this reduction in collagenase inhibitor the collagen degradation would proceed at an increased rate resulting in a decrease in tissue bulk.

Although no signs of cell death were observed in the dermis of the pressure treated scars, the number of cells in any given area of the tissue slowly reduced. (Baur et al, 1975). This reduction appeared to take place over a long period of time. During the active phase of the scar the myofibroblast population remained relatively constant. This could account for the tendency of the hypertrophy to recur if pressure therapy was discontinued before the end of this stage. (Baur et al, 1978). When the scar was no longer active the pressure treated dermis of the scars showed a noteable reduction in myofibroblast population.

The number of mast cells found in pressure treated scars was found to be similar to that in normal mature scar. This reduction in numbers was first noted in the papillary layer and mid dermis, suggesting that pressure therapy had its earliest effects at this depth. (Kischer, 1978). It has been suggested that one immediate tissue response to oedema is a degranulation of the mast cells. The acid mucopolysaccharides in the cell granules bind the water produced by the oedema, to form a mucinous ground substance. Based upon the finding of an increased water content in hypertrophic scars Kischer and Bailey (1972) postulated that this ground substance could provide a favourable matrix for the formation of new collagen. Baur and colleagues (1976) found no degranulating mast cells in pressure treated scars and concluded that the pressure therapy, which reduced the water content of the tissue, therefore inhibited the degranulation of the mast cells which in turn inhibited the subsequent formation of the mucinous ground substance.

A mucopolysaccharide assay was carried out on two

hypertrophic scars. (Kischer et al, 1975). After three months of treatment the chondroitin sulphate A level had decreased, and the hyaluronic acid level increased, towards that of normal skin. Although these results indicate that the accelerated maturation of the scar tissue by pressure involves a change in the mucopolysaccharides, more quantitative analyses have yet to be reported.

Although the pressure therapy caused the hypertrophic scars to regress, the fibroblasts within the pressure treated tissue still showed dilated and plentiful rough endoplasmic reticulum, suggesting a high level of synthesis. It was concluded, however, that the enzyme products synthesised had altered to those required for nodule degradation and that pressure therapy altered the distribution and/or effectiveness of these enzymatic products to favour tissue remodelling.

CHAPTER 3

HYPERTROPHIC SCAR TISSUE MICRO-ARCHITECTURE

PATIENT/SPECIMEN RECORD

SECTION 1, PATIENT HISTORY

Name/Hospital number

Sex

Race

Date of Birth

Date of Injury

Cause of Injury

Extent/severity of injury

Initial Treatment

Previous Treatment

Present Surgical Procedure

Fig. 3.1., part 1.

3.1. Preparation of Specimens

During the surgical remodelling of uncosmetic or disabling scars pieces of unwanted tissue are frequently excised and discarded. These pieces of tissue provided the specimens for the analysis of the fibrous architecture of the hypertrophic and keloid scars.

3.1.1. Patient/specimen record. Where possible a detailed record of the specimen's history was kept (Fig. 3.1.). This can be divided into two main sections. The patient's relevant case history and the specimen's orientation and subsequent preparation for microscopy. Section one lists those aetiological factors which could affect the microstructure. The influence of age, race, and cause of injury have been discussed in section 2.5.

The extent and depth of the original injury will have an effect on the extent and depth of the scar tissue. Due to the process of contraction, apparently normal epidermis surrounding the visible surface scar will overlie fibrous scar tissue. The degree of severity of the original injury will also influence the level to which the scar tissue will extend into the dermis.

A record of any previous treatment is important, particularly a note of any previous grafting or surgery.

It was not possible to take into account all variables involved, but by being aware of them any unexpected abnormalities in the microstructure could perhaps be explained.

Section two of the specimen record lists those factors involved in the specimen preparation protocol, all of which are under the control of the experimentor.

3.1.2. Specimen excision and fixation. A record of the

SECTION 2, SPECIMEN RECORD

Scar Site/Orientation

Fixation

Cleaning

Cutting

Section number and orientation

Fig. 3.1., part 2

specimen's orientation was kept at all times. At the beginning of the surgical operation the area to be excised was marked out with a sterile dye by the surgeon. If the specimen was large enough a surgical stitch was then inserted at a known position to act as a marker. If the specimen was too small to allow this a detailed scale drawing was made, marking any distinguishing features.

Where possible the incisions were made at right angles to the surface of the skin to avoid obtaining a wedge shaped specimen with its associated small area of intact dermis. Care was taken at all times to distort the specimen as little as possible. To prevent the specimen from curling up in the fixative, immediately after excision the sample was placed epidermis uppermost on a piece of porous card. Small specimens adhered to the card with no further problem, larger specimens were pinned to the card around their boundaries. The specimen attached to the card board was then immersed in a copious volume of fixative. The fixative used throughout was 10% formol saline buffered with phosphate. This fixative was selected as it is particularly suited to the accurate preservation of the relations between tissue layers (Brown, 1971). It gives tissues a firm consistency without excessive hardening. The fixation time varied from three days to several weeks, depending upon the size of the specimen. The fixative was changed frequently (at least every 14 days) to prevent the build up of sediment.

3.1.3. Sectioning techniques. The specimens of scar tissue obtained came in all shapes and sizes, varying from 5 m.m. wide to 5 c.m. wide. Because of this size variation no set pattern of tissue sections could be adopted. Instead each sample was cut to give as much information as possible.

The method used to cut a tissue specimen is important. Artifacts can be produced during sectioning, and these can give rise to misleading results. Because of the variation in thickness of the specimens transverse sections were first prepared. This allowed the thickness of the different tissue layers to be measured so that horizontal sections could be prepared at appropriate levels if needed. The majority of the pieces of fixed scar tissue were firm enough to handle without any further support. It was therefore decided to cut the specimens by hand using a dermatome blade. The dermatome blade has a wedge profile, chamfered on both sides, so that from one piece of tissue two complementary transverse sections were produced, either or both of which could be further prepared for the S.E.M.

Before use the dermatome blades were cleaned by immersion in a 2:1 water/chloroform solution to remove any grease, and then allowed to dry under a dust cover.

The tissue to be cut was placed, epidermis uppermost on several thicknesses of blotting paper and then immersed in fixative. The knife was then placed at right angles to the surface in the correct orientation and a steady force was applied. This force was maintained until the knife had cut through into the blotting paper. Sawing action of the knife blade was avoided to prevent unnecessary distortion. A fresh portion of the cutting edge was used for each tissue section.

An unexpected advantage in using this method of tissue sectioning was discovered. As the knife blade was pushed through the specimen, components of the tissue gave slightly before being cut. The degree of 'give' depended upon the compactness or hardness

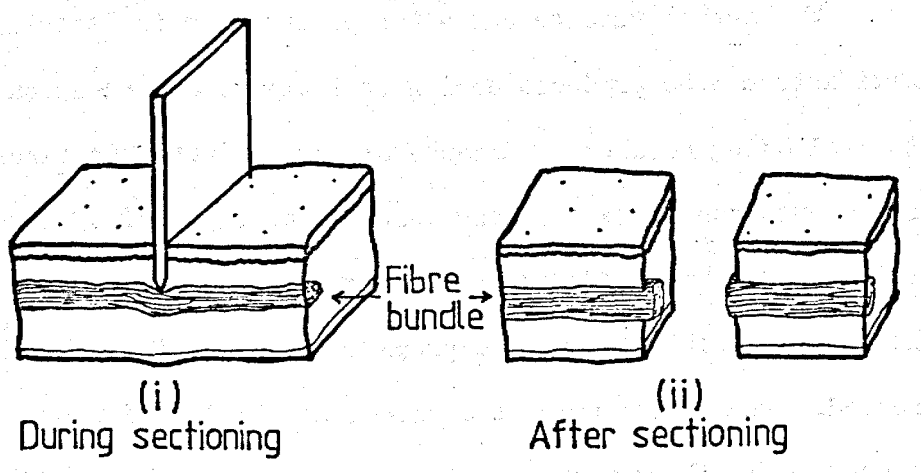


Fig. 3.2. Wet sectioning of specimens, resulting in a highlighting of the fibrous micro-architecture.

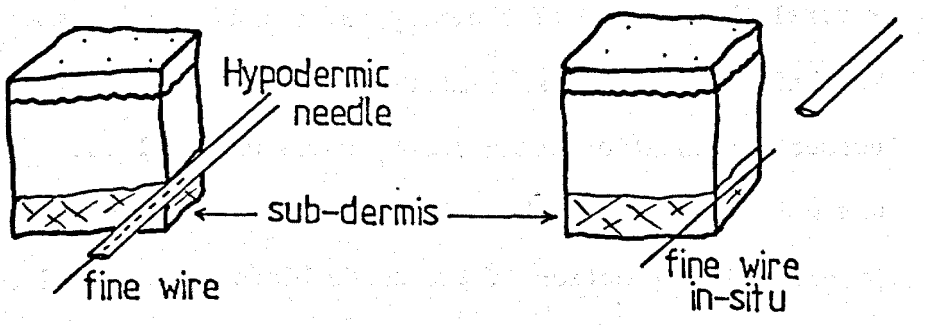


Fig. 3.3. Insertion of copper wire marker to record specimen orientation.

of the tissue. In the dermal scar tissue large bundles of collagen fibres were observed. When the knife edge met one of these, the fibre bundles resisted slightly before being cut. After the blade had passed through the tissue this resulted in the cut end of the fibre bundle being left protruding very slightly from the exposed surface (Fig. 3.2.). The protrusion was so slight that if the specimen was viewed perpendicularly the surface of the tissue appeared to be level, but when viewed at an angle (as is usually the case when using an S.E.M.) a slight shadow formed around the fibre. This method of tissue sectioning, therefore, helped highlight the gross characteristics of the fibrous microarchitecture.

At all times a record of the specimen orientation was kept (see section 3.1.1.). In most cases this involved a sketch of the tissue, marking any distinguishing features. Some specimens however, had no distinctive shape or features. With these an alternative method of recording the orientation was used. A fine hypodermic needle (Gillette 25 G x $^{15}/_{16}$) was passed through a corner of the sub-dermal tissue of the specimen. A fine piece of copper wire (diameter $^{6}/_{1000}$") was then inserted into the needle which was then withdrawn leaving the wire in-situ (Fig. 3.3.). Once the transverse section had been observed under the S.E.M., any horizontal sections needed were prepared. This time the specimen was cut frozen using a Leitz sledge microtome, Type 1300. The tissue sample was placed in the middle of the cold stage of the microtome in a drop of fixative. Carbon dioxide was then passed through the cold stage, freezing the tissue and at the same time adhering the specimen to the stage.

The microtome produces planar sections whose thickness can be

pre-selected, and the adjustable tilt of the specimen clamp permits the choice of specimen inclination. A 240 x 44 x 11 m.m. blade with a wedge profile plane on both sides was used as this was recommended by the manufacturers, Jung, for frozen tissues. Optimum sharpness was maintained throughout by sharpening the blade before each batch of specimens in a Shanden Elliott Automatic Microtome Knife Sharpener Mk. II.

The blade was clamped in the microtome such that the free angle was 5°. A smaller angle could have caused the knife to slide off the specimen, whilst too steep an angle could cause it to dig into the tissue. The place of interest was exposed by removing 20μm thick sections. These thin sections were discarded since they did not have sufficient tissue bulk to prevent distortion of the fibrous architecture during subsequent preparation for observation.

The protrusion of any large fibre bundles was not so apparent in specimens that had been cut whilst frozen. Freezing the tissue made its components much more rigid and decreased their ability to 'give' with the cutting edge. Records of the specimen orientation were kept as before.

With both methods of sectioning the appearance of the sections was good with little tearing of the collagen fibres.

3.1.4. <u>α-amylase treatment.</u> If fixed, "un-cleaned" tissue is observed with an S.E.M., the resolution of fibre detail is limited, except in very young tissues, by the adherence to the fibres of an amorphous material, presumably ground substance. These deposits take the form of globular masses or films on the surface of the fibres.

A considerable improvement in fibre detail can be obtained by

prolonged incubation of the fixed specimens in phosphate buffer, with 'intermittant agitation'. However, the addition of crude - α- amylase (C.B.A.) from "Bacillus Subtilis" to the buffer results in improved penetration and cleaning, and gives a higher resolution of the fibrils within a fibre. It has been shown that the C.B.A. does not damage the fibril, but acts on the glycoprotein component of the fibre (Finlay et al, 1970).

The standard treatment consisted of incubating the specimen in 0.2 M phosphate buffer, pH 5.4, containing 0.3% w/v C.B.A. for three days at room temperature with occasional agitation. A small amount of chloroform was added to each solution to act as a bacteriostatic agent. The specimens were then washed twice in isotonic saline, for one hour in each wash.

3.1.5. Dehydration, mounting and coating. Initially, the specimens were taken directly from the saline wash and placed in acetone. This resulted, however, in a crystalline substance, presumably salt, being deposited on the tissue surface. The artifacts produced completely obscured the fibre detail of the specimen. To prevent this happening the specimen was placed in 10% acetone for one hour after the two washes in saline and the specimen was then dehydrated by immersion in three successive baths of 100% acetone, each for 24 hours. Drying was completed using a Critical Point Drying (C.P.T.) apparatus. (Polaron, Type E3).

After complete dehydration the specimen was mounted on an S.E.M. specimen stub with 'Evo-Stick' adhesive and left for 24 hours under a dust cover for the adhesive to dry.

The specimen was coated in a Polaron sputter coater (Diode Sputtering system, Type E5000) with gold. A coating time of two

minutes had been established by previous experimenters as giving an optimum coating thickness. After coating, a conducting material ('Silver Dag') was painted around the specimen, next to the stub, to complete the conducting path to earth.

3.2. Photomicography

Specimens were viewed using a Cambridge Stereoscan Mark 2A electron microscope. During the early stages of the project micrographs were recorded on Ilford FP4 film 120 size, by a Zenza Bronica - S single lens reflex camera set on a time lapse exposure with an f-8 aperture. This camera was later replaced by an Exa 1A - single lens reflex camera and Ilford FP4 film 35 size was used thereafter. The disadvantage in using 35 m.m. size film was that the contact prints made were too small to allow much detail to be observed. This necessitated enlarging the majority of negatives, a process both time consuming and costly.

The quality of the micrograph depends on the condition of the specimen, aperture size, accelerating voltage of the beam, photo-multiplier level, black level, condenser setting, gamma control, and recording video controls. The following meter settings were used routinely.

Aperture size 200

Accelerating voltage 20 kV

Recording video controls - frame period of 40 seconds

The remaining parameters were varied according to the quality of the specimen to obtain optimum results.

3.3. Generalised Micro-architectural Picture

The micro-architecture of hypertrophic scar tissue is under the influence of a large number of variables, i.e. the age and race of the patient, and the nature and extent of the injury (see section

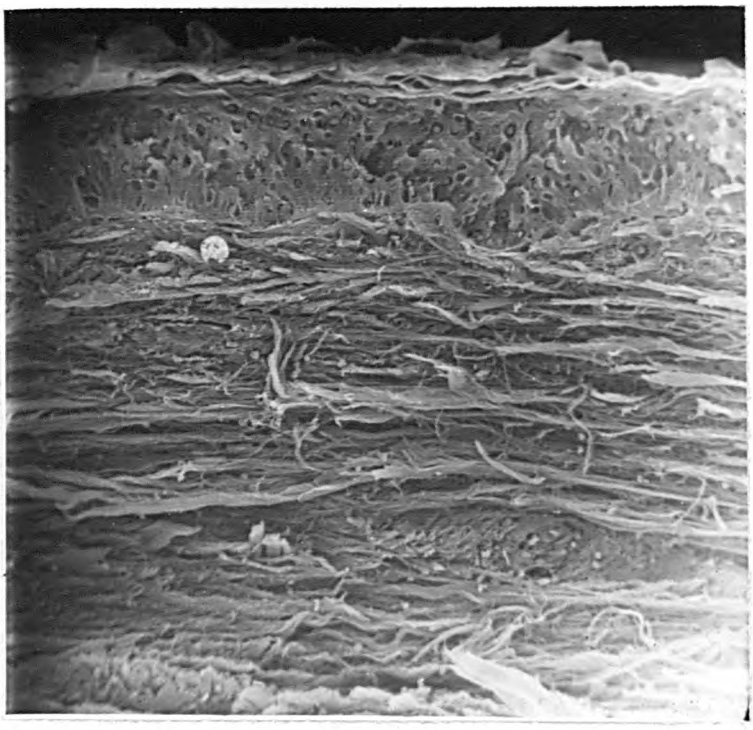


Fig.3.4.⟵⟶ 50μm. Dermo-epidermal junction lacking regular rete ridges and papillae. (Transverse section (TS)).

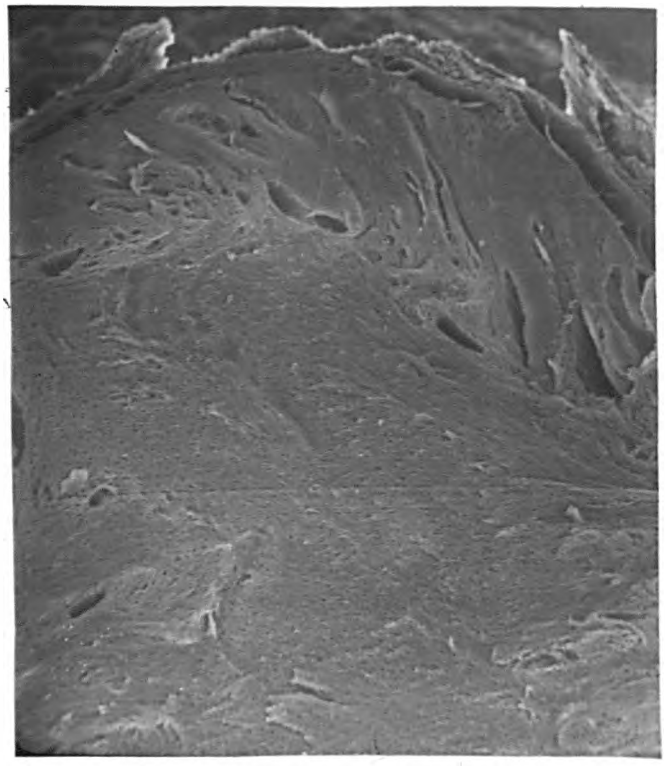


Fig.3.5.⟵⟶ 500μm. Dermo-epidermal junction following irregularities in scar tissue surface. (TS).

3.1.2.). Because of the variation in influencing parameters it was not possible to build up a particular structural picture of the hypertrophic and keloid scar tissue. Since no one individual specimen demonstrated all the characteristics found in the various scar samples three representative scars have been chosen from the 32 scars studied. The specimens obtained from these three different patients demonstrated the range of different fibre patterns that differentiate hypertrophic and keloid scar tissue from 'normal' scar tissue and normal skin.

A short summary of each patients relevant case history is presented in appendix A. In the succeeding discussions the patients will be referred to by their case numbers only.

As can be seen from the case histories the age of the patients at the time of injury ranged from 5 years to 13 years, placing them in the age group which appears to be more susceptable to hypertrophic scarring (see section 2.5.2). Although the actual age of the scar specimens ranged from 4 to 19 months, all three scars were diagnosed as still being in the active phase. (see section 2.7).

The micrographs shown in the following sections are representative of the type of microarchitecture observed in the majority of specimens obtained from patients in the age range of 5 to 48 years old.

3.3.1. The epidermis In edge view the epidermis of the scars did not show the orderly series of rete ridges and papillae commonly seen in transverse sections of normal skin. In general the dermo-epidermal junction was flatter than in normal skin (Fig. 3.4), although where the outer scar surface was irregular the junction followed the same contours (Fig. 3.5). Other regions (sometimes within the same specimen section) showed grossly abnormal epidermal

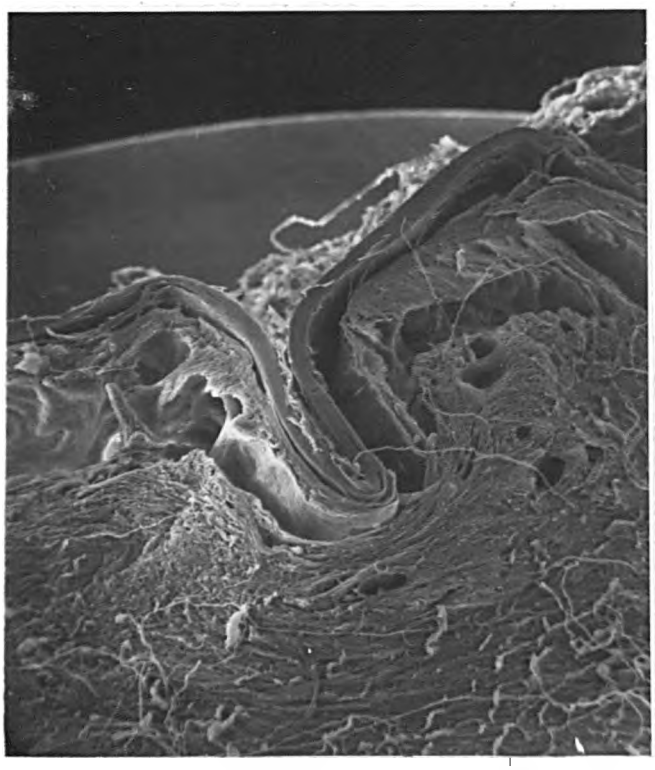


Fig.3.6. and..

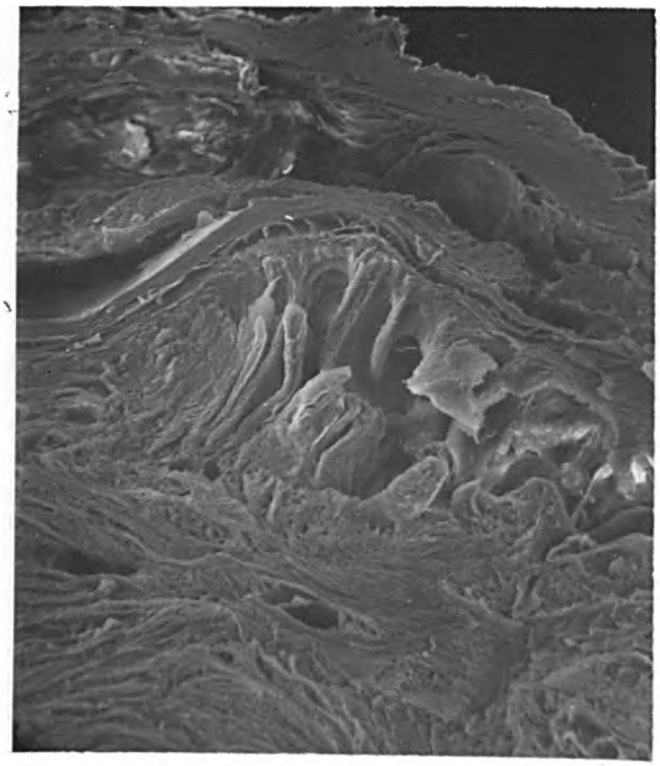


Fig.3.7._____ 500µm. Abnormal epidermal configurations with deep pits, folds and clefts in the cell layer.

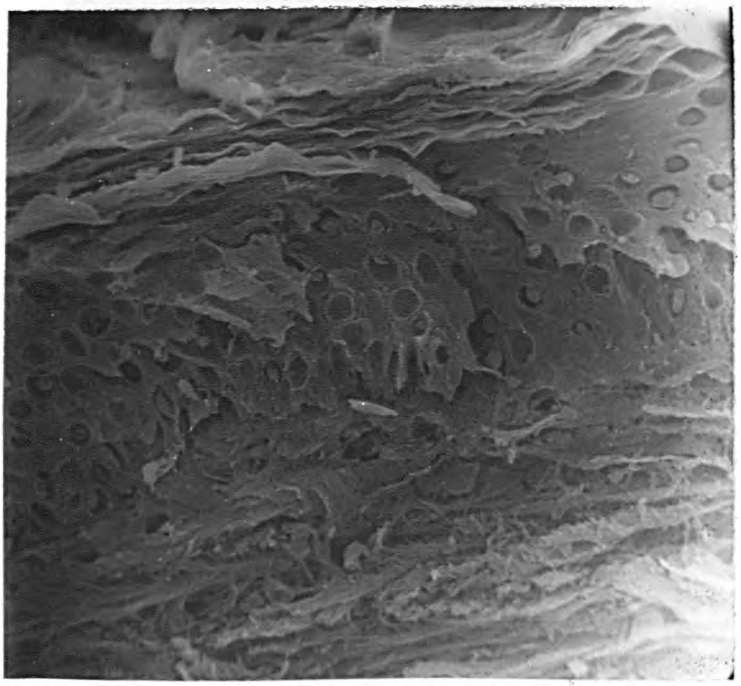


Fig.3.8. ——— 20μm. Normal appearing cell layer of hypertrophic scar.

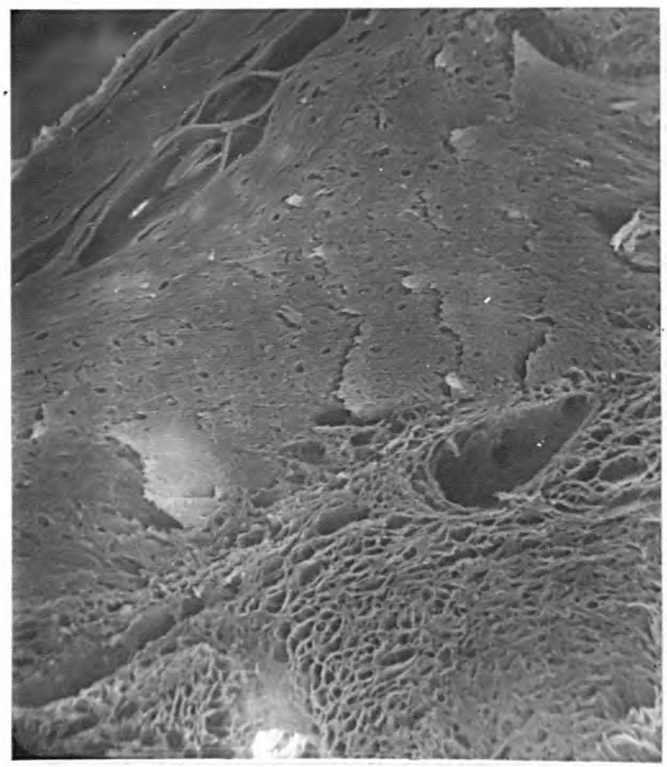


Fig.3.9. ——— 20μm. Thick cell layer of 'active' hypertrophic scar.

configurations, with deep pits, folds and clefts in the cell layers (Figs. 3.6., 3.7.). Figs. 3.5. - 3.7. are micrographs of sections from case no. 3 whose wound had originally been covered with a mesh auto-graft. It is possible that the specimens contained remnants of the mesh graft which had only partially taken, giving rise to the deep pitting of the epidermis. Other specimens viewed which had similar indentations in the epidermis nearly all had a record of having been grafted, with only partial 'take' of the graft.

The thickness of the cell layer varied (Figs. 3.8., 3.9.), being thicker in those scars diagnosed by their age, their inflamed appearance, and the symptoms of itchyness and tenderness, as being more physiologically active.

The orderly transition in cell shape from the rounded cells of the stratum spinosum to the flattened plaque like cells of the stratum corneum was observed in those sections of epidermis with a reasonably smooth appearance (Figs. 3.8., 3.9.). However, in some regions of the epidermis the cells of the stratum spinosum were elongated, giving the impression that they were strained. (See section 2.6.2.), (Fig. 3.10.).

Any sudden irregularities in the epidermal contour were also reflected in a change in orientation of the cell outlines. Fig. 3.11. is a micrograph of a region adjacent to the deep cleft shown in Fig. 3.6.

The surface of "active" hypertrophic scars, as seen with the unaided eye, has the appearance of being tightly stretched over the underlying structures. It is often red and shiny with flakes of stratum corneum peeling off. The patient often complains of how friable the scar surface is - the slightest trauma resulting in

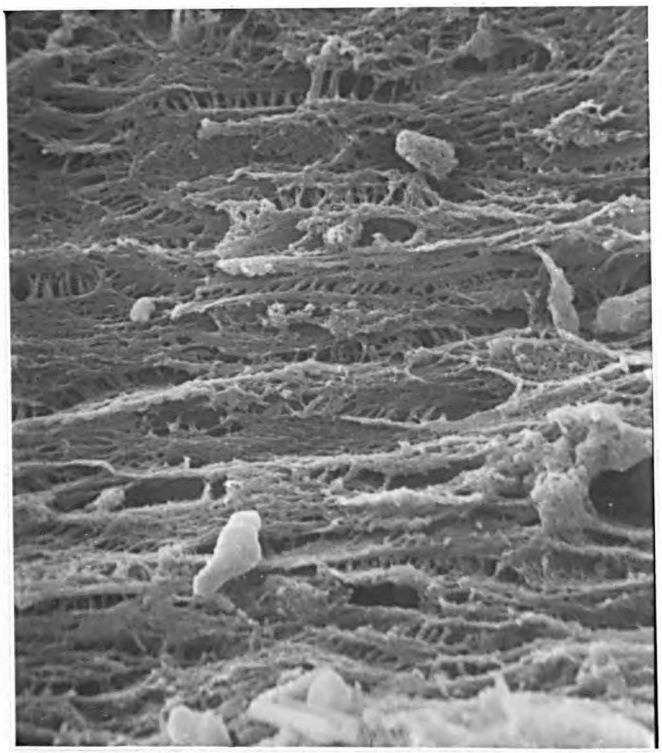


Fig.3.10. ⟵⟶ 5µm. Elongated, aligned cells of stratum spinosum giving the appearance of being under tension.

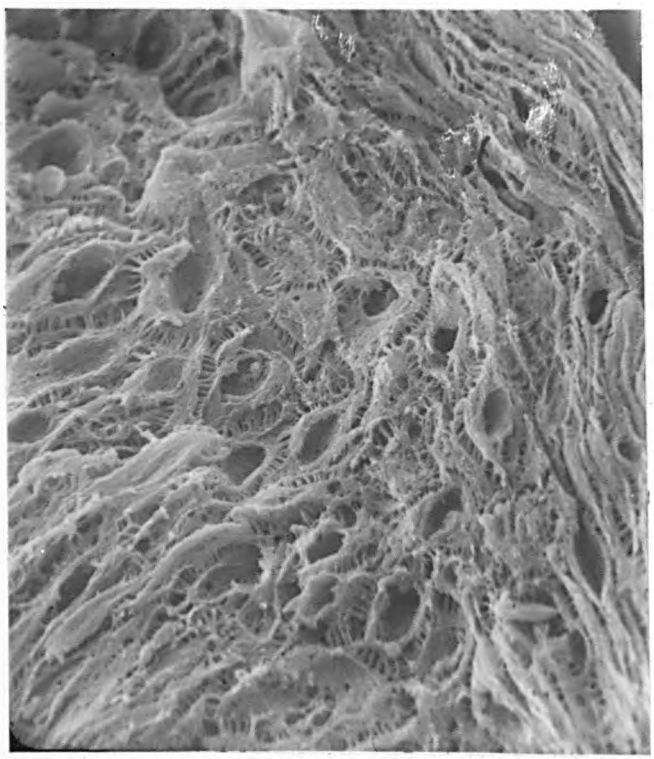


Fig.3.11. ⟵⟶ 5µm. Sudden irregularities in epidermal contour are reflected in a change in orientation of cell outlines.

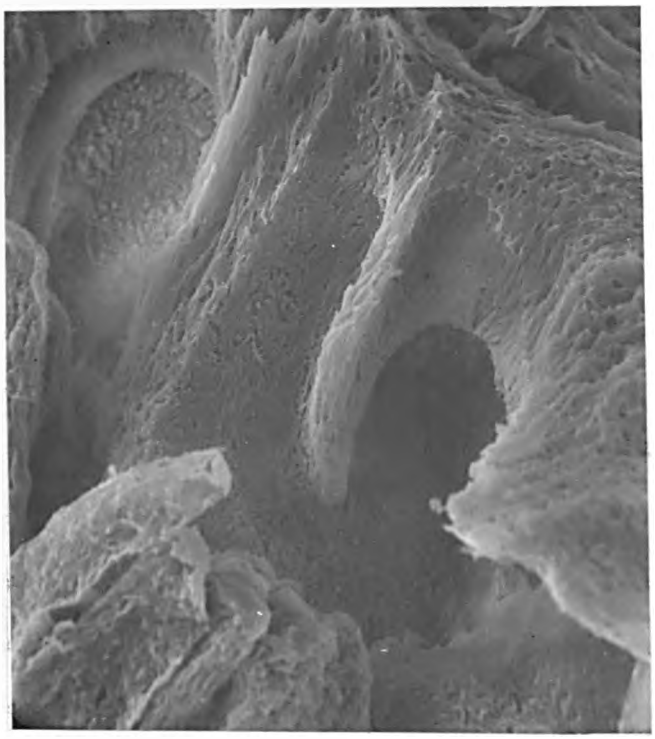


Fig.3.12. ⟵⟶ 50μm. Higher magnification of Fig.3.7. showing detachment of cell layer from tissue bulk.

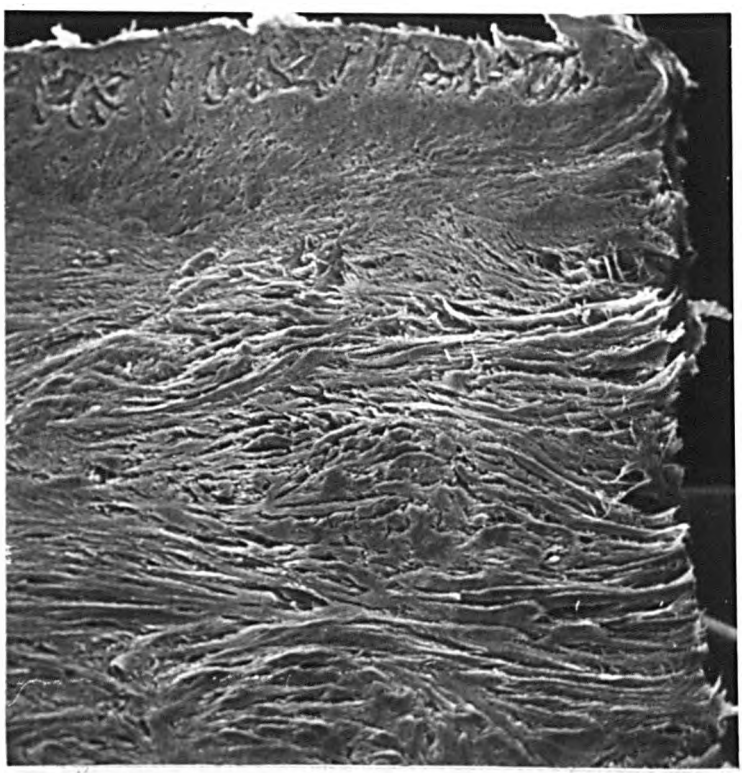


Fig.3.13. ⟵⟶ 200μm. Distinct layering of hypertrophic scar dermis.

a breakdown of the surface integrity. In the specimens of scar tissue studied the superficial layers of the epidermis were very prone to detachment from the tissue bulk. The layer involved was usually just the stratum corneum (Fig. 3.9.). But sometimes deeper layers detached as well. Fig. 3.7. is a micrograph of a grossly abnormal area of epidermis with deep clefts in its structure. Fig. 3.12. is a magnification of a region in Fig. 3.7. which shows cells of the stratum spinosum, and possibly even the cells of the basal layer, detached from the under-lying dermis. This detachment was possibly enhanced by the specimen preparation procedure used, but since detachment of the stratum corneum of specimens of normal skin prepared using the same protocol was rare, it does serve to highlight the friability of the scars epidermis.

3.3.2. The papillary layer of the dermis. The papillary layer in normal skin is made up of fine fibres typically 0.3μm to 3.0μm in diameter, distributed in a relatively open network in which no regular arrangement is apparent (see Fig. 2.4., section 2.1.5.). In edge view this fine fibre network is easily distinguishable from that of the under-lying mid-dermis.

In transverse sections obtained from specimens of hypertrophic scar tissue a distinct fibre layer immediately below and following the contours of the epidermis is discernable (Fig. 3.13.). The fibres vary in diameter between 0.2μm to 2.0μm, with the majority in the lower range, making them somewhat smaller than those found in normal skin. The most striking difference, however, is in the fibre network. The fibres are much more tightly packed, often making it difficult to distinguish the individual fibres. In some

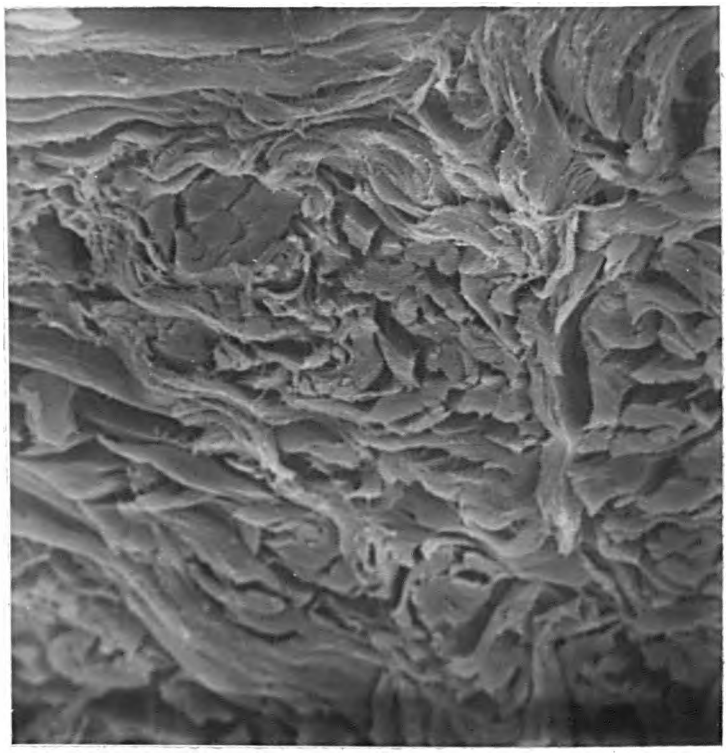


Fig.3.14. ⌊——⌋ 200μm. Horizontal section through papillary layer.

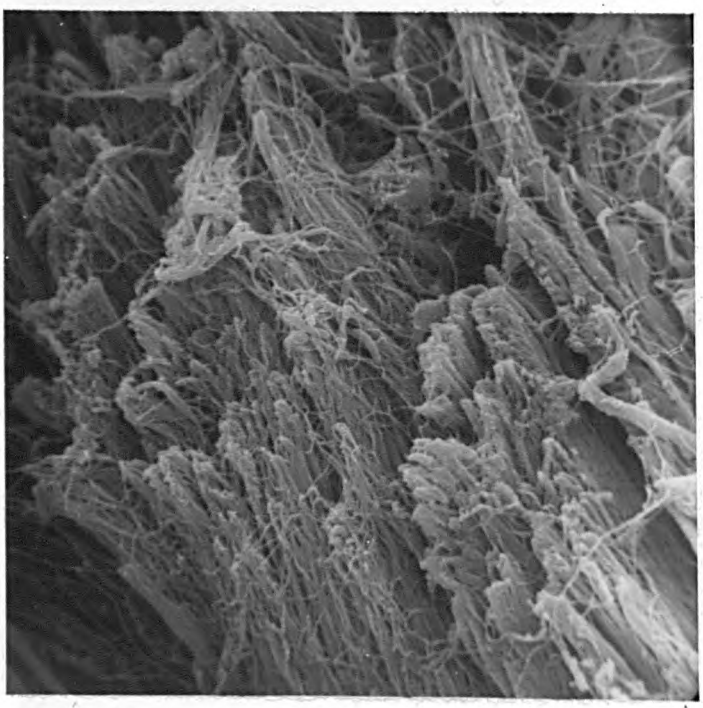


Fig.3.15. ⌊——⌋ 500μm. Higher magnification of fibre bundles within papillary layer showing small diameter fibres.

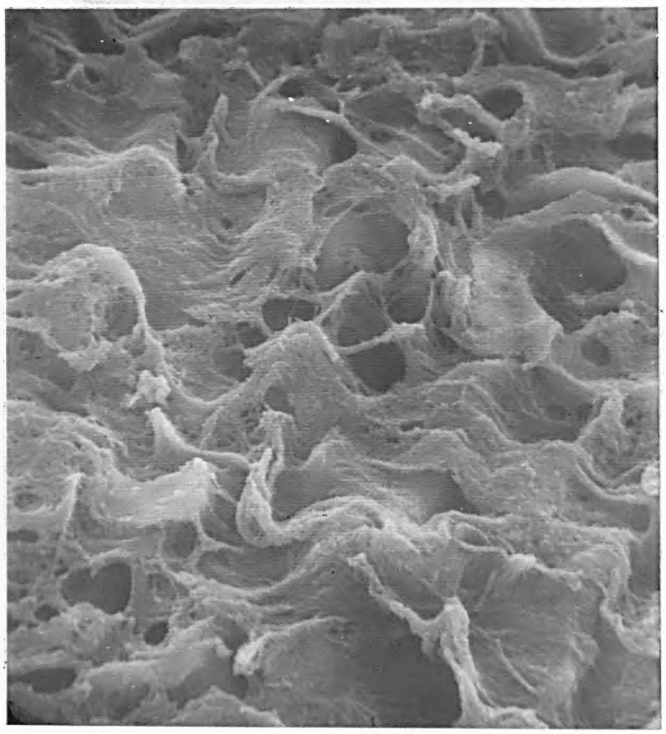


Fig.3.16. ——— 5μm. Papillary layer fibre orientation varies from the wavy pattern shown above to the straight fibres shown in Fig.3.4.

Fig.3.17. ——— 500μm. Transverse section of hypertrophic scar with distinct layers within dermis.

specimens this gives the impression that the papillary layer is made up of larger than normal diameter fibres. Fig. 3.14 is a micrograph of a horizontal section through the papillary layer which gives such an impression. However, on magnification the individual fibres making up the fibre bundles are visible (Fig. 3.15).[x]

The fibres and fibre bundles are much more orientated than in normal skin. In some regions the fibres are virtually straight, giving the impression that they are under tension (Fig. 3.4). In some specimens, although still aligned the fibres exhibit a definite wavy pattern (Fig. 3.16).

The papillary layer shown in Fig. 3.9, from an area adjacent to that shown in Fig. 3.16, demonstrates a more normal appearance which is probably a remnant of the original graft.

3.3.3. The mid-dermis In the mid-dermis of normal skin the fibres range from 10μm.to 40μm.in.width, and are arranged in a series of layers, parallel to the skins surface but with many fibres running at a slight angle between adjacent layers (see Figs. 2.6-2.9, section 2.1.5.).

The fibre arrangement in the dermis of hypertrophic and keloid scar tissue can take a variety of forms; from that seen in "normal" mature scars to a distinctly abnormal arrangement of large collagen fibre bundles. However, the more striking deviations from normal in the micro-architecture occur in the mid-dermis.

Fig. 3.17 is a transverse section, at low magnification of a specimen taken from case no.1. A series of distinct layers is visible. Uppermost is the stratum corneum (slightly detached from the tissue bulk), beneath the epidermis is the papillary layer. The next two layers, which together constitute the mid-dermis stand

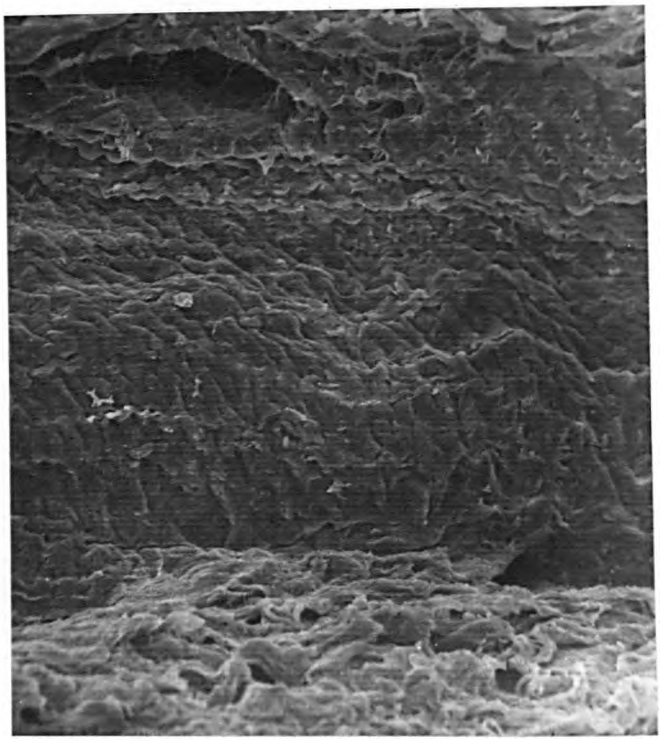


Fig.3.18. ⟵⟶ 50μm. Higher magnification of distinct orientated band in mid-dermis in Fig.3.17.

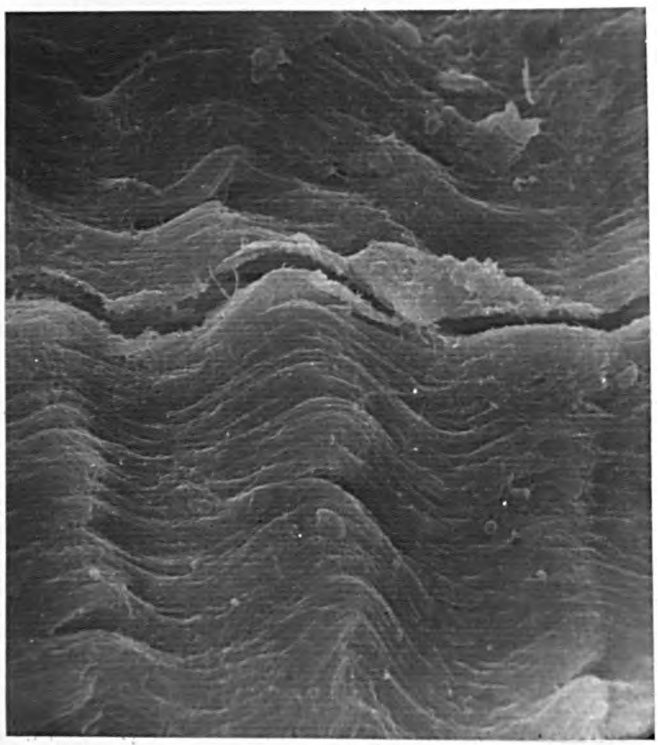


Fig.3.19. ⟵⟶ 5μm. Wavy pattern (λ=15μm.) of fibres within band shown in Fig.3.18.

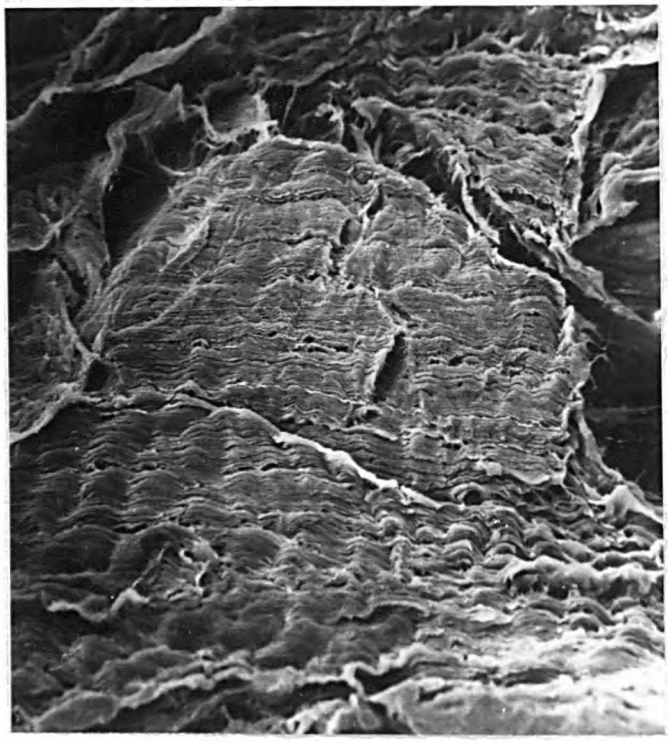


Fig.3.20. ——— 50μm. Higher magnification of layer above orientated band in Fig.3.17.

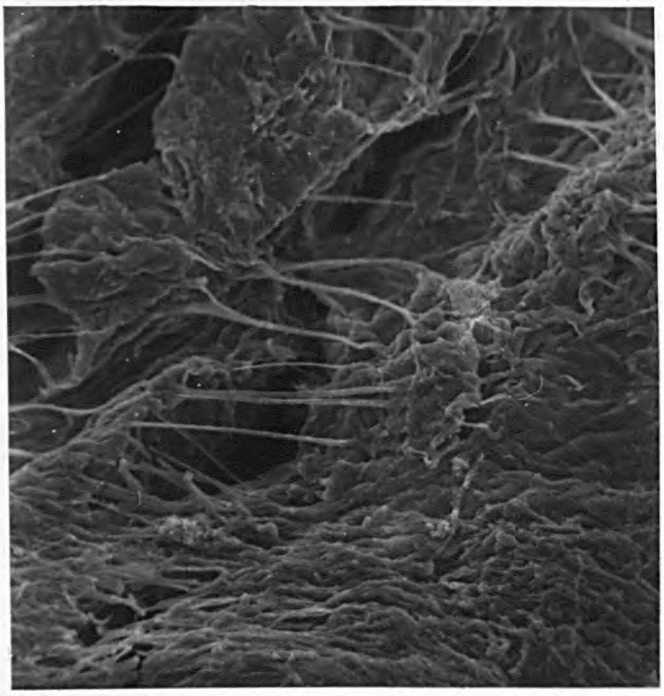


Fig.3.21. ——— 25μm. Cleft separating two fibre bundles shown in Fig.3.20.

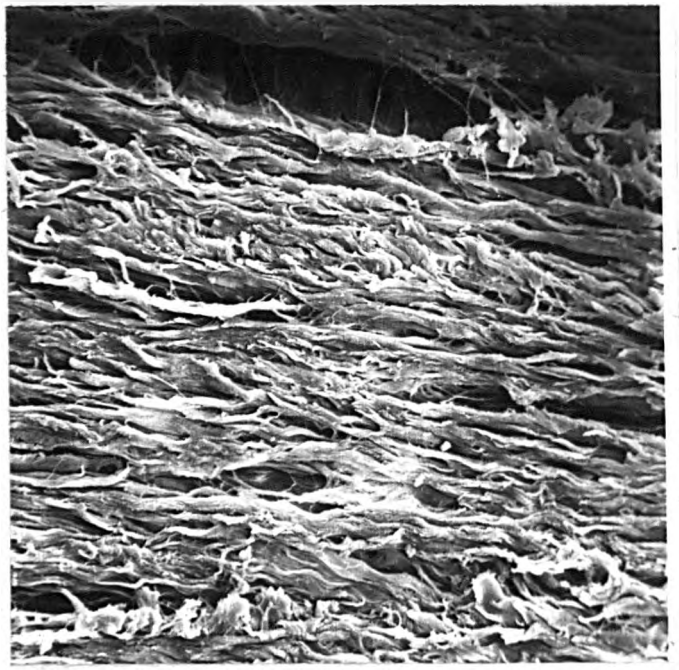


Fig.3.22. ——— 50μm. Horizontal section through layer shown in Fig.3.20.

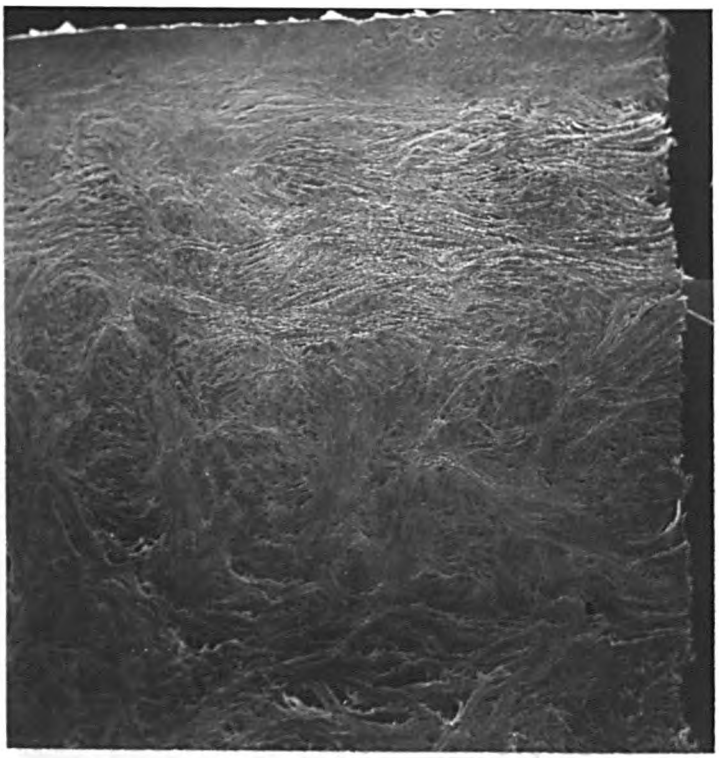


Fig.3.23. ——— 500μm. Transverse section. Tight packing of fibres within the dermis.

out very clearly. The lower of these two layers is made up of a band of highly aligned collagen fibres running in the caudio-cranal direction. This band is approximately 0.5 m.m. thick and on higher magnification presents a wavy appearance (Fig. 3.18.). This wavy pattern continues throughout the length of the band and has a fairly regular wavelength of approximately 15μm. (Fig. 3.19.).

The layer above the orientated band has a fibre arrangement that is more difficult to define. From Fig. 3.17. it gives the appearance of being made up of large fibre bundles running almost perpendicularly to the caudio-cranal axis, but on magnifying one of the large bundles (Fig. 3.20.) it appears to be made up of fibres running along the caudio-cranal axis, again with a wavy appearance although now the wavelength is approximately 28μm. The overall picture suggests that this layer is made up of large bundles of collagen fibres running at a slight angle to the plane of the section with the cut ends of the fibres having been spread slightly by the dermatome blade during the specimen preparation. The fibre bundles are separated by clefts in the network (which have probably been enhanced during the dehydration of the specimen) with straight, often interbranching fine fibrils bridging the gaps. The fibril diameters range between 5μm. and 10μm. (Fig. 3.21.).

Fig. 3.22. shows a horizontal section through the same layer, demonstrating the high degree of fibre orientation.

Fig. 3.23. is another transverse section, again at low magnification, of a specimen from case no. 2. The tight packing of the fibres is clear, and on higher magnification it can be seen that they are arranged in criss-crossing bundles of fibres running

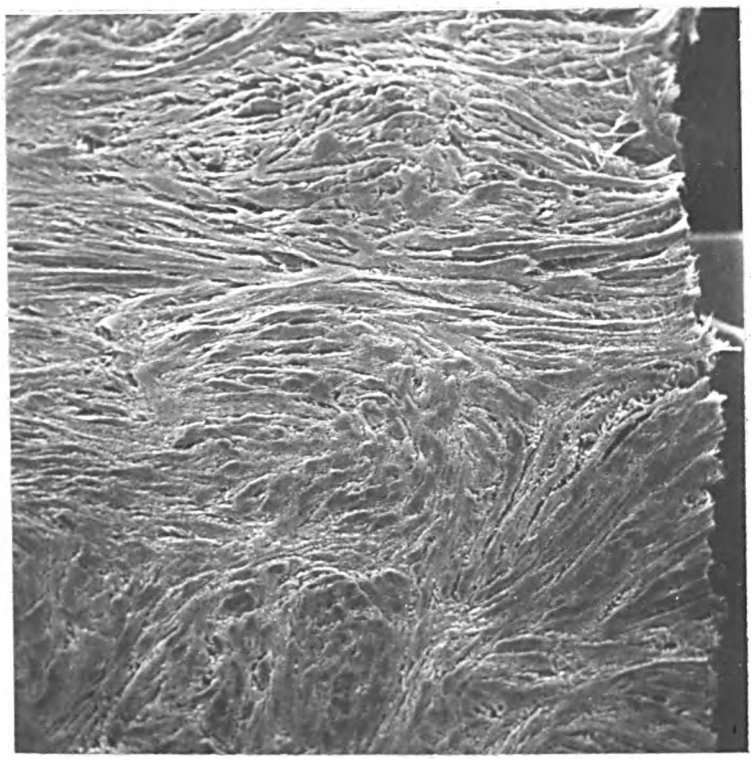


Fig.3.24. ——— 200μm. Magnification of Fig.3.23. showing criss-crossing bundles of fibres.

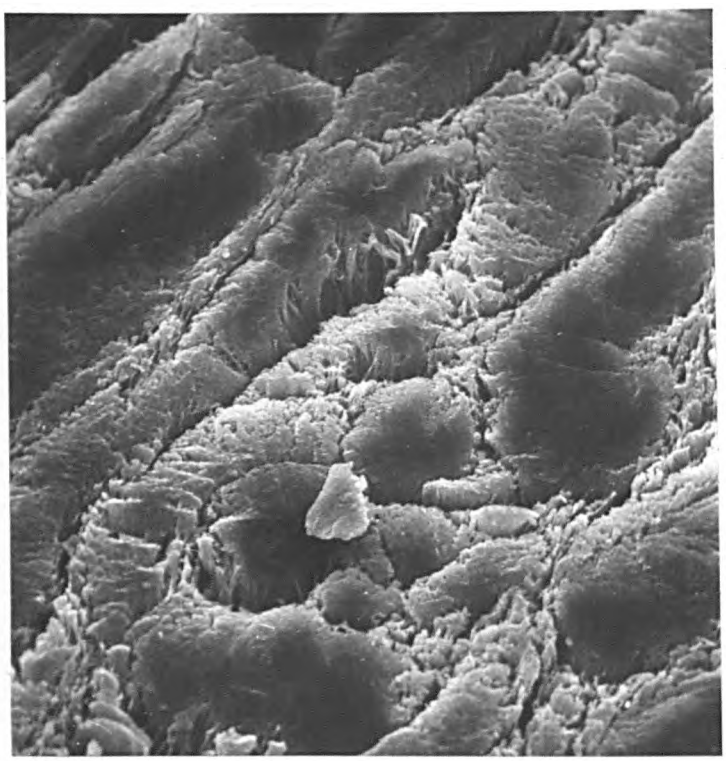


Fig.3.25. ——— 20μm. Transversely cut fibre bundles within dermis shown in Fig.3.24.

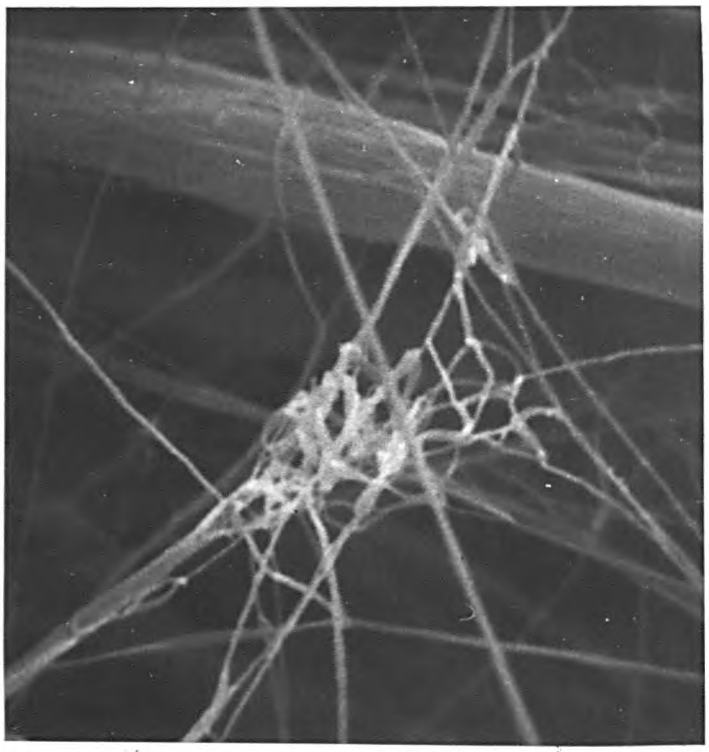


Fig.3.26. ——— 1μm. Coiled fibres within clefts between fibre bundles.

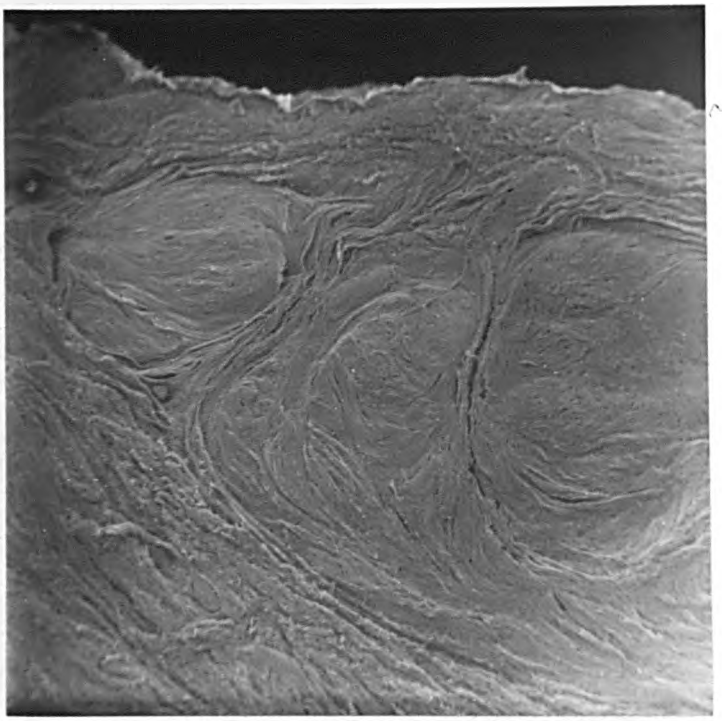


Fig.3.27. ——— 500μm. Transverse section of keloid scar, with extremely large collagen fibre bundles surrounded by septal-like bands.

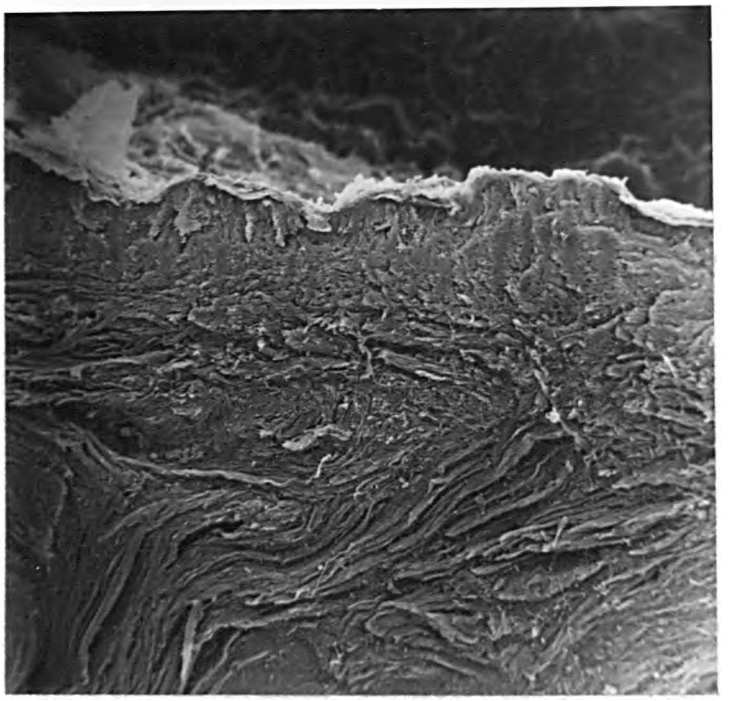


Fig. 3.28 ⌊——⌋ 200μm. Blending of septal-like band into the papillary layer.

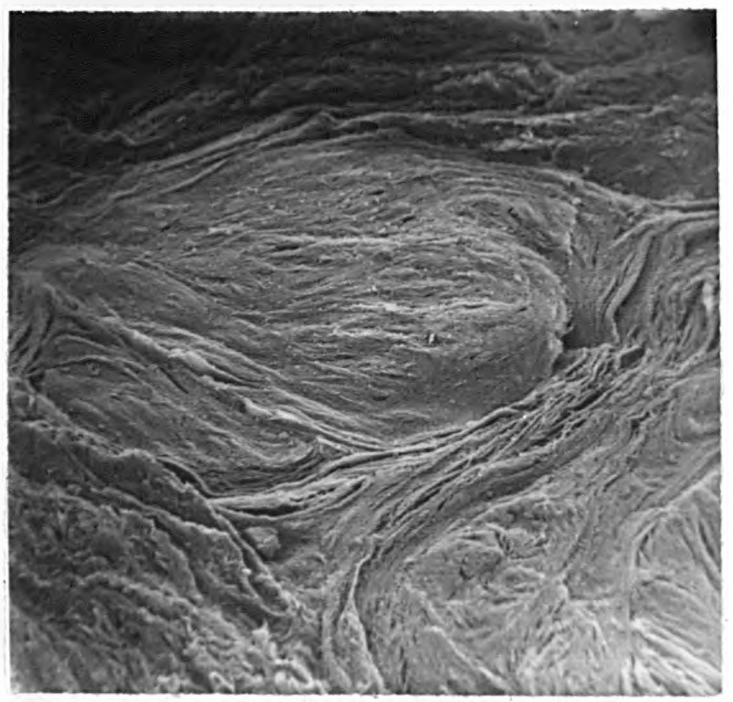


Fig. 3.29 ⌊——⌋ 200μm. Magnification of nodule shown in Fig. 3.27.

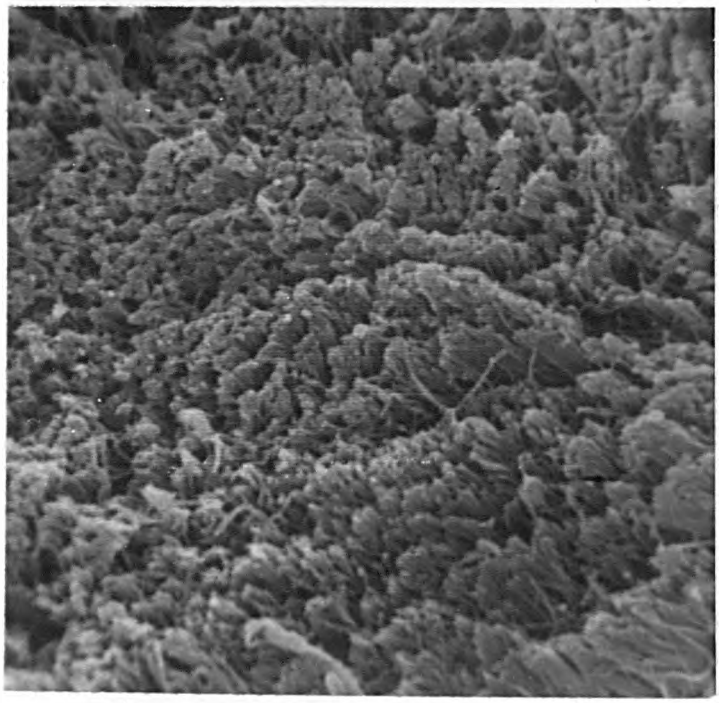


Fig.3.30 ——— 2µm. Transversely cut fibres within nodule shown in Fig.3.29.

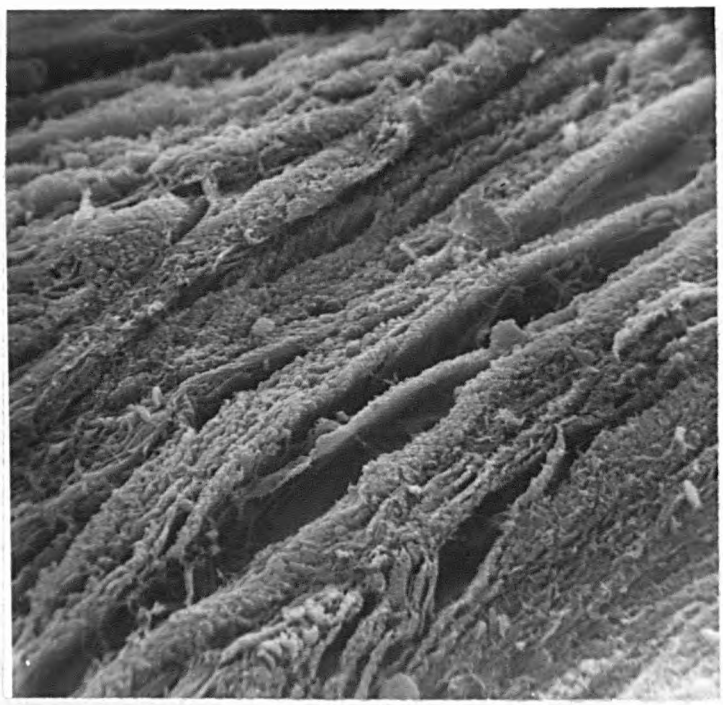


Fig.3.31. ——— 225µm. Flat sheets of fibres making up septal-like bands.

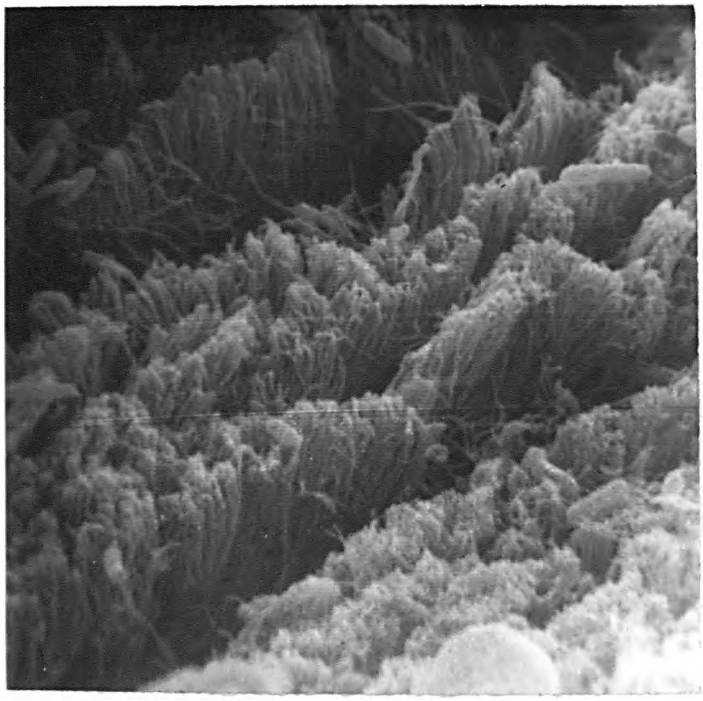


Fig. 3.32. —— 2μm. Magnification of fibres shown in Fig. 3.31.

in all directions. (Fig. 3.24). The criss-crossing bands of fibres enclose roughly circular structures, which are in fact bands of collagen fibre bundles cut transversely (Fig. 3.25). The resolution in Figs. 3.23-3.25 is poor due to the particular specimen preparation protocol used. Because of contamination to the dehydrated surfaces of the original specimens, fresh faces were prepaired by simply slicing through the specimen with a clean dermatome blade and re-coating. The fibre bundles being of different degrees of compactness cut at slightly different levels (see section 3.1.3). Thus although this salvaging technique tended to obscure details at higher magnifications; at lower magnifications the uneven surface thus prepaired highlighted the fibre bundle pattern.

Fig. 3.24 shows clefts between fibre bundles; similar to those described earlier, although in this specimen they are more sparse. These clefts usually occur in the thinner bands of fibres separating the large tranversely cut fibre bundles. The fibrils within these clefts are usually straight, giving the impression that they are under tension. Occasionally these fibrils are linked by thinner more coiled fibres, which could possibly be elastin fibres (Fig. 3.26).

An exaggerated form of the circular arrangements of collagen fibres described above, is shown in Fig. 3.27. This is a transverse section of tissue taken from the central region of the keloid scar excised from case no.2. It consists of extremely large groups of collagen fibre bundles (up to 1.5mm in diameter) separated by septal like bands of fibres. These septa have no well defined beginnings or ends, but seem to just blend into either the

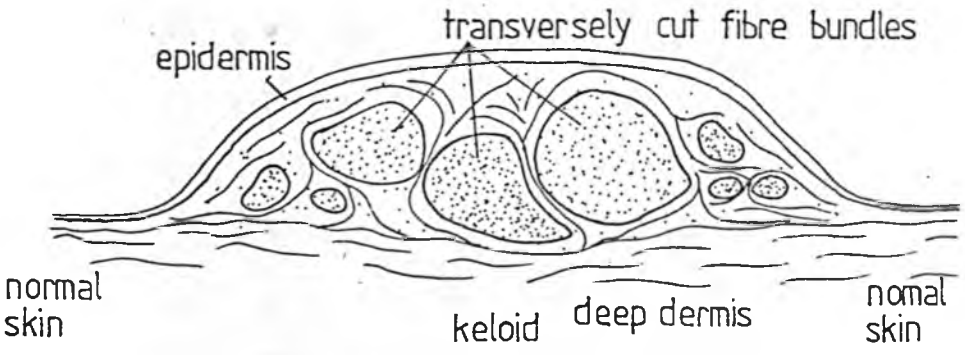


Fig. 3.33. Schematic diagram of a cross-section of a keloid scar. (Case no. 2)

papillary layer (Fig. 3.28) or the deep-dermis (Fig. 3.27). These circular arrangements will from now on be termed as Nodules, although they are not of the same form as the nodules described by Larson and his colleagues (see section 2.8.2).

Looking at the nodule on the left in Fig. 3.26 more closely. It is seen to be made up of tightly packed fibres (Fig. 3.29), grouped into fibre bundles running at right angles to the plane of the section (Fig. 3.30). The fibre diameters are of the same order as those found in the papillary layer ie 0.2μm to 0.5μm.

The septal like boundaries of the nodules are again made up of highly orientated fibres, but now they are aligned in flat sheets of fibres rather than cylindrical bundles (Figs. 3.31, 3.32).

Because of the restricted field of view of the SEM, and the large cross sectional area of the tissue specimen from case no.2, it was not possible to obtain a micrograph of the complete specimen cross section. However, a schematic representation is shown in Fig. 3.33.

The large nodules, such as those shown in Fig. 3.27, tend to be restricted to the central region of the scar. The septal band dividing the mid-dermis from the papillary layer and the deep-dermis is continuous throughout the section, although it is continually branching and re-forming other septa. Smaller nodules are found outside the central region (Figs. 3.23, 3.24) but these are of the same order of magnitude as those seen in transverse sections of hypertrophic scars.

The central nodules are not continuous along the long axis of the scar. A series of sections were taken through the tissue and the nodule cross-sectional shape was seen to be altered. The

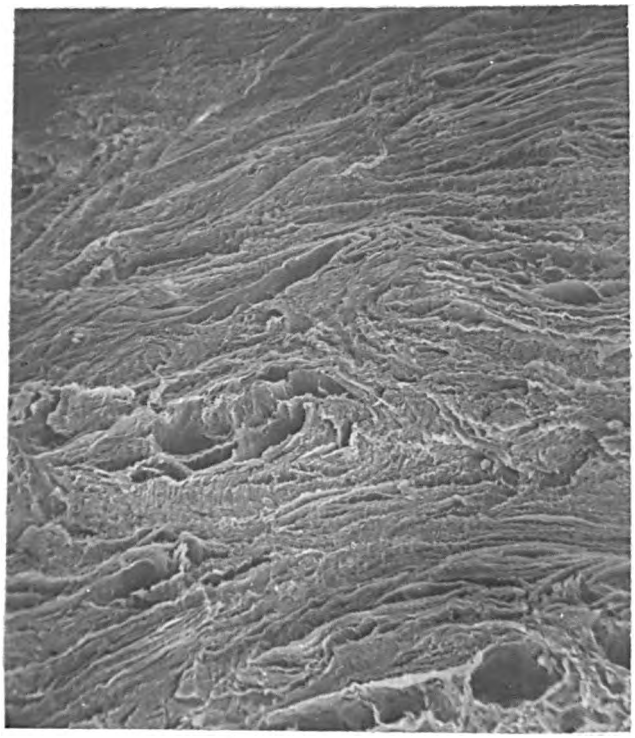


Fig. 3.34 ——— 200μm. Transverse section of deep dermis showing more open fibre bundle network.

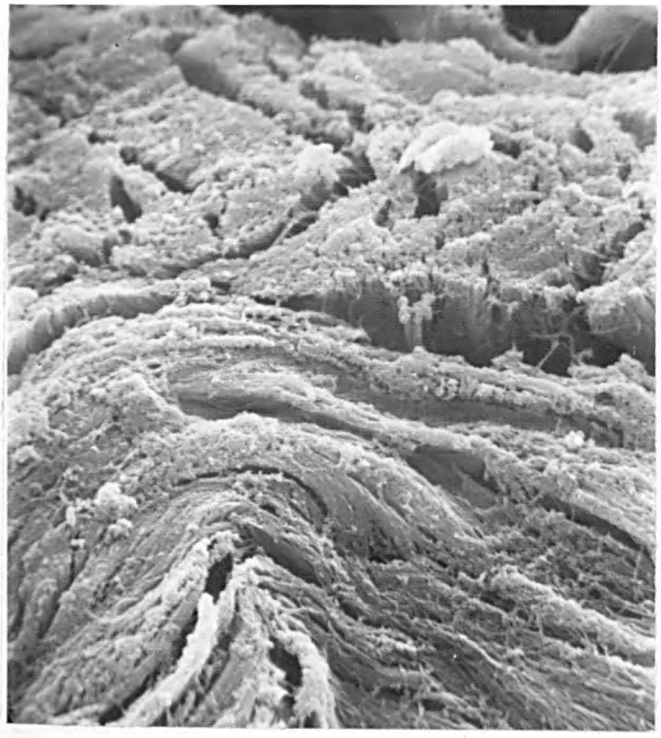


Fig. 3.35 ——— 5μm. Magnification of fibre bundles in Fig. 3.34.

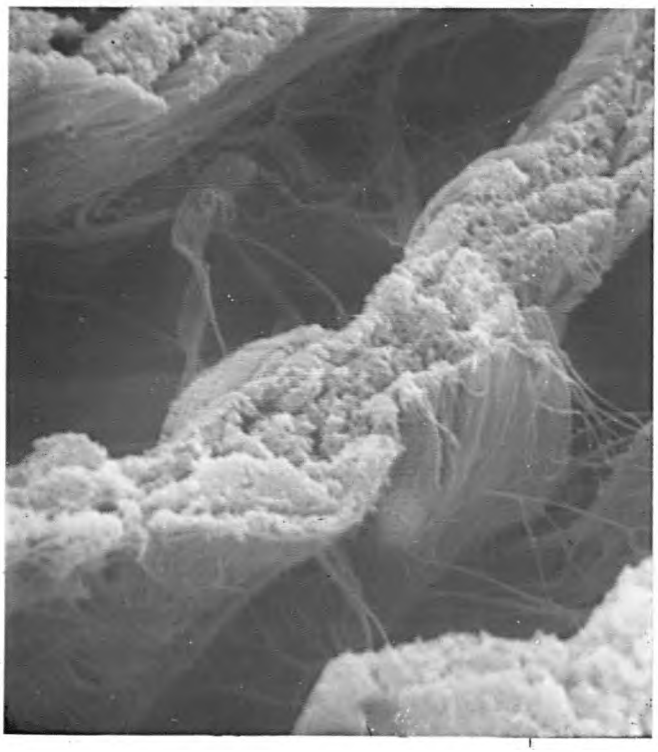


Fig.3.36.——— 2μm. Small diameter fibrils forming a loose covering network around fibre bundles in the deep-dermis.

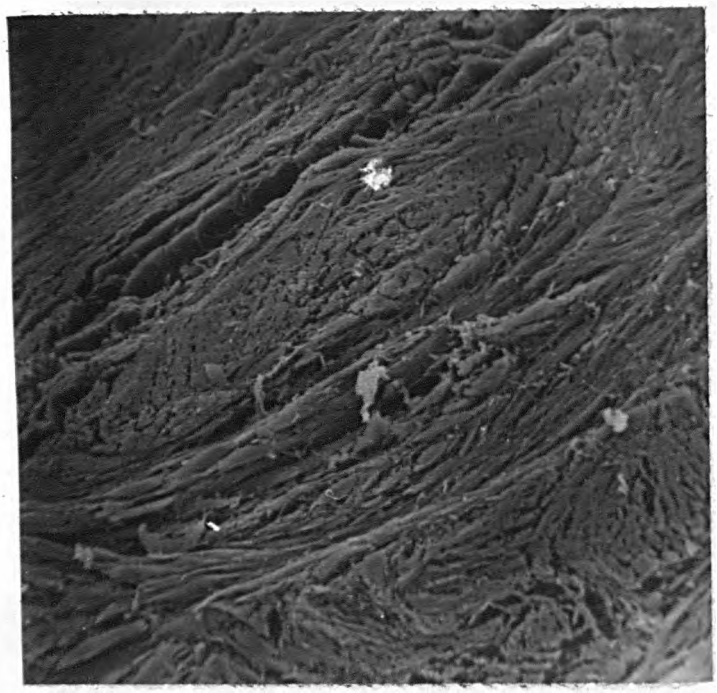


Fig.3.37.——— 100μm. Horizontal section through the papillary layer showing concentric arrangement of fibres around a possible blood vessel. (c.f. Fig.2.36.).

overall picture was that the large groups of fibre bundles (which in cross-section present the outline designated by the term nodule), branched and joined other bundles, just as collagen fibres do in the network of normal skin.

The micro-architecture of the mid-dermis of the keloid tissue is an exaggerated form of the micro-architecture of the mid-dermis of the hypertrophic scar tissue. The fibre patterns observed in the tissue samples taken from all cases in this study ranged from the parallel aligned, wavy fibres seen in mature scar tissue through the patterns shown in Figs. 3.17-3.26, to the extreme case of the keloid pattern in Fig. 3.27.

3.3.4. The deep-dermis Of all the layers of the hypertrophic scar tissue studied, the micro-architecture of the deep-dermis differed the least from that seen in normal mature scar tissue. The fibres themselves are still tightly packed, but now they are aggregated into more discrete bundles and sheets. These, in turn, are arranged in a more open network than that found in the mid-dermis (Figs. 3.34, 3.35). Small diameter fibrils, 0.06μm to 0.13μm in width, form a loose network covering some of the fibre bundles, and running between adjacent groups of fibres (Fig. 3.36).

3.3.5. Blood vessels Because it is not possible to effectively label the tissue components when working with the S.E.M., individual blood vessels are very difficult to distinguish in micrographs. No structures that could definitely be described as blocd vessels were observed in the tissue specimens obtained from theatre, although several objects that had similar characteristics to blood vessels were seen. Fig. 3.37 shows a concentric arrangement of fibres observed in a section parallel to

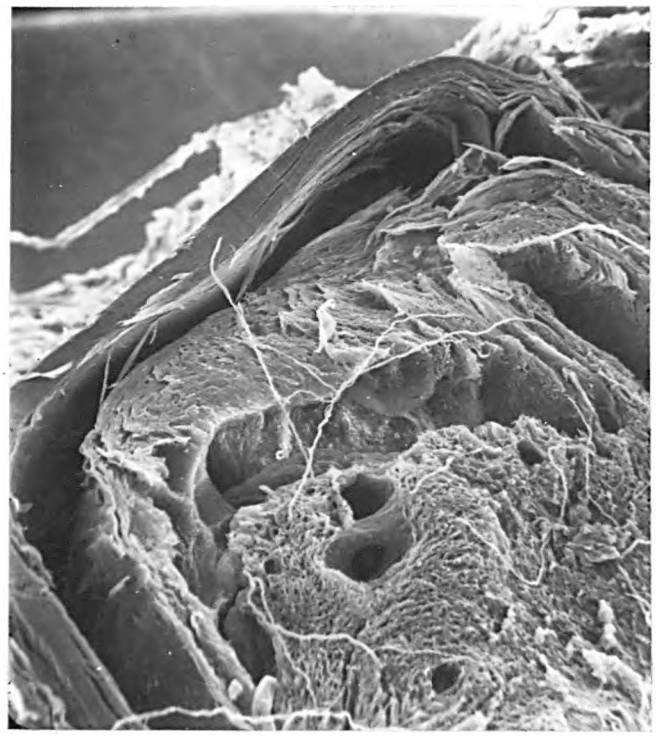


Fig.3.38.——— 150μm. Transverse section of epidermis and superficial dermis with possible blood vessels visible.

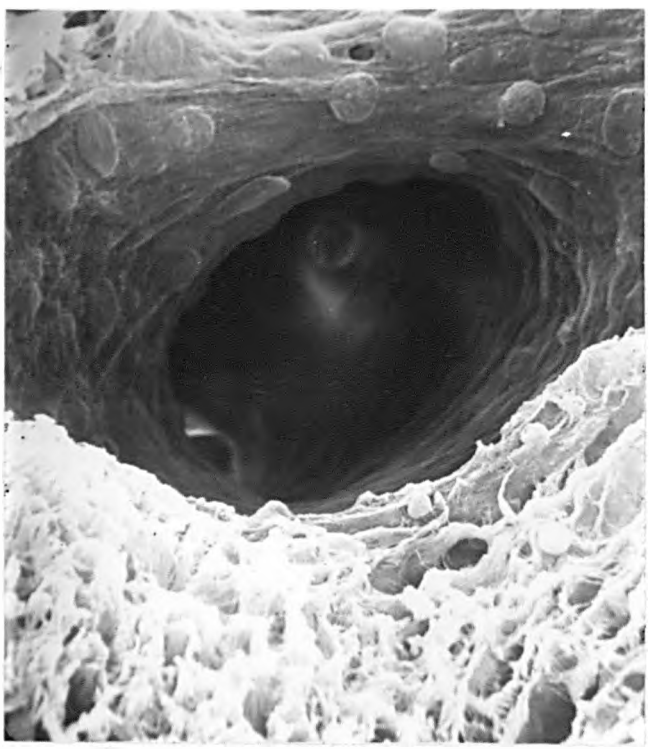


Fig.3.39.——— 15μm. Magnification of blood vessel in Fig.3.38. with red blood cells on vessel walls.

the scar's surface within the papillary layer, which could possibly contain a small blood vessel. Fig. 3.38 shows a transverse section of tissue from case no.3.

The "holes" in the papillary layer were observed in all the tissue specimens that made up a complete cross section of the scar tissue contracture band. They ranged from 25μm.to 50μm.in diameter, which would make them very large vessels to be found within the papillary layer of the tissue. On magnification, flattened cells of the same shape and size of red blood cells were observed on the walls of these "vessels" (Fig. 3.39).

With another series of specimens adjacent tissue sections were processed for either light or scanning electron microscopical observation. Using this method the tissue could be stained up to show individual components under the light microscope, which could then be located in the adjacent section using the S.E.M. Blood vessels, of the same order of magnitude in diameter as those described above, were observed in the papillary layer of scar tissue specimens. Further discussion of the blood vessel network within the hypertrophic tissue will be presented in Chapter 4.

3.4 Summary

The overall micro-architectural picture of the hypertrophic and keloid scar tissue is that of tightly packed collagen fibres, grouped into large bundles and sheet like arrangements. This fibre arrangement could account for the high degree of rigidity exhibited by the tissue.

The particular fibre pattern is different in different specimens of the hypertrophic scar tissue. The pattern ranges from wavy sheets of collagen fibres, similar to that seen in specimens of

normal mature scars, to extremely large fibre bundles surrounded by septal-like sheets of collagen.

The possible relationship between fibre bundle alignment and the mechanical properties of the scar tissue will be discussed in Chapter 5.

CHAPTER 4

PRESSURE THERAPY AND ITS EFFECT ON TISSUE MICRO-ARCHITECTURE

4.1. Introduction

The application of a sustained pressure to an 'active' hypertrophic scar does accelerate the resorption of that scar. Similarly pressure therapy will help to both prevent the formation of, or improve the characteristics of the already established contracture deformities often associated with these types of scars. The degree of success achieved with the therapy, however, is patient variable. Why the patient response varies, and how the actual pressure therapy works is not known. (For a review of the use and effectiveness of pressure therapy the reader is referred to section 2.9.5).

4.1.1. Study outline. The Plastic Surgery Unit of Canniesburn Hospital, Glasgow, Scotland has had successes in using pressure therapy to improve, both cosmetically and functionally, the hypertrophic scars of a series of patients. The ages of these patients ranged from approximately 18 months to 50 years. The majority of the scars treated were the results of burn injuries, and were of varying degrees of severity.

The results achieved with this therapy prompted the establishment of a research study which was designed to evaluate the effectiveness and mode of action of the pressure therapy in order that the techniques could be optimised to give the greatest benefit to the patients.

The study was divided into two main sections:

1) Monitoring of the pressures applied by different types of pressure wraps, and the patient/scar response to these. Investigating the effect of sustained pressure on, the rate of collagen synthesis within the tissue, the vascular response, and the water content of the hypertrophic scars.

2) Observing the micro-architecture of hypertrophic scar tissue as seen with the S.E.M. Obtaining a series of tissue biopsies from patients undergoing pressure therapy and observing the changes in micro-architecture with the sustained pressure. Investigating the inter-relationship between the mechanical characteristics and the micro-architecture of specimens of hypertrophic scar tissue in-vitro; and monitoring the changes in mechanical properties with pressure therapy for two patients, in-vivo.

The work involved in section 1) was carried out by a colleague and the results obtained by him will be presented in detail in his thesis (Naismith, 1980). The two sections of the study are interconnected and therefore a brief summary of Naismith's findings with respect to the pressure monitoring will be presented in the following section.

4.2. Pressure Monitoring

Initially the patients were fitted with tailor made pressure garments supplied by Jobst (Jobst Institute, Toledo, Ohio, U.S.A.). This particular form of pressure dressing was chosen initially since other researchers had already reported on its effectiveness. (Larson et al, 1971, 1974). A representative of the Jobst company measured the affected area of the patient's body, and these measurements were then used by the company to produce the required pressure garment, for example a glove for a scar of the hand, which was claimed by the manufacturers to apply the correct degree of pressure needed to accelerate the scar's remodelling.

The pressure exerted on the scar surface by the garment was measured by means of an instrumentation system developed by Naismith (1980). This comprised a circular parallel-plate

Fig.4.1. Capacitive bridge pressure monitor and set of pressure transducers.

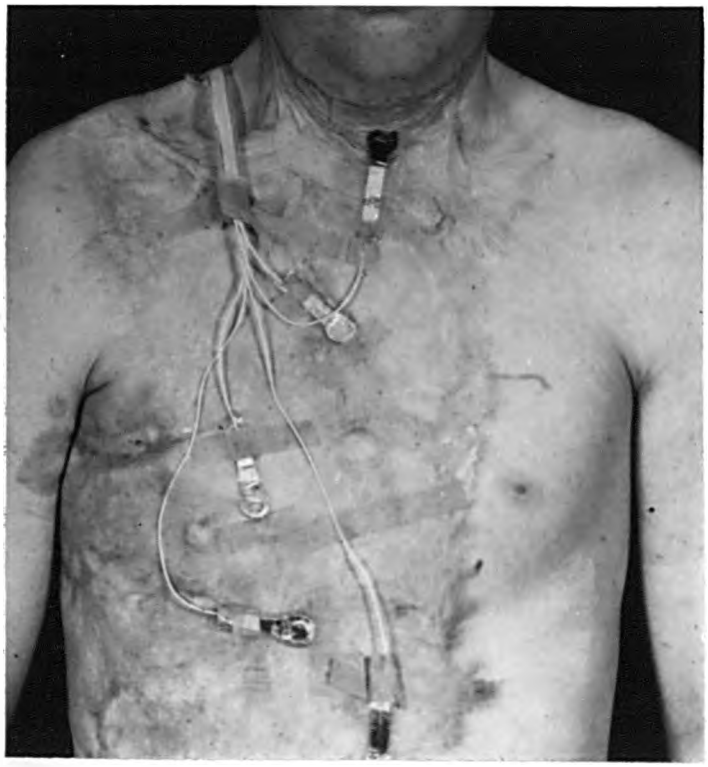


Fig.4.2. Pressure transducers in-situ, showing that they do not sensibly alter the scar surface contour.

capacitive pressure transducer 1cm^2 in sensing area and approximately 0.2mm thick, a capacitance bridge pressure monitor, and a pneumatic calibration chamber. (Fig. 4.1). The system resolution was 1 Torr (133.32 Pa) and the accuracy $\pm$ 2.5 Torr. Before use the pressure transducers were first calibrated, and a calibration curve of monitor output (mV) against pressure (Torr) was obtained. The pressure transducers were then placed on the scar surface and held in position by lightly taping the connecting leads to the skin. The transducers were of such a thickness that they did not sensibly alter the scar surface contour once in position (Fig. 4.2.). The pressure garment was then donned over the transducers and the changes in transducer capacitance were measured and displayed by the capacitance bridge pressure monitor. The monitor output in mV was then converted to pressure (Torr) using the previously obtained calibration curve. The transducers could be made up in sets (up to 5 in each set) enabling the pressure exerted on different regions of the scar surface by the garment to be measured.

Within a few days of commencing to wear the pressure garment the colour of the scar surface paled and the patients commented that the scar tissue felt less stiff. Another beneficial effect of the pressure therapy was that the severe itchyness of the scars was relieved. This was of particular importance in young children since they often scratched the scars so much that the surface broke down causing a further aggravation of the symptoms.

With time, and sustained pressure the scar tissue thickness reduced and the extensibility of the tissue improved. Whilst the scar remained in its active phase it was essential that the pressure garment was worn continually. Patients were supplied with

two garments so that one could be worn whilst the other was being washed. When the garment was removed the patients described a "tingling" feeling in the scar, similar to "pins and needles", which was relieved as soon as the pressure was re-exerted on the tissue. In one patient who unavoidably had to stop wearing his pressure dressing for a period of approximately three weeks, the scar tissue resumed its red and hard appearance and the symptoms of tenderness and itchyness returned.

With continuous maintenance of pressure the resorption of the scar tissue eventually reached a plateau, with no further change in cosmesis and/or function being noticeable to the patient. However during the early stages of this plateau "phase", removal of the pressure resulted in a gradual recurrence of the hypertrophic scar symptoms. This was felt to be due to the fact that although the scar had effectively been remodelled by the pressure therapy, its characteristics had not yet stabilised enough to maintain this improvement. If, after a period of one month or more there was no further improvement in the scar's characteristics the patient was advised to leave off the pressure garment for increasing periods of time and to note any changes in sensation. If the tissue was found to be fully stabilised the pressure therapy was discontinued.

The Jobst pressure garments were commercially tailored for the individual patient. This was a costly process and often resulted in long delays before a suitable garment was manufactured. The possibility of using alternative types of pressure dressings was therefore also investigated. An elastic bandage, manufactured in the form of a continuous cylinder and supplied under the trade name of "Tubigrip" (Seton Ltd., Oldham, England) was found to be equally

effective. At the time of the study this was available in a variety of diameters and could easily be fitted to the trunk and the limbs. The same company have recently started manufacturing a range of pressure garments, made of the same material as Tubigrip, which again come in a variety of sizes enabling the patient to be fitted "off the peg" with a suitable garment at a far lower expense and with a considerably smaller time delay.

The results of monitoring the pressure therapy over a period of 2 years showed that although pressure was effective in accelerating the resorption of the scar tissue, there appeared to be no critical combination of pressure magnitude and duration to which scar resolution could be attributed. Some patients responded very well to low pressures being applied to the scar tissue, (considerably lower than the critical 25mm Hg reported by Larson and colleagues (1974)) whilst other patients needed the application of much higher pressures before any improvements were noted.

The magnitude and duration of the pressures developed by a pressure garment were found to be dependant upon the following variables: 1) the mechanical properties of the materials, 2) the mechanical properties of the tissue and 3) the anatomical location of the scar.

It was found that the pressures exerted by the pressure garments supplied by Jobst were not constant either spatially or temporally, and that satisfactory results could be achieved with the less expensive Tubigrip. With both types of dressing it was found that the mechanical properties of the materials resulted in a permanent stretching of the garments after a period, at the maximum, of 6 weeks continuous wear. Because this resulted eventually in

only negligible pressures being applied to the scar, patients were frequently provided with new garments.

The mechanical properties of the scar tissue, before commencing treatment, also had an effect on the pressures developed by the pressure garments since an originally hard, rigid scar would supply a much firmer base for the dressings than a softer scar. The changes in the mechanical properties of the scar tissue with pressure, measured in-vivo will be discussed in Chapter 6.

That the pressures developed were dependant upon the anatomical location of the scar was to be expected, since it was much more difficult to apply pressure to scars located over soft regions of the body i.e. the stomach; or over regions with a concave curvature i.e. the axilla.

For a complete discussion of the results of pressure monitoring, and the changes in the biochemical properties of the hypertrophic scars the reader is referred to the Ph.D thesis of Naismith (1980).

4.3. Serial Biopsies

To be able to observe the effect of the pressure therapy on the fibrous micro-architecture of the scar tissue necessitated taking a series of tissue biopsies. These tissue biopsies had to be large enough to permit meaningful observations of micro-structure to be made, and yet small enough to give an acceptable level of patient discomfort. It was decided that a 3m.m. diameter core of tissue was of sufficient size to enable the necessary histological observations to be made, whilst being small enough to heal spontaneously without the necessity for suturing. At the time when the first biopsies were required a biopsy punch that could give an undistorted core of scar tissue was not available on the commercial market and

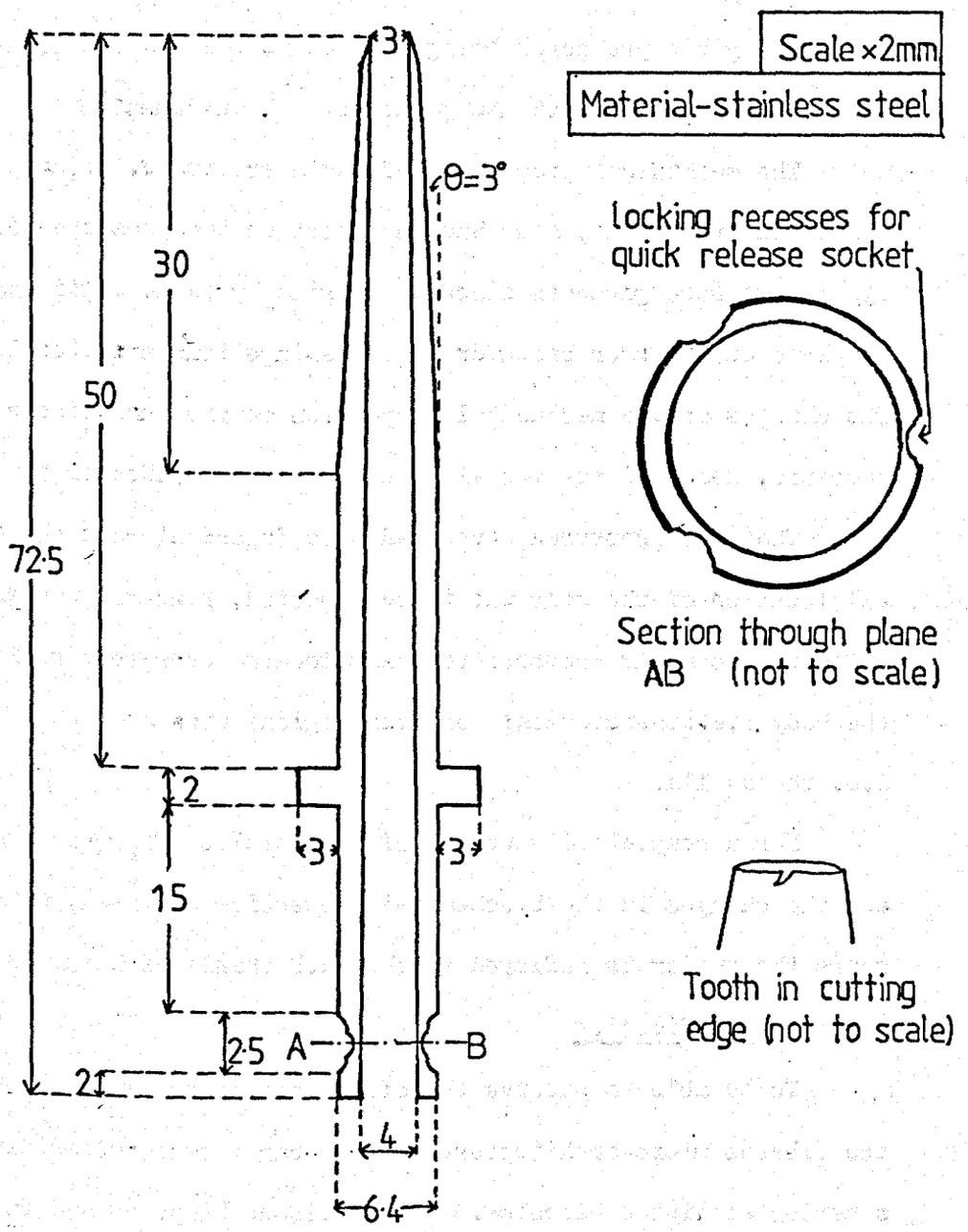


Fig. 4.3. Scale diagram of rotary biopsy punch cutting bit

therefore a suitable device had to be designed and constructed.

4.3.1. Rotary biopsy punch. The prime requirement for the design of the biopsy punch was that it should produce a full thickness core of tissue with minimum distortion of the specimen and minimum discomfort to the patient.

A very sharp cutting edge is needed to cut either skin or scar tissue without at the same time distorting its fibrous structure. It was not possible to manufacture a circular blade that was sharp enough to cut through the scar tissue when turned by hand, and it was therefore decided to construct a motor driven cutting device. By turning the blade at speed a less sharp cutting edge was required.

There were reports of people using modified dentist's drills, and even commercial power drills to try and obtain small cores of skin, but these rather crude techniques invariably resulted in gross distortion of the biopsy rendering it useless for the observation of fibrous micro-architecture.

The design of the rotary biopsy punch could therefore be divided into two main sections: the design of a cutting bit that produced an undistorted core of tissue; and the choice of a suitable motor and power source such that the torque exerted on the tissue, and the speed of rotation of the cutting bit allowed the controlled taking of a tissue biopsy.

A scale drawing of the developed cutting bit is shown in Fig. 4.3. The bits were machined out of rods of Fh american AISI 4/20 high carbon stainless steel, which could be sterilised by either immersion in an antiseptic solution or by auto claving. The centre hole was machined using a tapered drill bit so that once the tissue had passed inside the cutting edge it was not in close contact with

the revolving walls of the bit. The outer wall of the cutting device was also tapered to allow easy passage of the blade through the tissue. From trials carried out on pieces of excised scar tissue the blade was found to pierce the epidermis with less distortion if one small tooth was cut into the edge of the blade (Fig. 4.3) This tooth did not cause any tearing of the dermis of the tissue core. The final edge was not put onto the cutting blade until the bits had been hardened by placing them in an oil quench at 980°C followed by tempering at 180°C. (This gave an average hardness of 600 on the Vickers hardness range.)

The cutting bits were mounted onto the shaft of the chosen motor by means of a quick release socket and a special collar. The quick release socket was of the type used to connect hydraulic grips to an Instron testing machine (Instron Ltd., High Wycombe, England) and was used to enable the cutting bits to be changed quickly and aseptically. (Appendix B, Fig. B1).

Once a trial arrangement of cutting bit, motor and power source had been assembled, 'in-vitro' tests were carried out to find a method of stopping the biopsy punch once the full thickness of the scar tissue had been cut through. The thickness of the hypertrophic scars varies considerably. With normal skin an estimate of skin thickness can be obtained by measuring with calipers the thickness of a skin fold. However, hypertrophic scar tissue is much too rigid to allow a fold of tissue to be measured, making it difficult to judge the full depth of a scar. The idea of using a mechanical 'stop' on the cutting blade was therefore discarded.

In the 'in-vitro' tests, it was found that as soon as the cutting blade had passed into the fat layer beneath the scar the

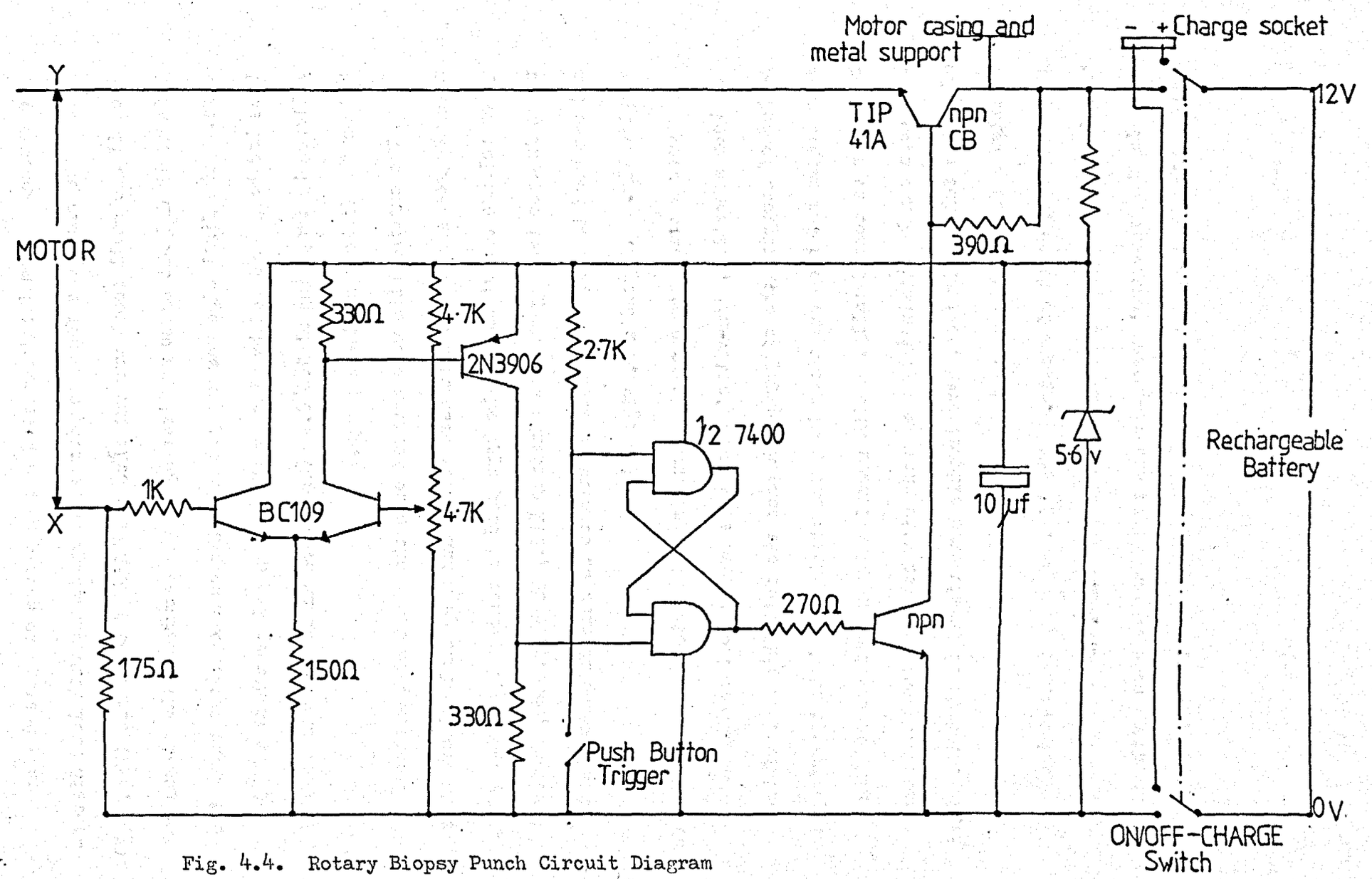


Fig. 4.4. Rotary Biopsy Punch Circuit Diagram

current required to drive the motor increased. This increase in current was due to the increase in torque exerted on the cutting bit by the tissue once it had passed into the fat layer. The tissue of the scar dermis was firm enough to be cut by the blade with little torque required, however the semi-liquid fat layer just gave against the cutting edge, the fine fibrils between the fat cells wrapping themselves around the bit resulting in a high torque being exerted on the blade. By incorporating an adjustable current limiting device (C.L.D.) into the circuitry supplying power to the motor it was therefore possible to stop the motor once the torque/current had passed a certain level. Using this C.L.D. the biopsy punch could be made to stop automatically as soon as the fat layer was reached. The punch was then removed leaving the tissue core in-situ, attached by the thin fibrils within the fat layer. The loosely attached specimen could then be withdrawn from the dermis with forceps and the attaching fibrils cut with a scalpel.

The circuit diagram for the biopsy punch is shown in Fig. 4.4. The motor was activated by means of the double pole double throw ON/OFF/CHARGE switch. As soon as the current drawn from the batteries reached the set level the motor automatically stopped. The motor could only be restarted by using the push button trigger to bypass the C.L.D., this safety measure ensured that the punch did not start to rotate again as it was withdrawn from the scar. The current cut-off level was adjustable.

The biopsy punch was used in-vivo for the first time, in theatre, on a region of scar tissue that was to be excised during the surgical correction of a contracture deformity. Biopsies were taken with the current cut-off level set at a variety of values, and the mean value

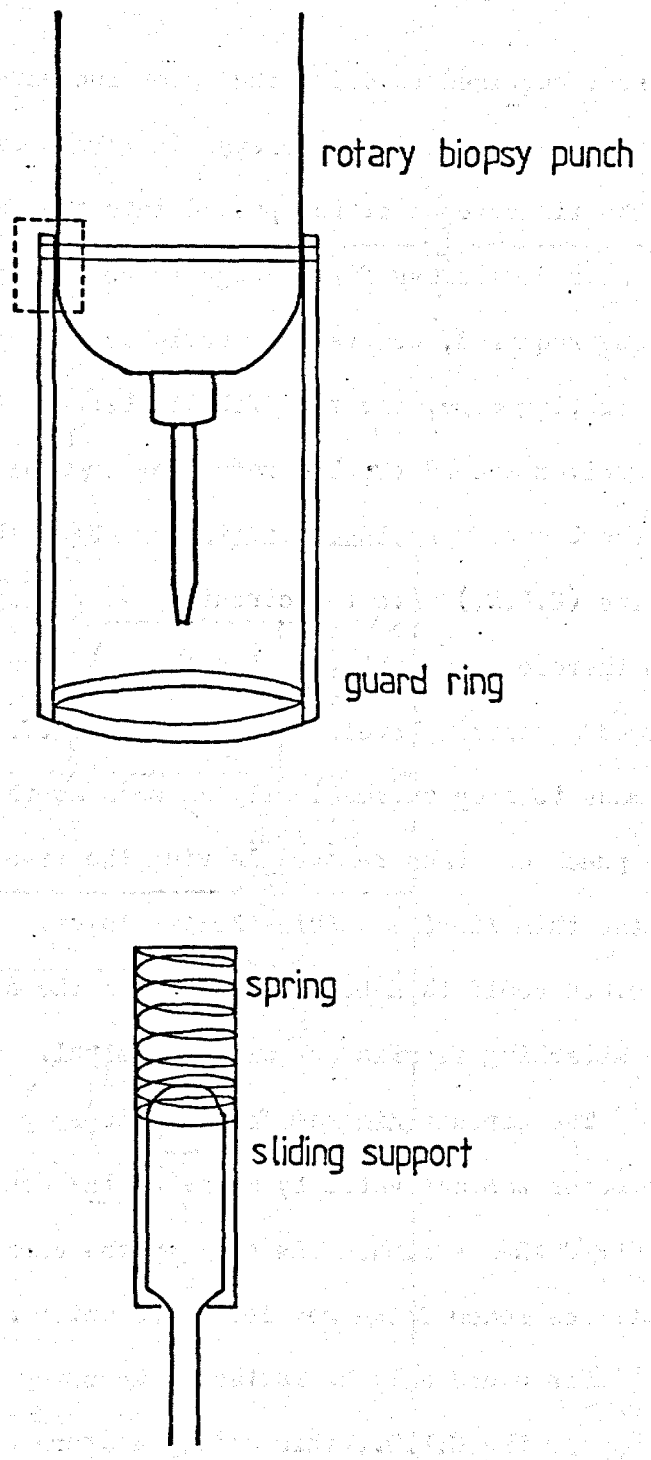


Fig. 4.6. Diagram of spring loaded guard ring for rotary biopsy punch, designed to prevent sliding of the cutting bit.

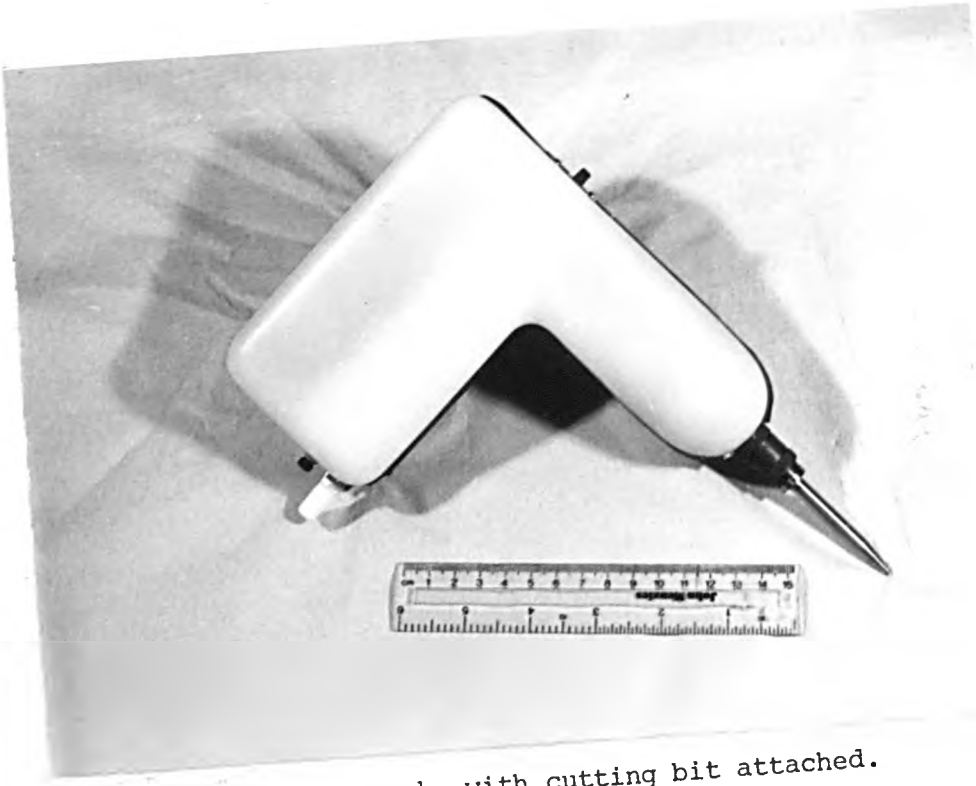


Fig.4.5. Rotary biopsy punch, with cutting bit attached.

that permitted both a full thickness tissue core to be obtained and the motor to stop automatically on reaching the fat layer was marked on the C.L.D. adjustment screw. This mean cut-off value was obtained for an average scar "thickness" of 4mm - 5mm (where the "thickness" was taken as the height of the scar tissue above the level of the surrounding normal skin). When the rotary punch was used in the outpatient clinic, with a local anaesthetic, the operator could adjust the current cut-off level from the mean value depending upon the thickness of the scar.

The power source consisted of a 12 volt stack of re-chargeable Nickel Cadmium button cells (supplied by Varta, Great Britain Ltd., Somerset, England). The motor used in the prototype operated at 3,000 r.p.m., 0.006 H.P. from a 9-12 volt D.C. source (R.B. Pullin & Co. Ltd., London, U.K.). This motor was used because it was readily available and had the required electrical specifications. It was however bulky so that together with the circuit board and stack of batteries the assembled biopsy punch was also very bulky. Fig. 4.5. shows the assembled punch with cutting bit in place. The casing was vacuum formed out of thermo plastic to enable the complete device to be wiped down with an antiseptic solution.

One problem when taking the biopsies was that the cutting bit tended to slide across the scar tissue unless the device was held very firmly. A spring loaded guard ring was designed that could have been attached to the device (Fig. 4.6.). The guard ring would be placed on the scar tissue and the cutting bit advanced by pushing against the ring, this would have held the cutting bit at right angles to the tissue and eliminated the initial slippage problem.

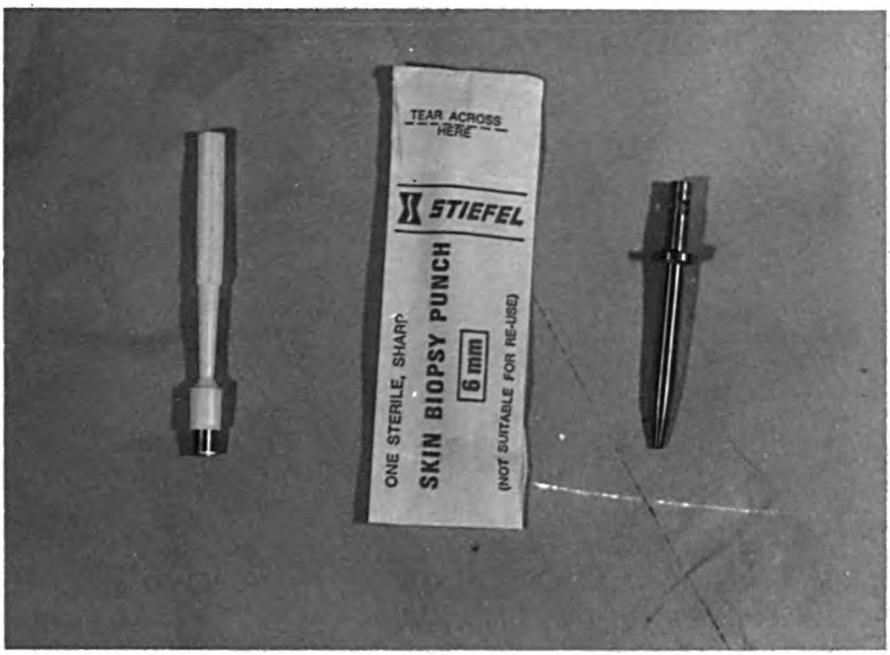


Fig.4.7. Stiefel skin punch and rotary biopsy punch cutting bit.

The prototype rotary biopsy punch (without guard ring) was used successfully in-vivo to obtain the initial tissue biopsies. However because of its large bulk it was both difficult to handle and a little off-putting for the patient. A few weeks after its first use a commercial hand driven biopsy punch became available. (Stiefel Laboratories (U.K.) Ltd., Slough, England). (Fig. 4.7.) This device had several advantages to the rotary biopsy punch designed. It was supplied already sterilized and was disposable. The cutting edge was scalpel sharp, enabling it to be used by hand. A variety of cutting blade diameters were available and the overall device was small and easy to handle.

The rotary punch designed in this study could have been refined to equal the efficiency of the commercial hand operated punch, however it was felt that the resources, and particularly the time, required would have been insufficient to allow this. Therefore, since the main objective of the research study was to observe the tissue micro-architecture and characterise the effects of pressure therapy further modifications to the rotary punch design were shelved. Instead the Stiefel skin punch was used throughout the remainder of the tests.

4.3.2. Tissue biopsy protocol. Since the patient was required to attend the pressure monitoring clinic and other outpatient activities at frequent intervals, and have a series of tissue biopsies taken, a high degree of patient cooperation was needed. The choice of patient was further limited to those who were of an age to consent to, and fully understand, what was involved in the tests.

Initially a biopsy of normal skin as well as that of the scar tissue was taken to give a "within patient" comparison. The normal

skin biopsy was taken from an area as close to the scar tissue as possible, to minimise any further scarring. However this threw some doubt onto the "normality" of the specimens since it was difficult to define the original wound boundaries due to the contraction of the wound edges. Ideally the initial pair of biopsies were taken before the patient was fitted with a pressure garment, however, a number of patients had already been receiving pressure therapy for several weeks before a suitable biopsy punch was available. In these cases biopsies were taken as soon as possible. The biopsy site was chosen from the area of scar tissue that it was felt would obtain the greatest benefit from the pressure. The biopsy site was noted in the patients case notes so that later biopsies were not taken from the same place.

After numbing the area with local anaesthetic, the surgeon removed a core of tissue using a 3mm Stiefel skin punch. The standard of biopsy obtained varied. Because the surgeon had to judge by feel when the full thickness of the tissue had been pierced some of the biopsies obtained were not full thickness. Other biopsies were greatly distorted by the forceps used to remove them from the scar. Consequently the standard of a lot of the biopsies taken in the early stages of the study was poor and this limited the tissue available for the subsequent microscopy.

4.4. Specimen Preparation

To attempt to obtain as much information as possible from the tissue biopsies a combined light, scanning electron and transmission electron microscopy (T.E.M.) régime was planned. The facilities and technical support were readily available for the light and scanning electron microscopical investigations, unfortunately

difficulties arose when an attempt was made to prepare and observe sections using the transmission electron microscope.

4.4.1.. Transmission electron microscopy. A review of the ultrastructure of hypertrophic scar tissue is given in sections 2.7.2. and 2.8.3. The author's original objective was to attempt to verify the presence of the abnormal collagen filaments (abnormal in terms of diameter and cross-section) and the high concentration of fibroblasts with markedly dilated r.e.r. described by Linares and colleagues (1972, 1973).

Although the tissue processing protocols to prepare specimens for observation with a T.E.M. appear straightforward, a high degree of skill is required to obtain worthwhile and meaningful results. Unfortunately this skill was lacking by the author and no experienced technical assistance was readily available. A large amount of time was spent obtaining ultrathin sections of the hypertrophic scar tissue biopsies and some T.E.M. micrographs of collagen fibres were eventually obtained. However, these were of such a standard that no meaningful observations could be drawn from them and no T.E.M. results will be presented in this thesis.

Although no definite results were obtained the experience gained was beneficial, and the tissue preparation protocols finally established are shown in Appendix C for future reference.

4.4.2. Combined Light and Scanning Electron Microscopy Specimen Preparation. As stated in section 2.1.5. it is not possible to effectively label tissue components for viewing with an S.E.M. However by taking adjacent thin and thick sections from a specimen and preparing the former for light microscopy and the latter for S.E.M., tissue components can be labelled in the thin section, their

location noted and the corresponding component located in the S.E.M. section. This was the proceedure used to observe the micro-architecture of the scar tissue biopsies.

After fixation in 10% phosphate buffered formol saline the specimen was washed in distilled water overnight. After which it was prepared for embedding in paraffin wax using a Histokinette (Hendrey Relays and Electrical Equipment Ltd., Slough, England). This device automatically passes the specimen through a series of baths which, firstly dehydrates the specimen by immersion in graded alcohols, secondly replaces the alcohol in the specimen with an oil capable of dissolving paraffin (ie, xylol) and finally saturates the specimen in melted paraffin wax, the entire process taking 48 hours. The paraffin saturated tissue was then ready for embedding.

The specimens were placed on a thin film of semi-liquid paraffin in a small mold and orientated to allow transverse sections to be obtained. The mold was then filled with liquid paraffin which was allowed to cool and harden. The embedded specimens could be stored at this stage.

The sections were cut using a Rotary microtome (Lab. Tek. Rechert, Austria) with the microtome knife set at a cutting angle of 5°. The knife was sharpened before each batch of specimens in a Shanden Elliot Automatic Knife Sharpener MkII. The tissue block face was prepared by removing a series of thin sections. As soon as two good quality thin transverse sections (8μm) of the specimen were obtained the section thickness control was altered and a thick transverse section (25μm - 30μm) of the tissue was cut. The two sections were floated out on a bath of warm water and then picked up on separate microscope slides. (Adhesion of the sections to the

microscope slides was ensured by first coating one side of the clean slides with a thin film of egg albumin). Initially only two pairs of thin and thick sections were cut, the remaining tissue being stored for future reference. The mounted sections were then left to dry on a warm tray.

After drying the two thin sections were stained up and prepared for observation using standard histological techniques. One tissue section was stained with Hematoxylin and Eosin (H & E) and the other section was stained with Acid Orcein and Giemsa Stain (AO & G). The staining procedures used are shown in Appendix C.

Hematoxylin and Eosin is a stain commonly used for labelling sections of tissue. It results in the collagen being stained pink, the cell nuclei blue and the cell cytoplasm pink. Acid Orcein and Giemsa is a recommended stain for labelling elastin fibres. In sections stained with AO & G the elastin fibres are dark brown or black, the collagen rose-pink, the cytoplasm of epidermal cells light blue and the nuclei deep blue. (Pinkus, 1969).

The thick section of tissue was prepared for observation with the S.E.M. using a different technique to that described in section 3.1. The tissue was first de-waxed by immersion in 2 successive baths of xylol, for 5 minutes in each, after which they were placed in a bath of acetone. Prior to insertion in the S.E.M. all specimens require to be mounted on small metallic pedestals called specimen stubs. These are circular aluminium plates approximately 1 cm in diameter, which have a small projection on one face which is used to locate the stub in the specimen stage of the microscope. The specimen stubs were prepared for receiving the specimens by painting the surface of each stub with conducting Silver Dag 915

(Archeson Colloids Ltd., Plymouth) which was then allowed to dry. Whilst the tissue sections were immersed in the acetone they were detached from the microscope slides using a good quality artist's paint brush. The specimen could then be picked up on the paint brush, removed from the acetone and positioned on the Silver Dag. Adhesion was obtained in a few seconds by the evaporation of the acetone carried over. There was a drawback to this procedure: if too thick a coat of Silver Dag was applied to the stub, or it was not allowed to dry completely, the tissue specimen would become impregnated with the silver. This obscured the finer details of the tissue fibre arrangement and made direct comparison with the thin stained sections difficult.

After the acetone had completely evaporated from the tissue, the specimen was coated with gold/paladium in the same way as that described in section 3.1.5., and could then be viewed with the S.E.M.

4.5. Tissue Biopsy Microscopy Results

Ten sets of serial, scar tissue biopsies were obtained from patients undergoing pressure therapy. Because of the limited time period available and the high degree of patient cooperation required not all of the sets were complete in as much that they included pre-, during and post-pressure therapy scar tissue biopsies. Only one patient successfully completed the course of pressure therapy during the period of investigation, although several patients had reached the plateau phase. (See section 4.2.)

A training in histology/pathology and much experience is required to fully interpret sections prepared for light microscopy. However, the major features of collagen orientation, vascularity and degree of cellularity can be discerned by the novice and

Fig.4.8.——— 250µm. 8 week old hypertrophic scar, partial thickness showing friable epidermis and orientated mid-dermis fibres.

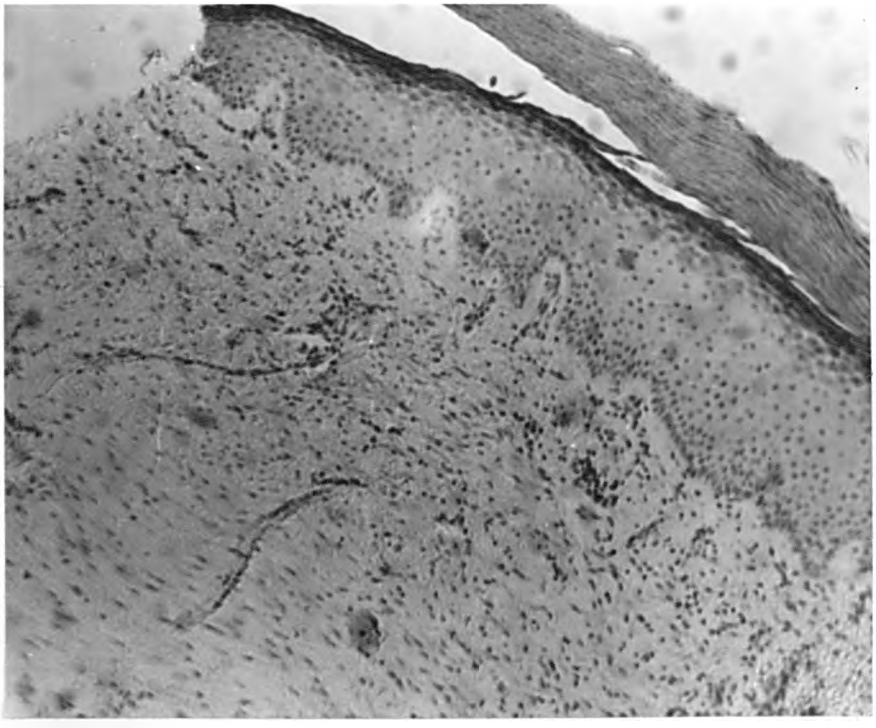


Fig.4.9.——— 100µm. L.M. of scar shown in Fig.4.8., showing high concentration of cells within the dermid. (H&E).

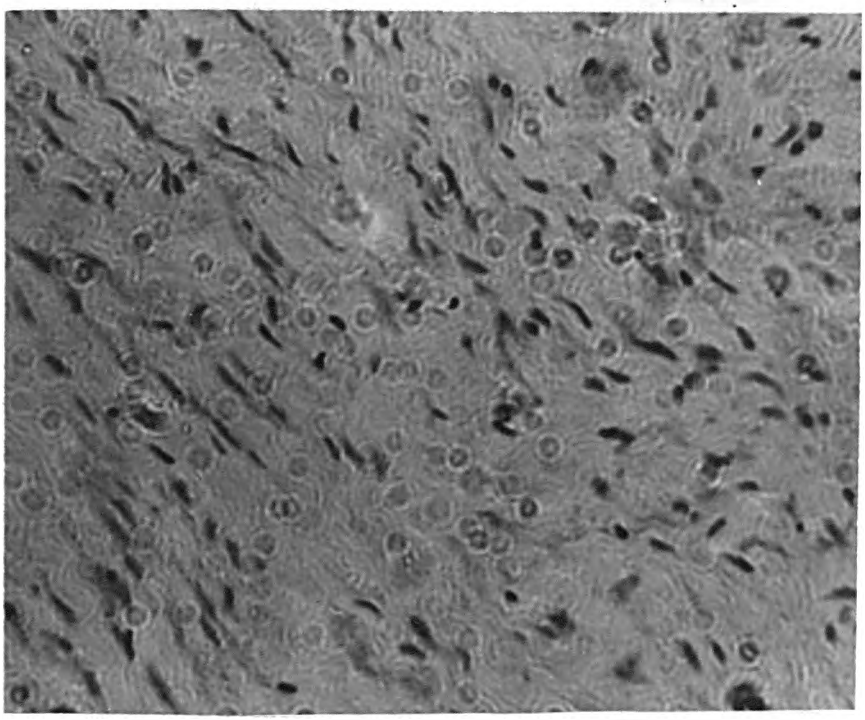


Fig.4.10.——— 25μm. Magnification of mid-dermis in Fig.4.9. showing spindle shaped fibroblasts in orientated band of collagen fibres. (H&E).

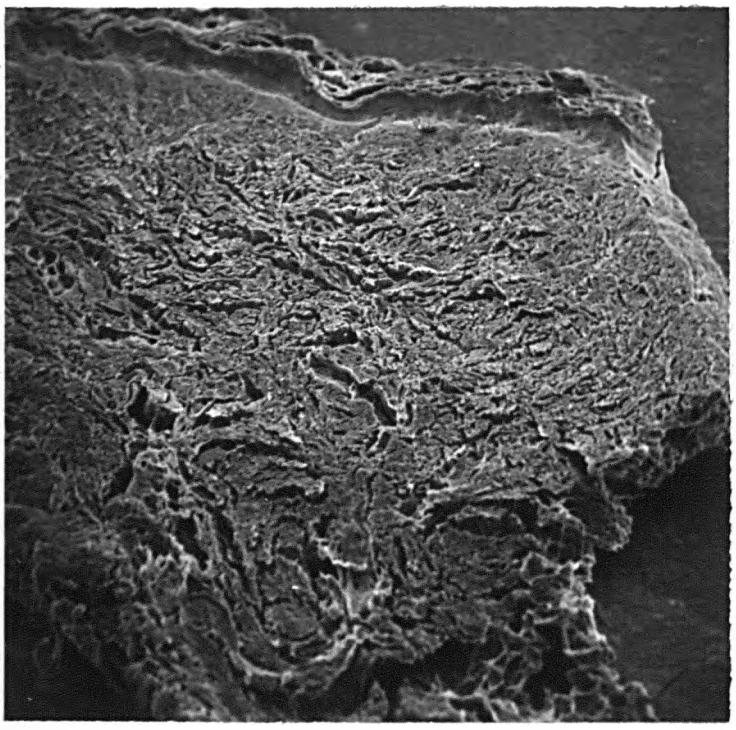


Fig.4.11.——— 250μm. S.E.M. of normal skin, patient 1, with fat cells in the deep-dermis.

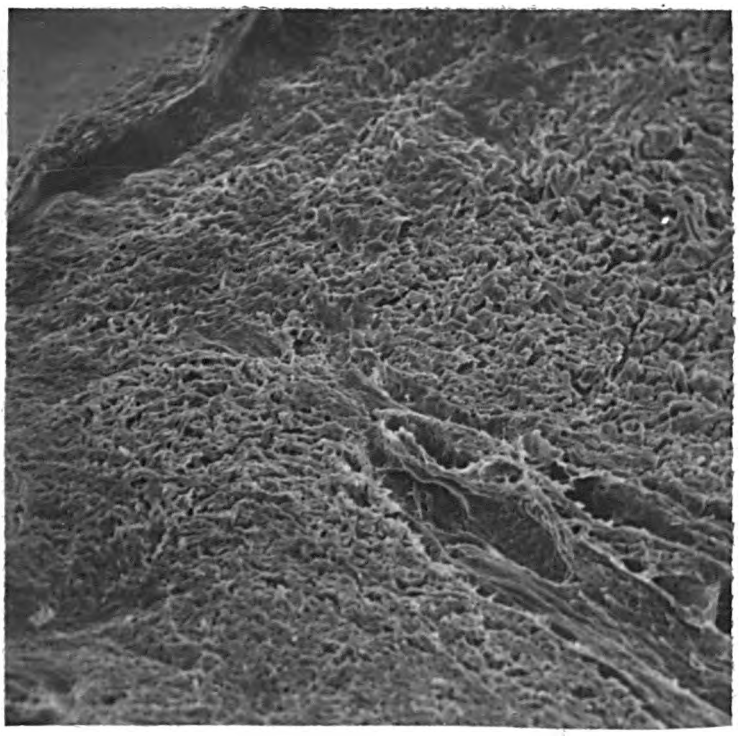


Fig.4.12. ——— 100μm. S.E.M. of normal skin, patient 2, showing a section of a sebaceous gland.

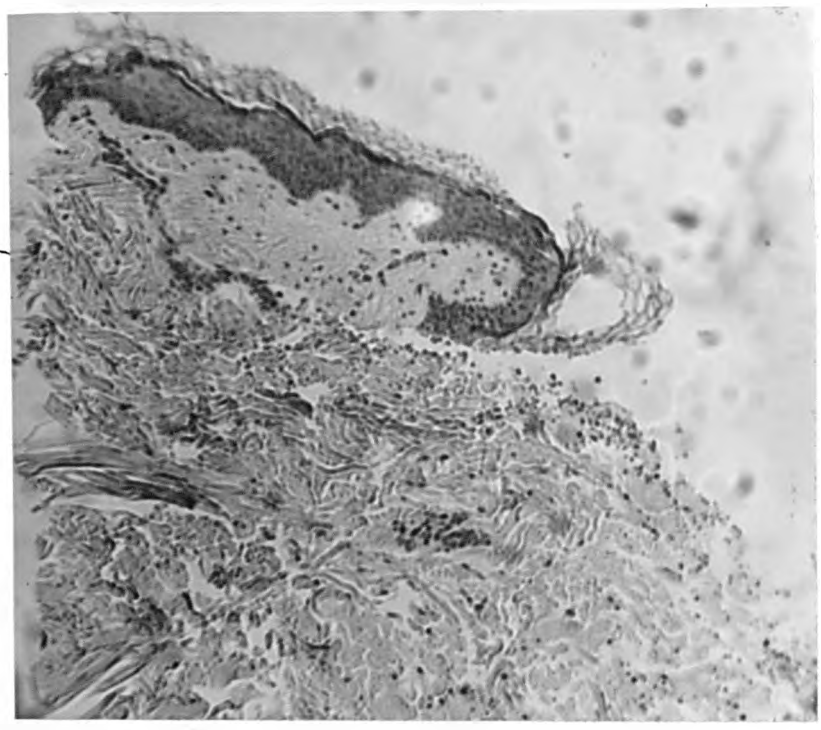


Fig.4.13. ——— 100μm. L.M. of normal skin, section adjacent to Fig.4.12. (H&E).

observations of this type have been made by the author and used to help interpret the scanning electron micrographs of the scar tissue.

The relative age of the scars studied (ie, age from the time of injury) varied from several weeks to approximately one year. Only one "true" keloid was studied - the scar tissue having exceeded the original wound boundaries.

All micrographs shown are of transverse sections. Due to operator difficulties, not all of the biopsies obtained were of the full thickness of the scar tissue.

4.5.1. Tissue micro-architecture before commencement of pressure therapy. Fig. 4.8. shows a partial thickness biopsy of an eight week old, un-treated hypertrophic scar as seen with the S.E.M. The friable epidermis has partially lifted away from the dermis. A band of highly orientated fibres is visible beneath the papillary layer.

Figs. 4.9. and 4.10. are light micrographs of an adjacent section stained with H & E. The epidermis is thicker than in normal skin and again the stratum corneum has partially separated. The tissue exhibits a high degree of cellularity and the spindle shaped fibroblasts are easily visible in the band of orientated collagen fibres (Fig. 4.10). Blood vessels travelling perpendicularly towards the skin's surface are also visible with diameters of the order of 9μm.

4.5.2. Micro-architecture of normal skin. The micrographs presented in the following three sections are taken from two patients: patient 1, fitted with a pressure glove for a hypertrophic scar of the back of the hand eight months after injury; patient 2, fitted with a pressure vest for hypertrophic scars on the chest

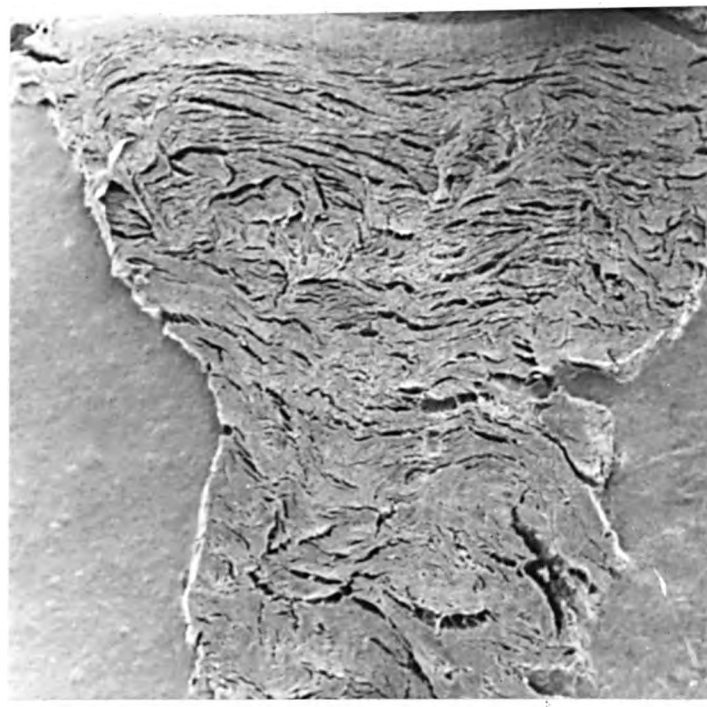


Fig.4.14._____, 250μm. S.E.M. Hypertrophic scar after two months pressure therapy, patient 1. Tight packing of fibre bundles.

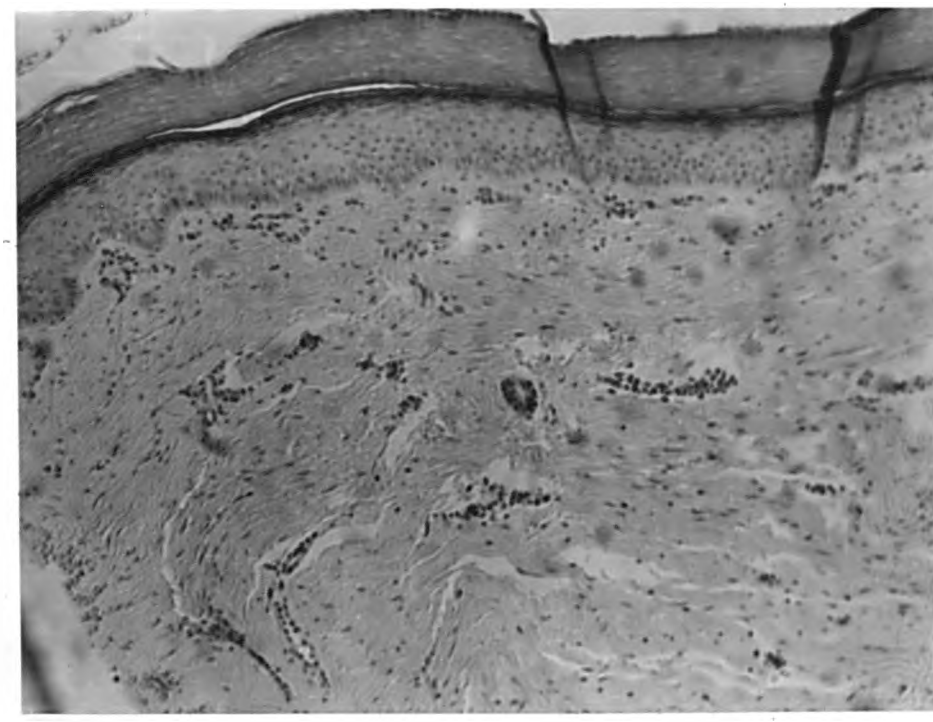


Fig.4.15._____, 100μm. L.M. of adjacent section to Fig.4.14. showing thick stratum corneum and large diameter blood vessels. (H&E).

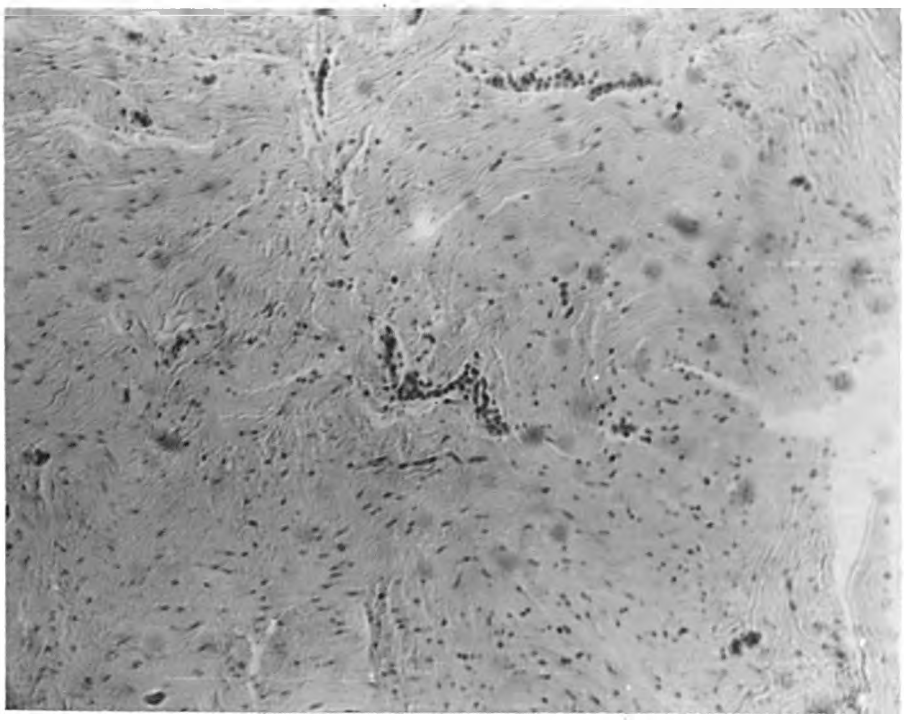


Fig.4.16. ——— 25μm. Magnification of dermis in Fig.4.15. (H&E).

Fig.4.17. ——— 250μm. S.E.M. Hypertrophic scar after 3 months pressure therapy, patient 2, showing nodular like arrangements in the papillary layer.

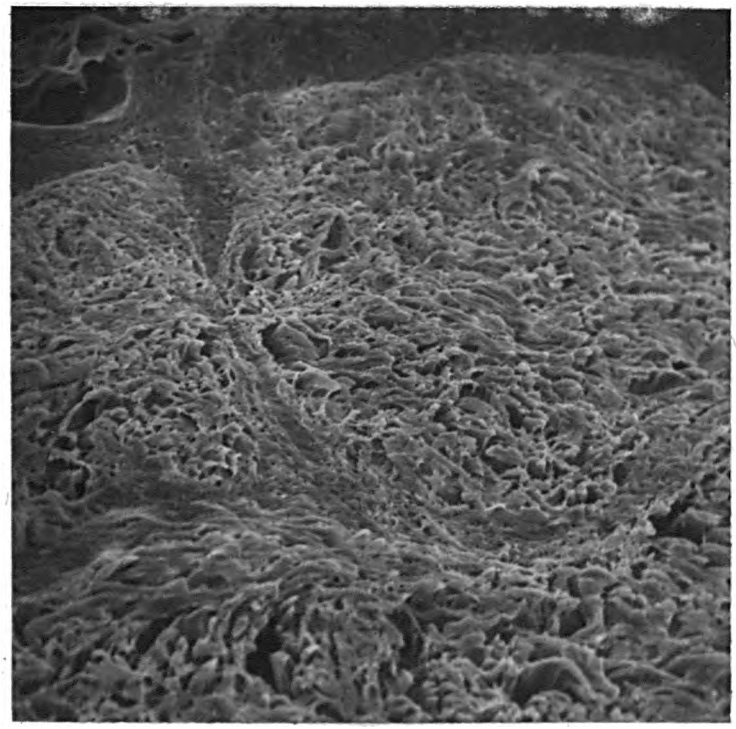


Fig.4.18. ⟵⟶ 50μm. Magnification of nodular-like region in Fig.4.17., showing possible blood vessels separating nodules.

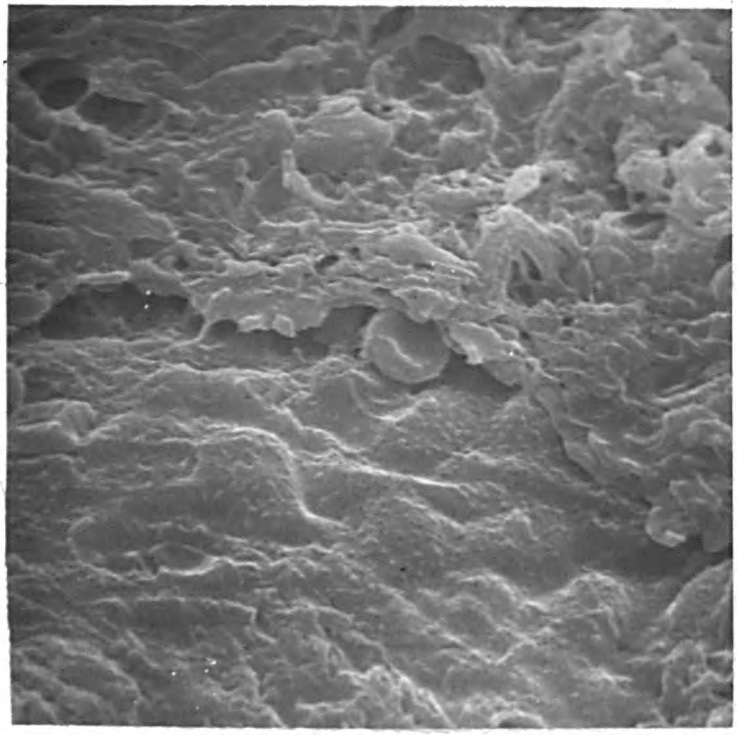


Fig.4.19. ⟵⟶ 5μm. Magnification of vessel in Fig.4.18. with red blood cell attached to wall.

fourteen months after injury.

Figs. 4.11. and 4.12. show S.E.M. micrographs of biopsies of normal skin taken from patients 1 and 2 respectively. The adjacent section to that shown in Fig. 4.11., stained with H & E is shown in Fig. 4.13. The fine network of fibres making up the papillary layer are visible in all three sections, as are the more coarse randomly arranged fibre bundles of the dermis. Fig. 4.11. also shows some fat cells of the hypo-dermis, and a section of a sebaceous gland is discernable in Fig. 4.12.

4.5.3. Early stages of pressure therapy. Fig. 4.14. is the S.E.M. micrograph from patient 1, two months after commencing pressure therapy. Figs. 4.15. and 4.16. are light micrographs from an adjacent section. Note the thick stratum corneum, slightly separated from the remaining epidermis. The fibres of the mid- and deep dermis are arranged in tightly packed bundles with a predominant orientation in the plane of the section. Large blood vessels (in the order of 13μm.diameter) can be seen in the clefts between the fibre bundles.

Micrographs of patient 2, three months after starting pressure therapy are shown in Figs. 4.17. - 4.19. (S.E.M.) and Fig. 4.20. (L.M.) Here the tissue has been sectioned at an angle to the preferential direction of orientation of the collagen fibres. Of particular interest are the nodular like arrangements in the papillary layer (Figs. 4.17., 4.18.). Using the S.E.M. it was not possible to identify these channels although occasionally red blood cells were found adherring to the walls (Fig. 4.19.). The sections stained for light microscopy, however, showed that these were blood vessels with diameters of up to 25μm.

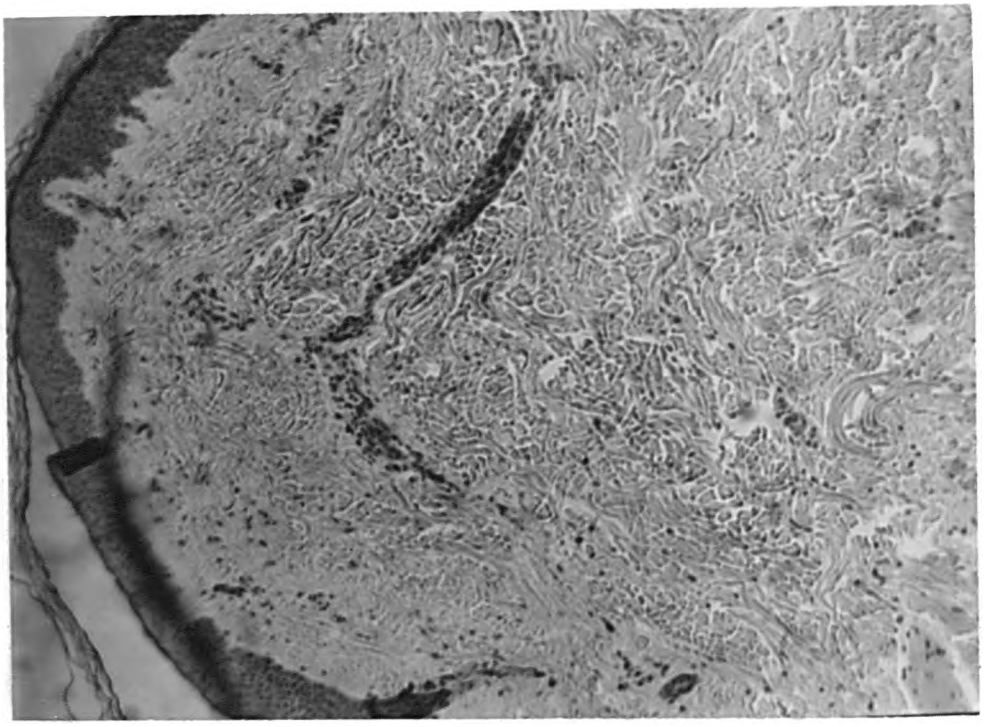


Fig.4.20. ——— 100μm. L.M. adjacent section to Fig.4.17., showing channels in superficial dermis are in fact large diameter blood vessels. (H&E).

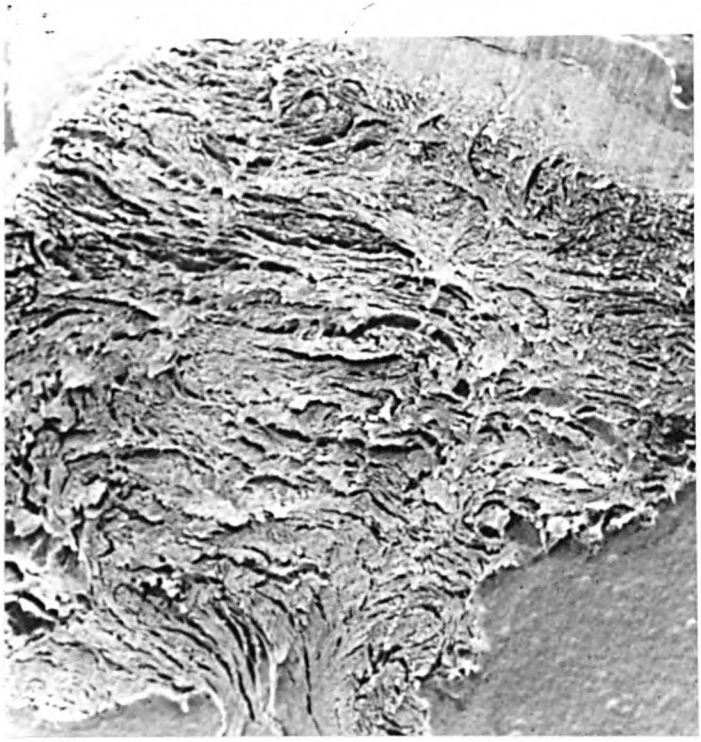


Fig.4.21. ——— 200μm. S.E.M. Hypertrophic scar after 8 months pressure therapy, patient 1. No significant change in dermal architecture.

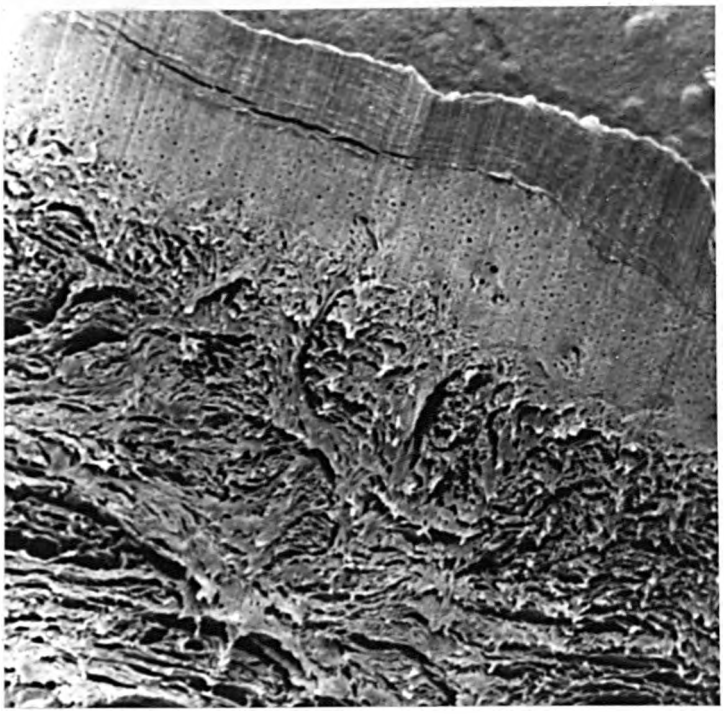


Fig.4.22. ⎵⎵⎵ 100μm. Magnification of epidermis and superficial dermis in Fig.4.21., showing large diameter blood vessels.

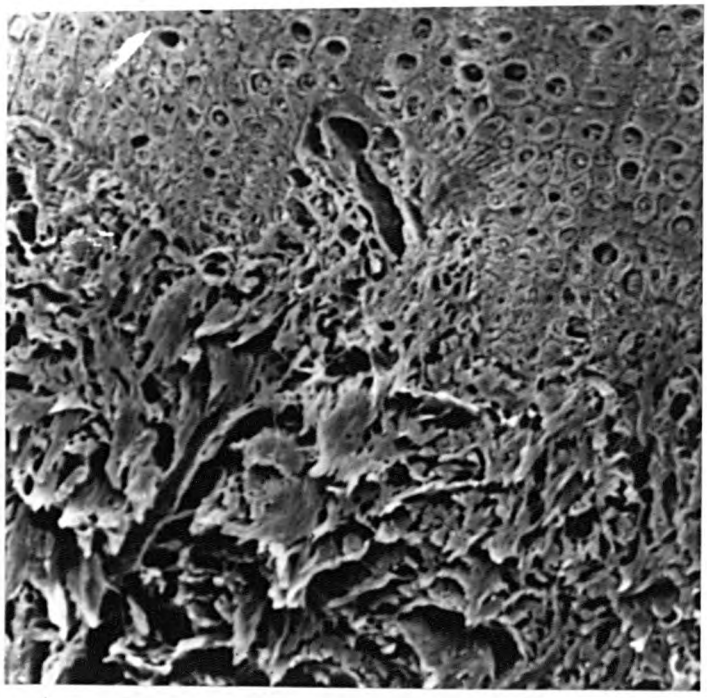


Fig.4.23. ⎵⎵⎵ 25μm. Blood vessel shown in Fig.4.21.

Fig.4.24. ——— 200μm. S.E.M. Hypertrophic scar after 8 months pressure therapy, patient 2. No significant change in dermal architecture.

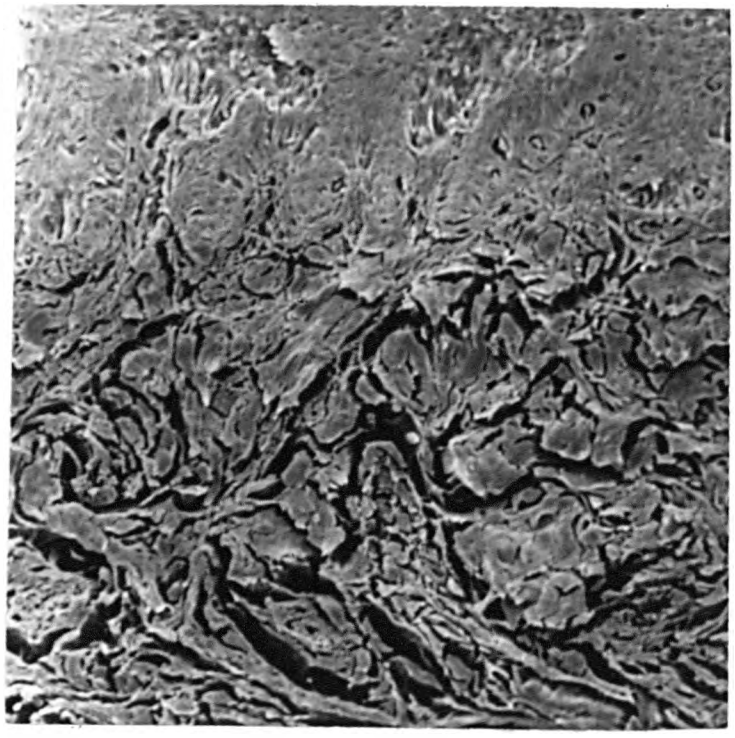


Fig.4.25. ——— 50μm. Magnification of epidermis and superficial dermis in Fig.4.24., showing similar blood vessel pattern as in Fig.4.22.

Although a large number of fibroblasts are still visible within the dermis they are less numerous than in the untreated scar tissue (Fig. 4.15. as compared with Fig. 4.9.).

4.5.4. Micro-architecture during intermediate/late stages of pressure therapy. Figs. 4.21. - 4.23. are micrographs of patient 1, eight months after starting pressure therapy. Figs. 4.24., 4.25. are micrographs of patient 2, again eight months after starting pressure therapy. No significant changes are visible in the dermal fibres micro-architecture, but both sections show large diameter (of the order of 20μm) blood vessels travelling towards the skin surface into dermal papillae where they change their direction to run parallel to the scar's surface. (Figs. 4.26., 4.27.). Similar blood vessels are also visible in the deep dermis (Fig. 4.28.).

4.5.5. Blood vessels. An extreme example of these large blood vessels is shown in Fig. 4.29., which is from a sixteen month old, untreated hypertrophic scar. The scar was clinically diagnosed as still being in the active phase with all the characteristic symptoms of tenderness and rubicundity. Fig. 4.30. is a magnification of the papillary layer of a section adjacent to that shown in the previous figure. The approximately 20μm diameter vessels are clearly visible travelling perpendicularly towards the epidermis where they then loop around to travel parallel to the dermo-epidermal junction for an unknown distance before presumably turning down into the dermis again. A micrograph taken at higher magnification of one of the vessels just beneath the epidermis is shown in Fig. 4.31. A section stained with AO & G to demonstrate the presence of elastin fibres shows a partial section of a blood vessel loop (Fig. 4.32.). (Because of the inexpertise of the author all sections stained with AO & G were over-stained so that it was not possible to positively

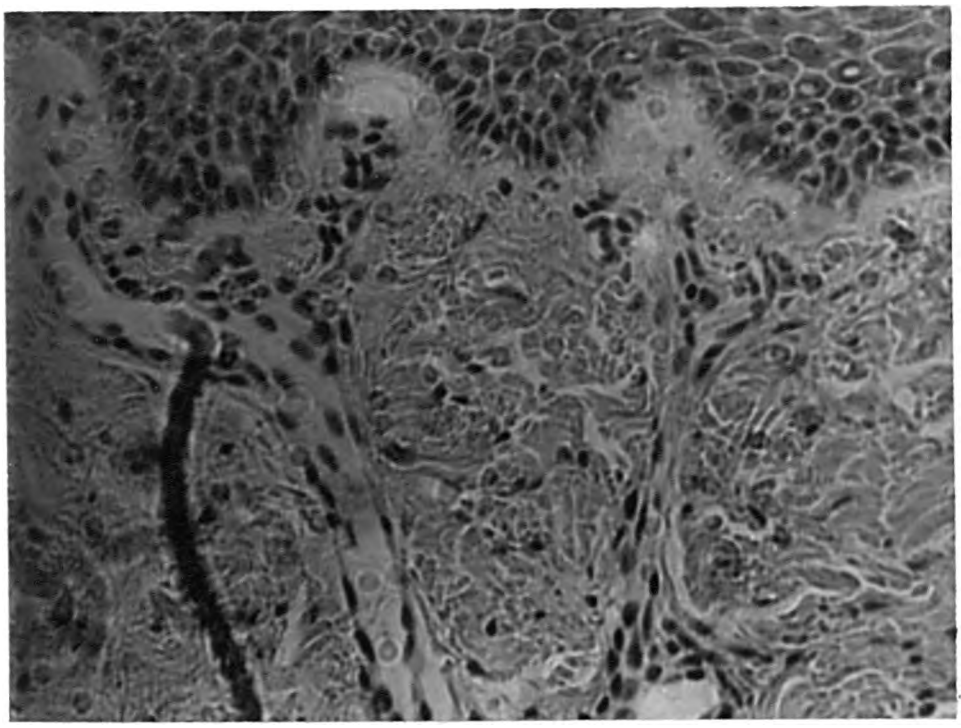


Fig.4.26. ——— 25µm. L.M. Blood vessels within papillary layer, section adjacent to Fig.4.21. (H&E).

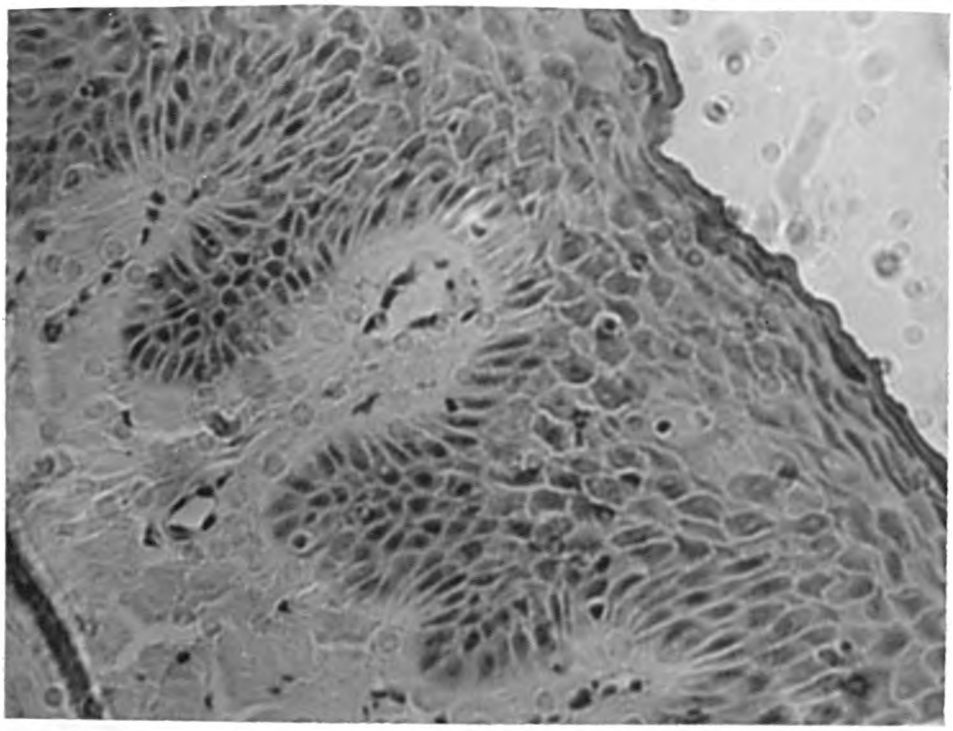


Fig.4.27. ——— 25µm. Similar blood vessels in distinct papillae within papillary layer, section adjacent to Fig.4.24. (H&E).

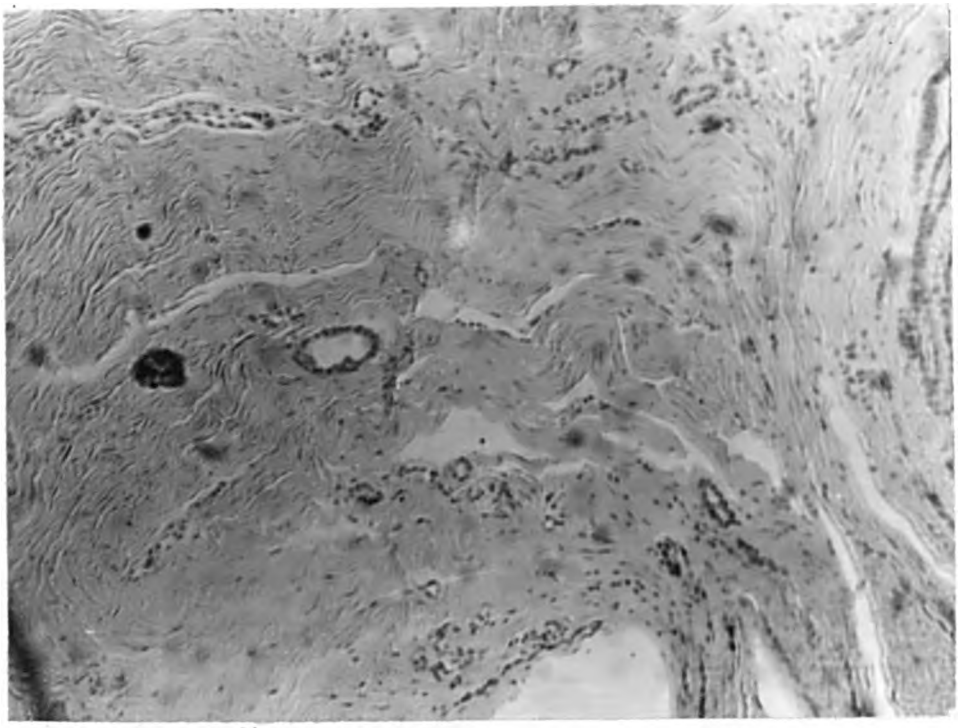


Fig.4.28. ⟵100μm. Dermis of 8 month pressure treated scar patient 1, Large diameter blood vessels also visible in the dermis. (H&E).

Fig.4.29. ⟵ 500μm. 16 month old untreated hypertrophic scar. (S.E.M).

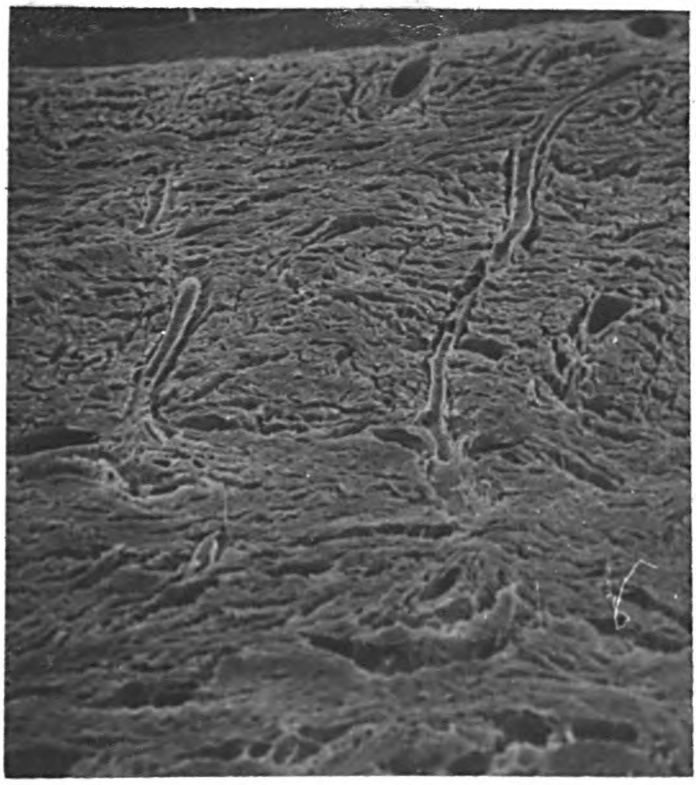


Fig.4.3o. ——— 100μm. Magnification of blood vessels in the papillary layer.

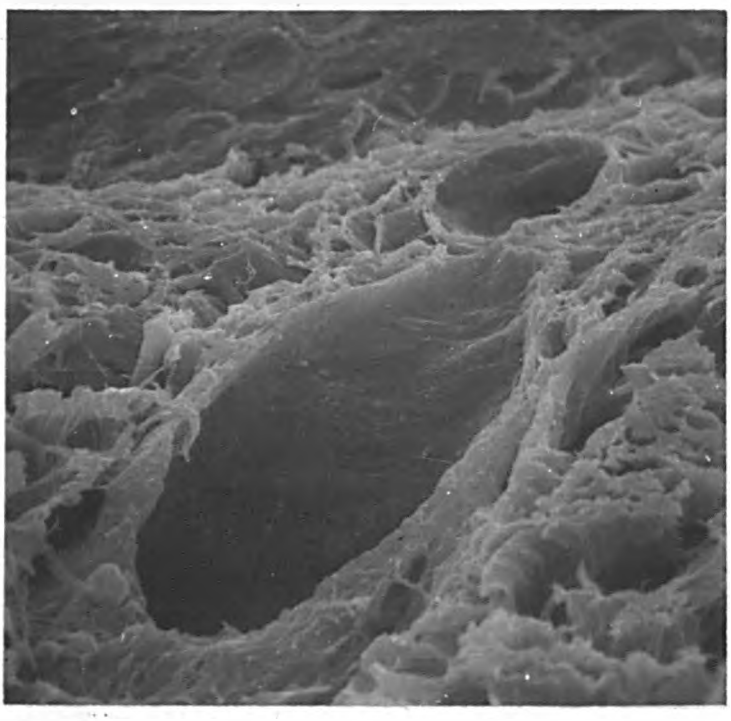


Fig.4.31. ——— 10μm. Blood vessels cut obliquely, just beneath dermo-epidermal junction.

identify elastin fibres).

The three-dimensional junctions between these blood vessels are shown in Figs. 4.33. and 4.34.

Although blood vessels were visible in the mid-, and deep-dermis they were neither so numerous or so well defined as those seen in the papillary or superficial layer. (Fig. 4.35.).

4.5.6. Micro-architecture post pressure therapy. As stated previously only one patient had successfully completed the course of pressure therapy during the limited study time period. This patient had hypertrophic scar tissue on both hands with associated webbing and contractures of the fingers. (Fig. 4.36.). After 24 months the scar tissue had remodelled to such an extent that the tissue was pliable and symptom free, and the contractures of the fingers were considerably relieved. (Fig. 4.37.). The patient has subsequently had no recurrence of the hypertrophic scar symptoms upon discontinuing the therapy.

The S.E.M. view of the successfully treated scar tissue is shown in Figs. 4.38. and 4.39. The large bundles of collagen fibres are no longer evident, in their place are smaller groups of fibres arranged in a less compact manner, similar to that seen in a normal mature scar.

There is no evidence of the profusion of large diameter blood vessels visible in the still "active" scars.

4.5.7. Micro-architecture of keloid scar tissue. Only one of the scars observed was diagnosed as being a true keloid. The patient suffered a minor burn of the deltoid region of the arm at the age of fifteen months. This healed spontaneously, leaving a scar approximately the size of one new penny. At the age of eight years the

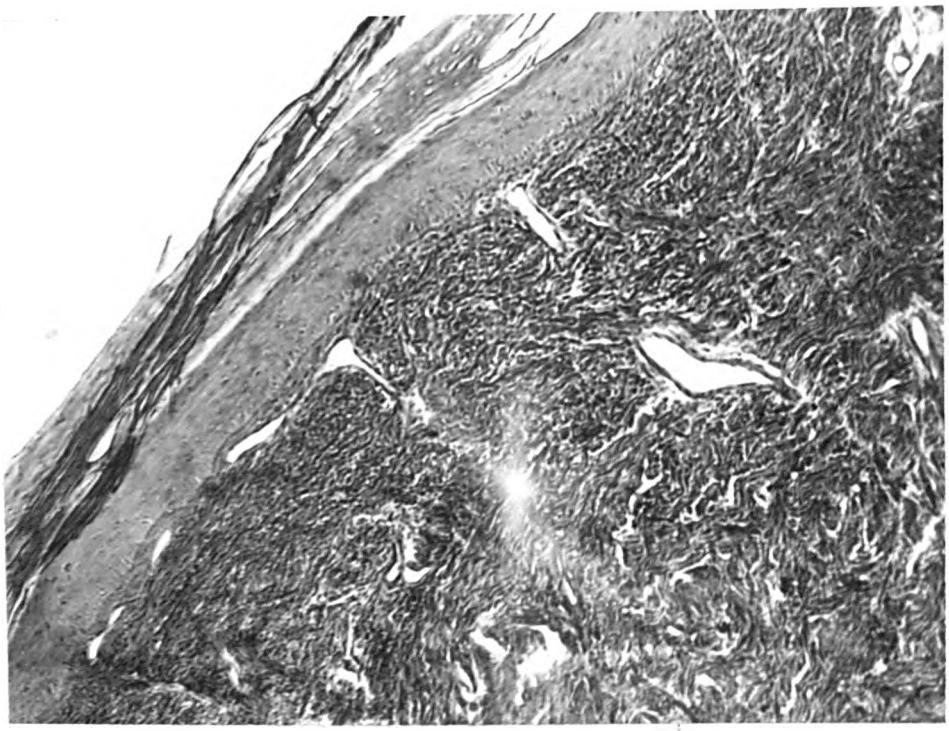


Fig.4.32. ——— 100µm. (L.M.) Partial section of blood vessel loops. (AO&G).

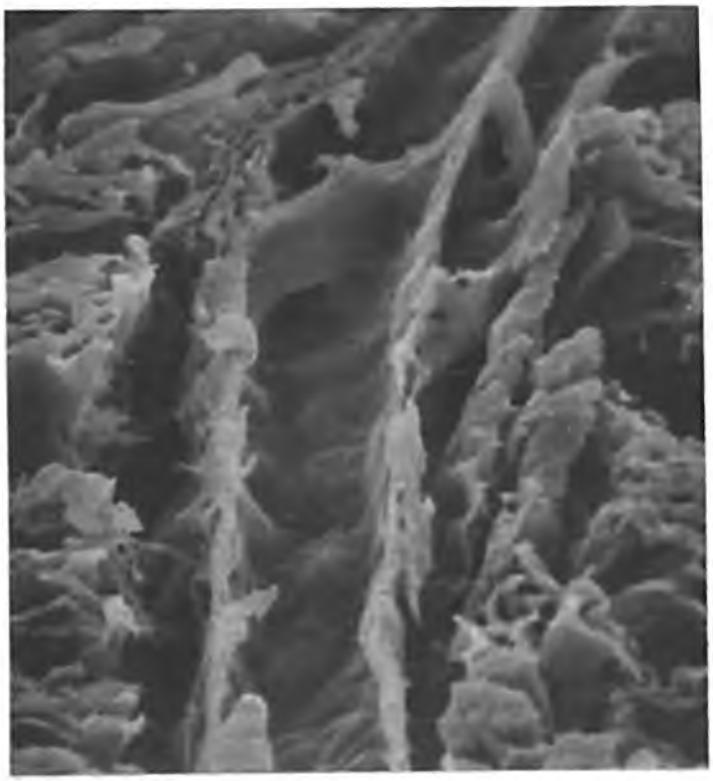


Fig.4.33. ——— 10µm. S.E.M. Branching of blood vessel.

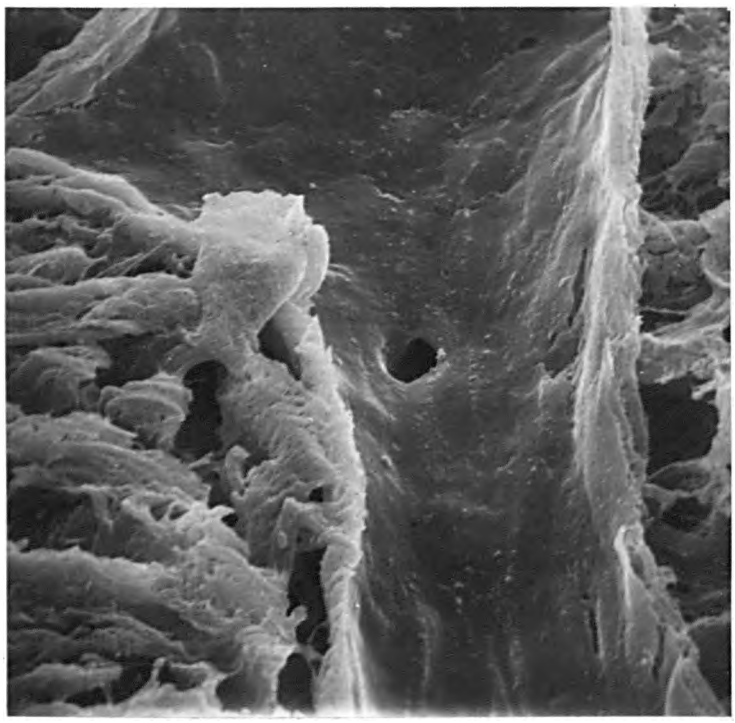


Fig.4.34.⟵⟶ 10μm. S.E.M. Three-dimensional branching of blood vessels.

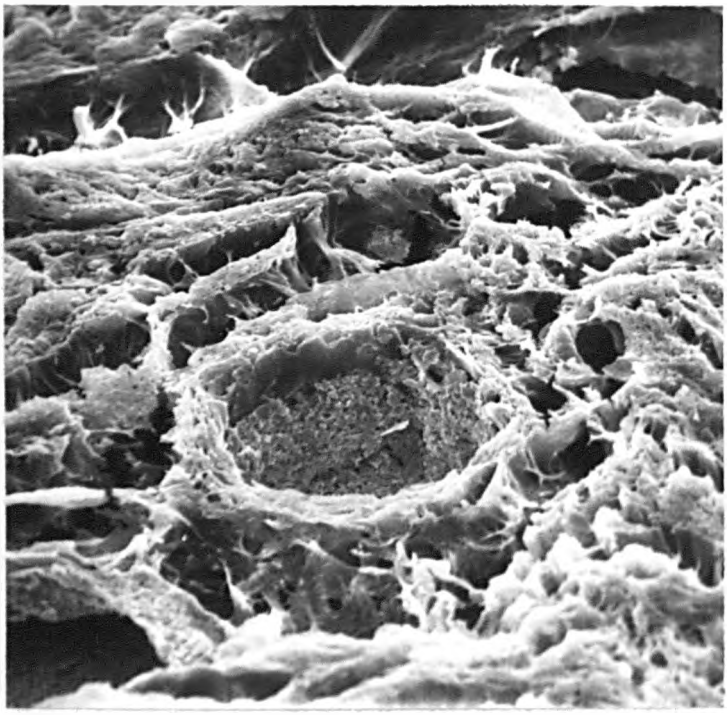


Fig.4.35.⟵⟶ 20μm. Blood vessel in the mid-dermis of section shown in Fig.4.29.

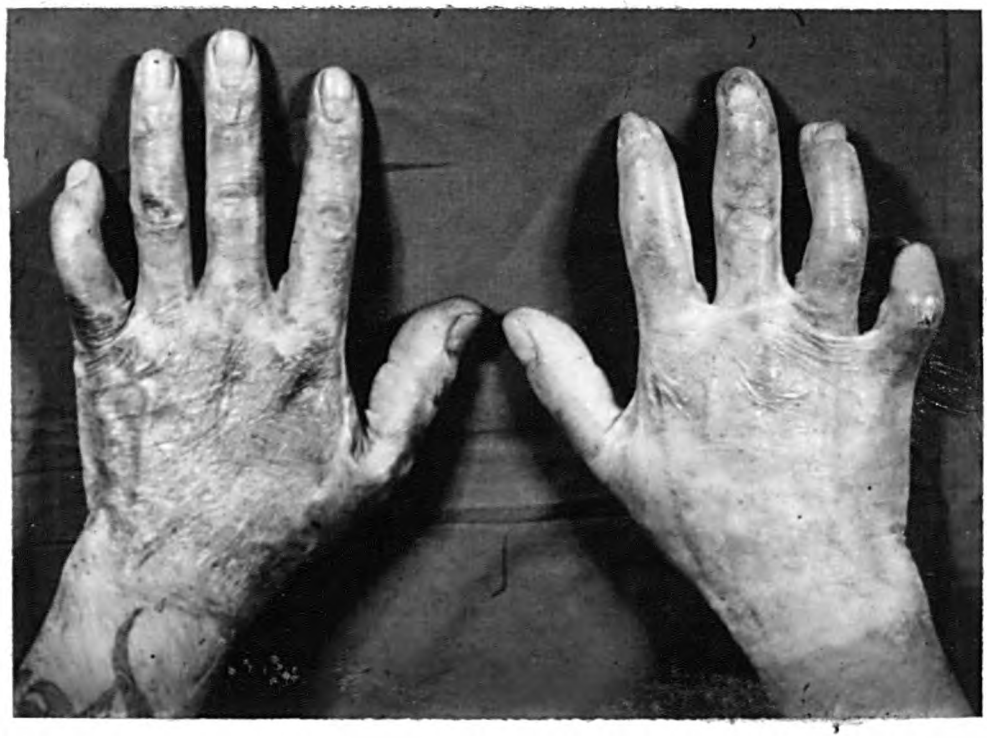


Fig.4.36. Hypertrophic scarring of both hands (untreated), scar tissue is inextensible and joint movement is severely limited by contracture webs between the fingers.

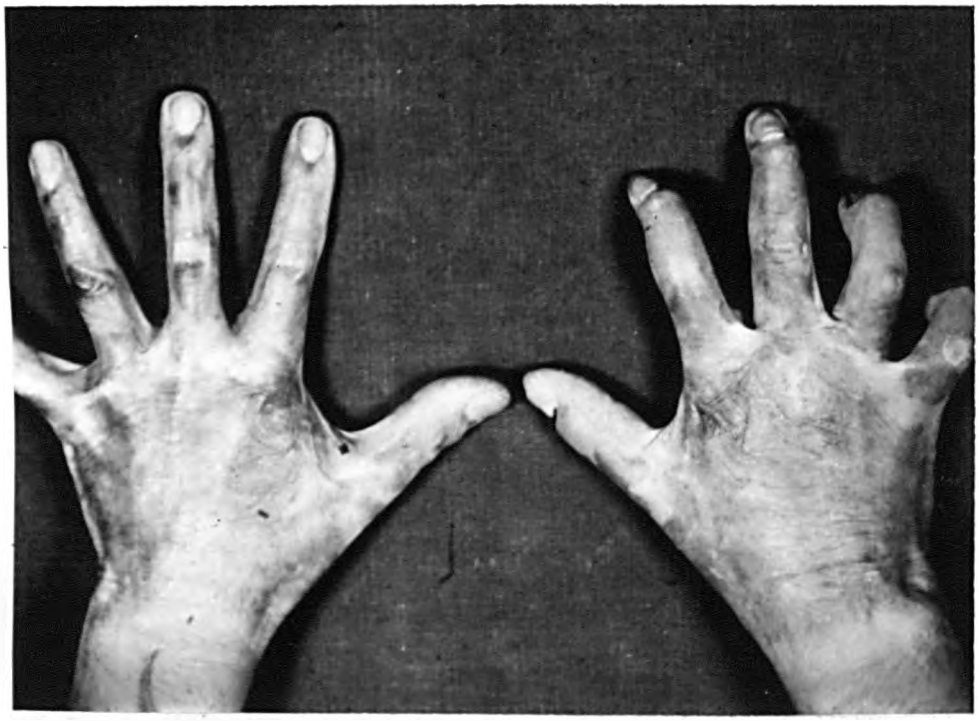


Fig.4.37. After 24 months of pressure therapy. Scar is pliable and stable, contracture webs have remodelled resulting in a considerable improvement in joint movement.

lesion was of such a size that it was excised, the resultant defect grafted and a course of Adcortyl cream prescribed.

The patient was first seen by the author four years after excision of the scar, during which time the scar had returned and increased in size until it was approximately 35 m.m. in diameter and 8 m.m. thick.

Only one biopsy, before commencement of treatment, was obtained from this scar, but the micrographs obtained have been included in this discussion since they show the only evidence of a foreign body (hair and keratinised material) reaction seen in the author's study of hypertrophic scar tissue. (See section 2.5.6.).

Figs. 4.40., 4.41. show a conglomeration of hair shafts and cellular material, buried beneath the epidermis of the scar. At least two hair fragments are clearly visible. (Fig. 4.41.).

The adjacent section viewed with the light microscope, unfortunately only showed a remnant of this abnormal formation (Figs. 4.42., 4.43.) with no hair shafts evident, but an accumulation of cells still visible.

The deep-dermis of the scar did not differ significantly from that of other, hypertrophic, scars.

4.6. Summary

A series of specimens of hypertrophic scar tissue were obtained from patients undergoing pressure therapy. Biopsies were taken initially using a purpose designed motor driven rotary biopsy punch, and later using a hand-driven commercial skin punch (Stiefel Laboratories (U.K.) Ltd., Slough.

Adjacent thick (25μm) and thin (8μm) sections were cut and prepared for viewing with an S.E.M. and a light microscope respectively.

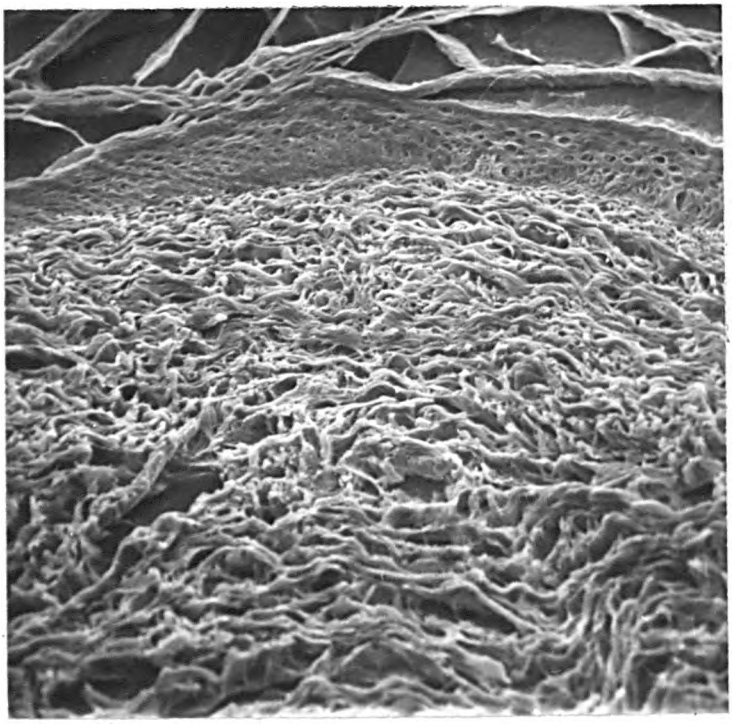


Fig.4.38. ——— 50μm. S.E.M. Hypertrophic scar post-pressure therapy. Epidermis and superficial dermis.

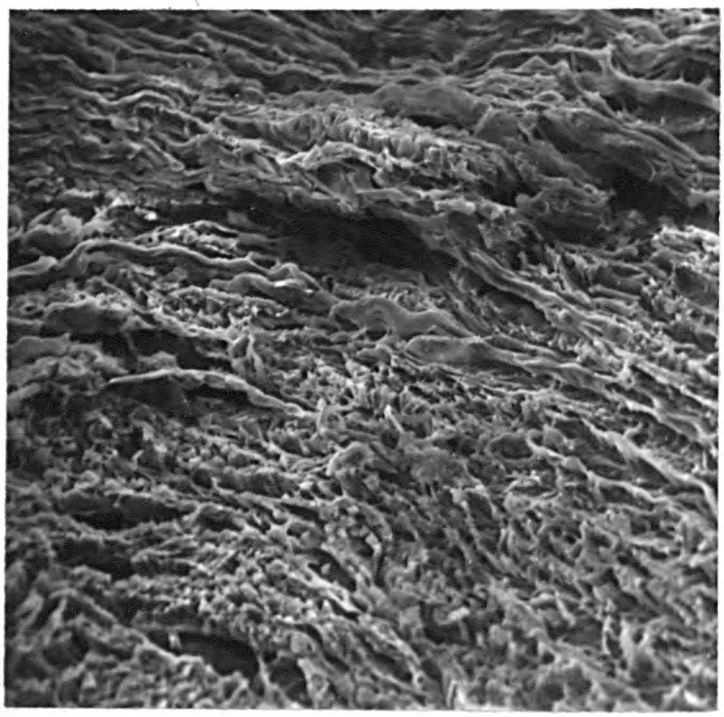


Fig.4.39. ——— 50μm. Mid-dermis of scar shown in Fig.4.38. Large fibre bundles characteristic of an 'active' hypertrophic scar have remodelled to give a micro-architecture similar to that of mature scar.

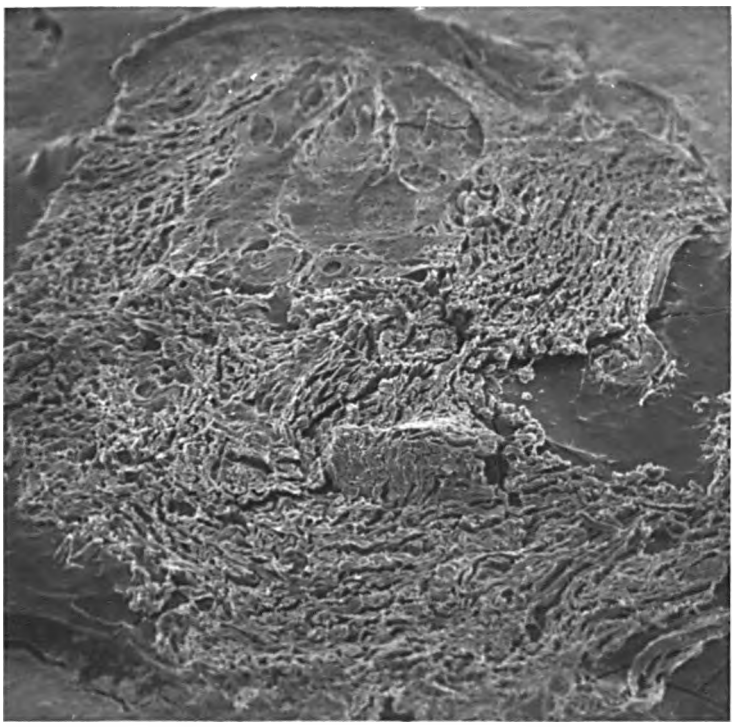


Fig.4.40. ——— 200μm. S.E.M. Keloid scar, with encapsulated hair fragments in the mid-dermis.

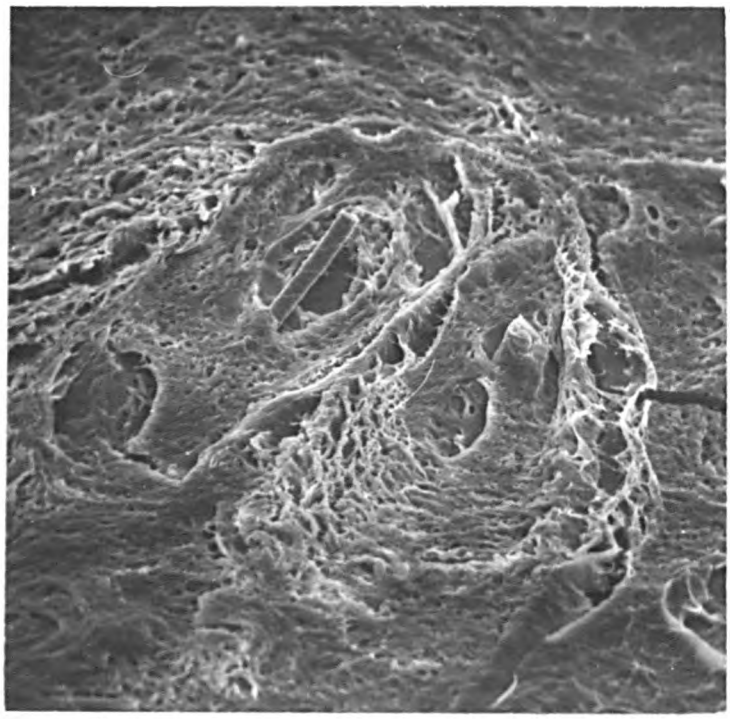


Fig.4.41. ——— 50μm. Magnification of region shown in Fig.4.40., showing hair remnants.

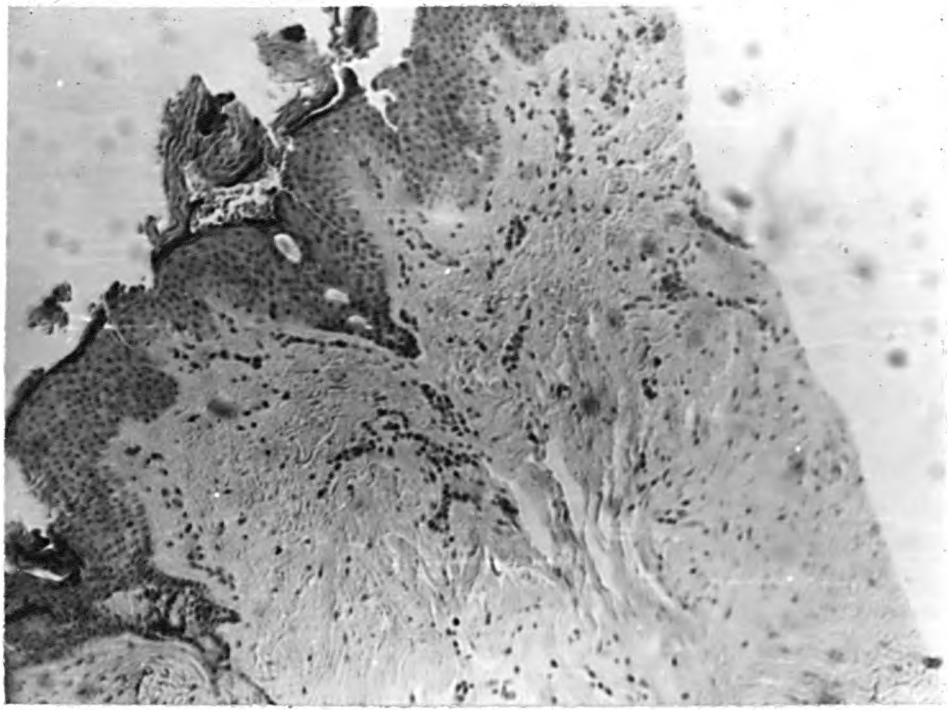


Fig.4.42. ——— 100µm. (LM). Adjacent section to that shown in Fig.4.40. Hair fragments are absent in this section, but accumulation of cells is still visible.

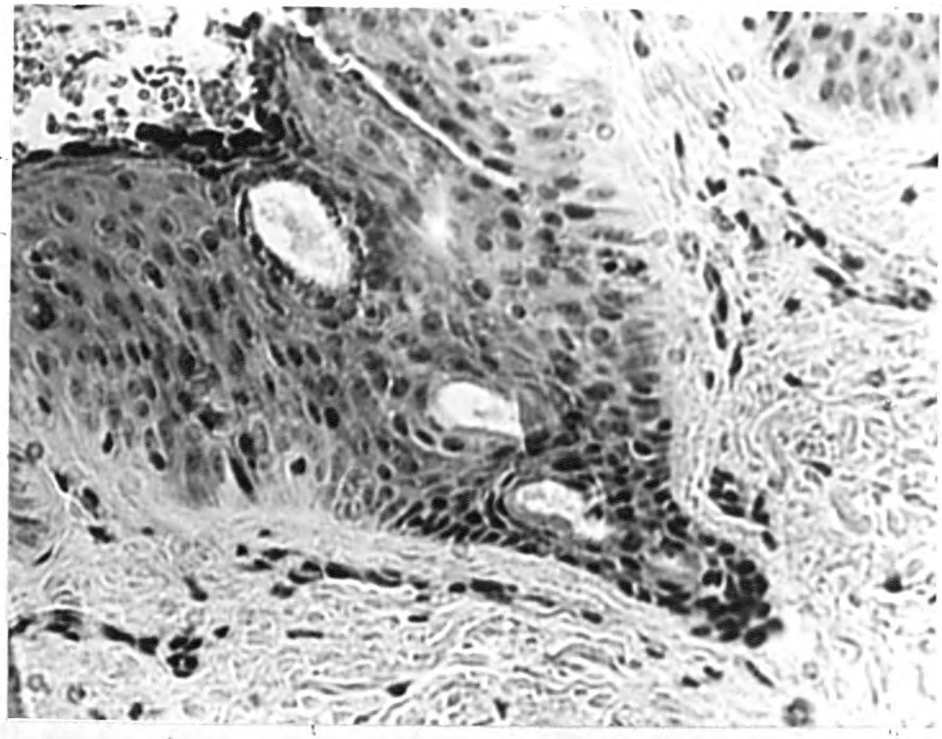


Fig.4.43. ——— 25µm. Magnification of region in Fig.4.42.

Sections of scar tissue taken before commencement of pressure therapy showed the friable epidermis and dense packing of the dermal collagen fibres indicative of active hypertrophic scar tissue. A far higher number of fibroblasts were visible in the scar tissue than in the normal skin.

Although there was no easily discernible change in the fibrous micro-architecture in the intermediate stages of the pressure therapy, the population of fibroblasts decreased.

Large diameter (in the order of 25μm) blood vessels were observed in the papillary and superficial dermal layers of the still active scars. These travelled perpendicularly either towards or away from the epidermis where they turned through 90^{o} to travel parallel to the dermo-epidermal junction for some unknown distance.

After a successful course of pressure therapy the fibrous micro-architecture was similar to that seen in normal mature scar tissue, and there was no further evidence of the presence of abnormally large blood vessels seen in the active scars.

CHAPTER 5

IN-VITRO MECHANICAL CHARACTERISTICS

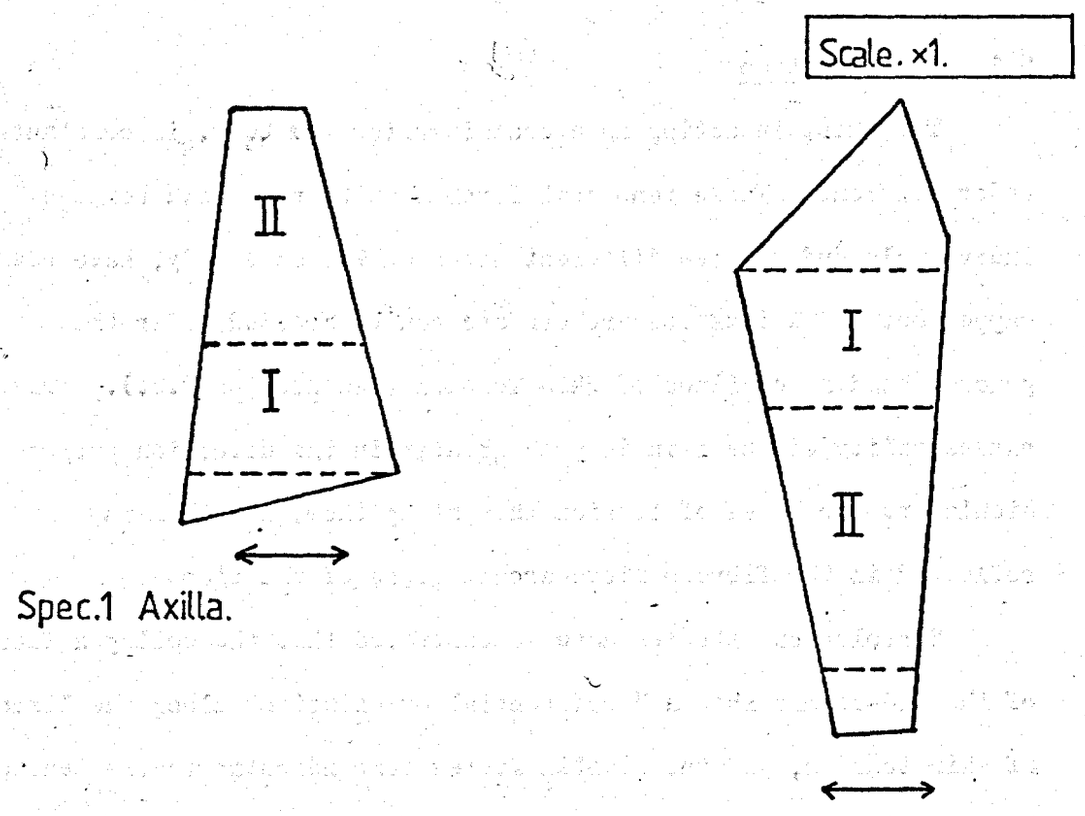
5.1. Introduction

The skin, in acting as a container for the body, is constantly under tension. These tensional forces, which vary both between individuals and between different sites on the same body, have been mapped out by various researchers and can be grouped under the general heading of Lines of Skin Tension (see section 2.6.). The extensibility of the skin is much greater in the direction perpendicular to the lines of tension than along them, and this is reflected in the fibrous micro-architecture of the tissue.

Histological studies have demonstrated that the collagen fibres of the mid-dermis show a "preferential orientation" along the lines of skin tension, and the elastin fibres perpendicular to the tension lines are arranged in loose coils in comparison with the more extended elastin fibres at right angles to these. (Flint, 1976).

The mechanical properties of the skin are dependent upon the physical properties of the extra-cellular materials and their peculiar micromorphology. Thus the extensibility of the skin is limited to that allowed by the deformation of the fibre network, which is in turn determined by the fibre configuration in the unstrained condition (Brown, 1973).

This interrelationship between mechanical properties and fibre configuration has been investigated by the author for hypertrophic scar tissue. Specimens of scar tissue have been subjected to known strains, at known strain rates, in order to obtain their stress/strain relationship; and then they have been chemically fixed whilst in the strained condition so that the response of the fibre configuration to the loads could be observed. The results of both the tensile tests and the subsequent histological observations are presented in this chapter.



Spec.1 Axilla.

Spec.2 Axilla.

Spec.3 Neck.

Spec.5 Neck.

Spec. 4 Axilla.

Fig.5.1 Scar specimen dimensions.(⟷ Contracture axis.)
(I, //el , II,⊥)

5.2. Specimen Preparation

It was decided that the minimum specimen area required to give meaningful measurements of the mechanical properties of the hypertrophic scar tissue was approximately a square, 3c.m. x 3c.m. This specimen area would have permitted two sets of tissue strips, at right angles to one another, to be prepared, such that a mean stress/strain curve with standard deviation measurements could be obtained for each scar specimen.

However areas of tissue of this size were not routinely excised unless the scar was causing considerable functional disablement to the patient. Therefore those scars that were excised tended to lie in the region of a joint, and to consist of a contracture web of tissue that could not have been effectively re-modelled by the surgical technique of performing a z-plasty.

5.2.1. Specimen excision. Five specimens of hypertrophic scar tissue of a suitable size were obtained. These varied in shape depending upon the original body site of the scar, and the severity of the contracture. Scale diagrams of the specimens are shown in Fig. 5.1. As with the specimens obtained for routine scanning electron and light microscopy, detailed records were kept of both the patients' relevant past case history and the specimen preparation protocol used.

When observing the mechanical properties of a viscoelastic material, the underlying requirement of the preparation procedure is that the material be handled in a non-traumatic standardised manner. The precise "state of strain" in a tissue is unknown as loading histories cannot be determined, but the majority of tissues exist in a state of pre-strain. It is essential to the study of

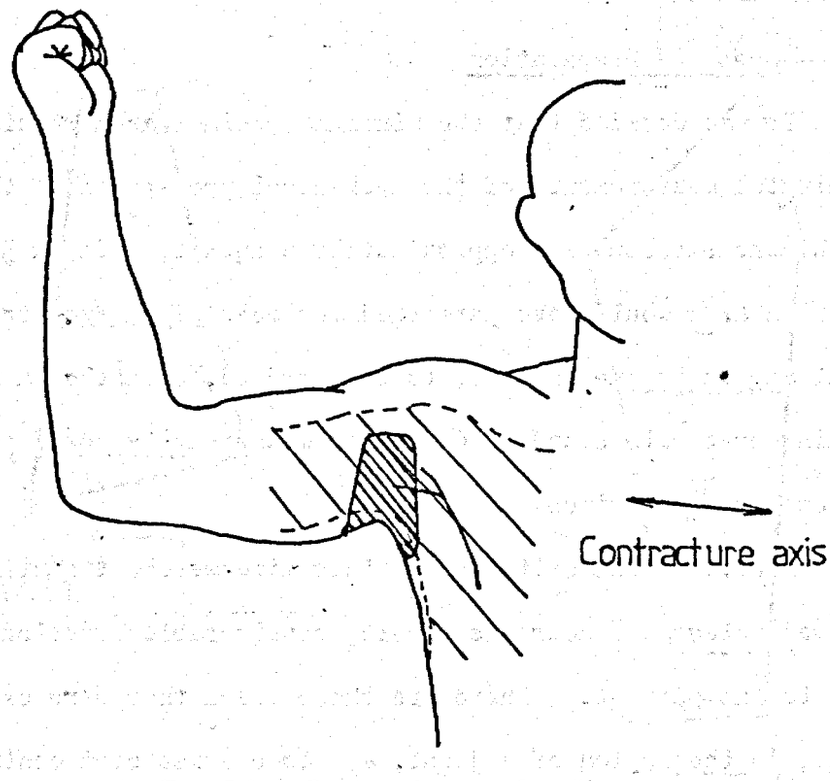


Fig 5.2a Contracture of axilla

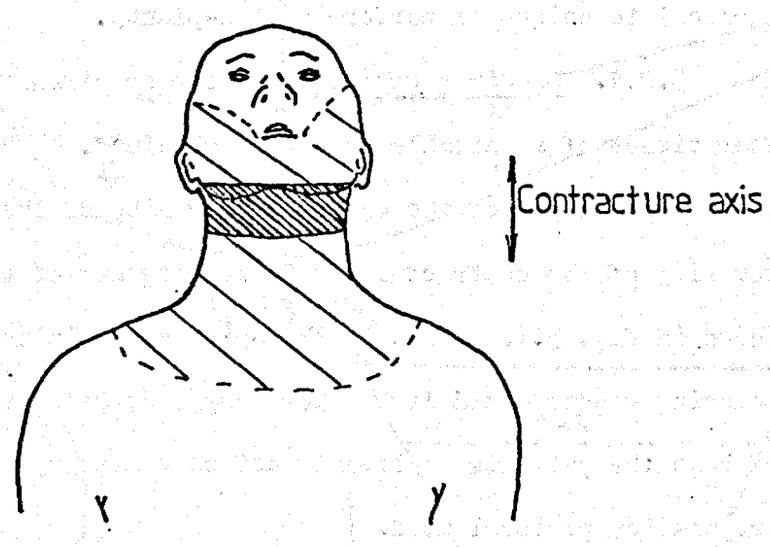


Fig 5.2b Contracture of neck

Possible area of scar tissue involved in contracture.

Site of excision

Fig 5.2 Excised specimen sites.

mechanical function that some measure of pre-strain be obtained.

The instantaneous value of pre-strain is dependent on body position. This is normally related to the "neutral" position where the body is prone with the anterior surface uppermost. This body position serves to define the resting state in skin.

Of the five specimens obtained, three were from the axilla and two from the front of the neck (Fig. 5.2.). Because of these body sites it was impossible for the scars to be excised whilst the patient was in the "neutral" position. Instead the neck, or arm of the patient had to be extended as far as the rigidity of the scar tissue would allow to permit access to the area of interest.

To measure the amount of pre-strain imposed on the scar by this positioning, before the area was excised a grid system of points (in a 1c.m. separation lattice) were tattooed on the scar surface using a flexible stencil and a sterile hypodermic needle which had been dipped in a sterile dye. The specimen was then excised and placed epidermis uppermost on a piece of porous cardboard and the distance between the tattooed points measured.

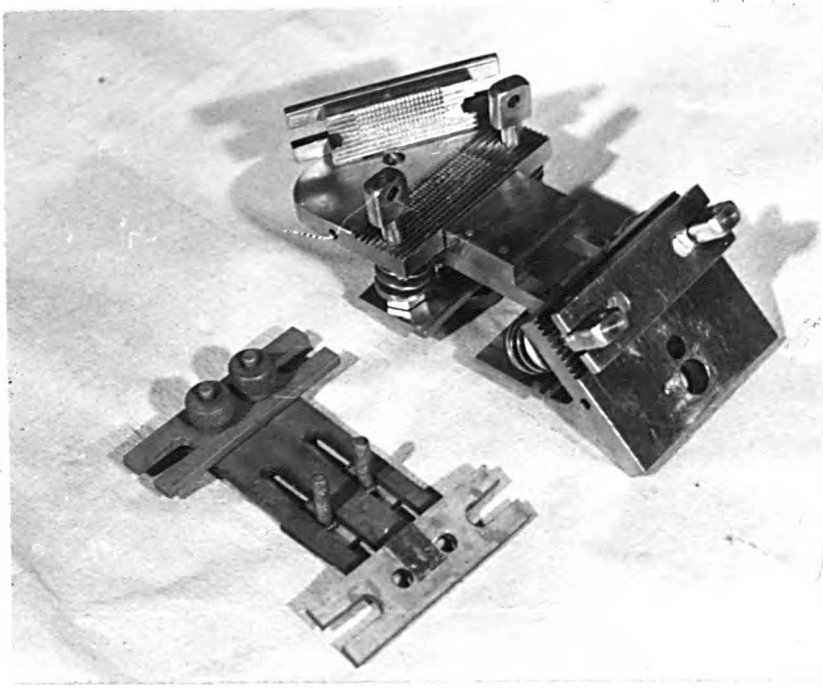
No appreciable contraction of the scar specimens was measured. This was felt to be due to the high degree of rigidity of the tissue and to the fact that where possible the contracture web had been excised intact with a surrounding border of either normal skin, or "normal" mature scar tissue.

The defect left after excision of the scar was larger than the area of tissue removed since the wound edges were able to retract upon the removal of the tension caused by the contracture band, and since the head/arm could be moved throughout its normal range. The wound was then resurfaced with an auto-graft.

Immediately after the measurements to determine whether the tissue had been under a pre-strain the specimen was covered with a moist swab and placed, still attached to the cardboard which prevented the specimen from bending, in a plastic bag. All the specimens were placed in a deep-freeze within one hour from the moment of excision, and stored at approximately -20°C until required for testing. (Maximum period of storage was one month. This method of storage had long been established within the Bioengineering Unit, and was felt not to give rise to any adverse artifacts within the tissue.)

5.2.2. Specimen cutting and mounting. Specimen dimensions of width 3m.m. and gauge length 10m.m. were chosen to allow the maximum number of strips to be obtained from each scar tissue specimen, whilst still giving meaningful results. The ends of the tissue strips were not flared out to give the maximum area for gripping since this would have severely cut down the number of specimens.

Two sets of specimens were prepared from each scar sample: one set (Set I) orientated in a direction parallel to the contracture band, and the other set (Set II) at right angles to this. The presence of a contracture band passing through the scar sample did mean that the thickness (epidermis to hypodermis) of the strips varied both between individual strips, and over the gauge length of those specimens prepared at right angles to the contracture band. This disadvantage, however, could not be avoided and since the object of the tests was not to absolutely quantify the stress/strain relationship for the hypertrophic scar tissue but rather to observe the inter-relationship between the hypo-extensibility of the tissue and its fibrous micro-architecture the observations for those strips



Jig I. Jig II.

Fig.5.3. Gripping apparatus. Jig I used to maintain initial and final gauge lengths. Jig II used to grip the specimen during the mechanical tests.

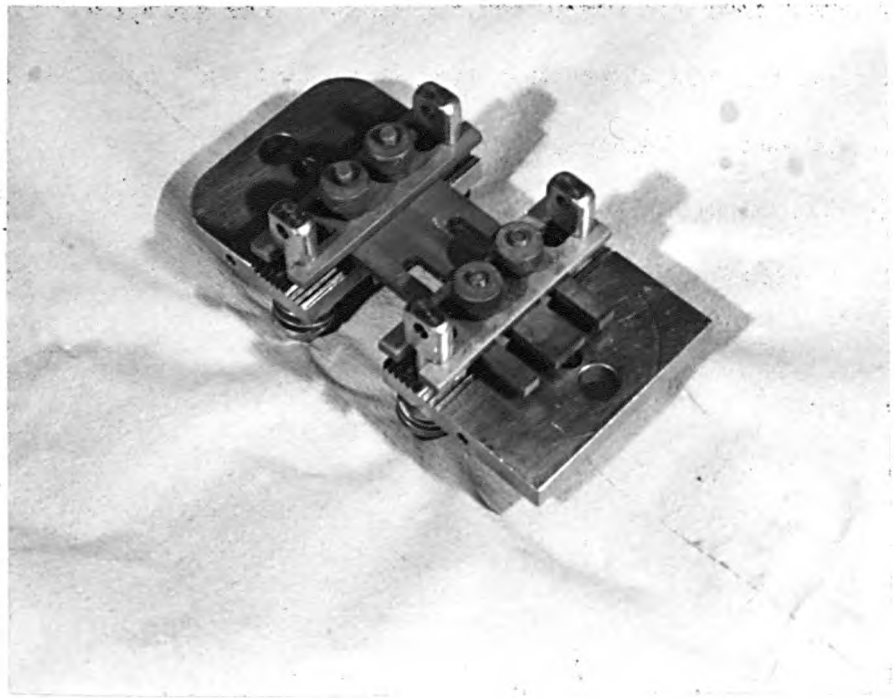


Fig.5.4. Jigs I and II assembled together as for a test.

of varying thickness are included in the results.

The number of tissue strips in each group was determined by the specimen's shape and dimensions (Fig. 5.1.). Set II were cut first, and their number was chosen to leave sufficient length of tissue for Set I to be cut. The tissue strips were cut all together using a set of dermatome blades mounted in a simple jig which kept the blades parallel to one another and separated by the chosen gauge width of 3m.m.

The scar specimens were removed from the deep-freeze and allowed to thaw at room temperature, being kept moist continuously. As soon as the tissue was soft enough to cut (i.e. before it had fully thawed and whilst it was still rigid enough not to distort under its own weight) the tissue strips were excised and then allowed to thaw out completely.

In general, connective tissues are difficult to grip and rarely can a slip-free attachment be obtained. The gripping of the hypertrophic scar tissue was made even more difficult by the thickness of the specimens. (Ranging from 3m.m. to 9m.m.). Purpose-designed grips have been evolved over the years in the Bioengineering Unit, and these had to be further modified to accept the scar tissue.

The grips form part of a test system designed to maintain the dimensions of the skin specimen both prior to experimental loading (i.e. in its unstressed condition), and after the loading programme has been completed (i.e. whilst the specimen is in a stressed condition.) The complete gripping apparatus consists basically of two separate jigs. (Figs. 5.3. and 5.4.).

The first jig (jig I), which is in direct contact with the tissue is used initially to grip the specimen and maintain its

dimensions, including the correct gauge length, prior to loading. Once the tissue has been mounted the complete assembly is placed onto the second jig (jig II) whose purpose it is to grip the specimen during the test. After the loading programme the first jig is then again used to grip and maintain the specimen in its stressed condition.

Pieces of extremely coarse, waterproof sand paper were glued to the inner faces of the grips of jig I to provide extra cohesion for the tissue.

Surplus pieces of scar tissue were used in trials to determine the best way to grip the specimens. Because of their high thickness to width ratio the samples tended to twist over as soon as pressure was applied to the grips. To prevent this distortion and to give enough rigidity to the tissue so that it could be easily handled, each tissue strip was immersed in liquid Nitrogen immediately prior to being mounted. The frozen sample could then be correctly positioned on jig I, and pressure could be applied gradually to the grips by tightening the knurled screws as the tissue thawed out. In this way the majority of the samples were firmly gripped with minimum twisting of the gauge length.

Jig I, complete with mounted scar specimen was then placed onto the second jig. The free grips of jig II were located over the clamping studs, and the normal force supplied by the helical springs (see Figs. 5.3. and 5.4.) transferred to them by rotating the knurled precompressing screws. The complete assembly was then mounted in the Instron test machine. (Instron Ltd., High Wycombe.)

Once mounted on the test machine the lower grips of jig I could be released to allow the specimen to extend with the applied load. (The grips of jig II now supplying the necessary pressure to hold

the specimen firmly.)

5.3. Strain Measurements

Strain measurements were taken using a non-contracting extensometric technique. Prior to mounting a grid was drawn on the specimen's epidermis, using a blunted scalpel blade and waterproof printers ink. Sequential photographs of the mid region of the specimen were taken during the test at known loads, and an accurate measure of strain could then be obtained from an analysis of the photographic negatives.

5.4. Mechanical Testing Protocol

Specimens were tested whilst being immersed in isotonic Ringer's solution at a temperature of $37^{\circ} \pm 1^{\circ}C$. An environment chamber attached to the Instron contained the solution. The chamber had plate glass sides through which the optical strain measurements could be made, and a storage tank in which the liquid could be heated. A pump in the tank enabled the chamber to be filled, and provided the circulation necessary to maintain the working temperature.

5.4.1. Loading and straining sequences. A constant strain rate of 0.3c.m. per minute was used throughout. (This gave an extension rate of approximately 30% per minute.)

Skin is a highly viscoelastic material and as such it possesses a memory. Deformations imposed on the tissue at the time of excision or subsequently, will influence the results of any mechanical tests. To attempt to standardise this memory of any prior loading, the specimens of scar tissue were "preconditioned" by repetitively loading and unloading the sample. Generally with repetitive loading the extension produced by a given load increases with each cycle

until an essentially stable characteristic is obtained - at which point the tissue is said to be "preconditioned."

Each scar specimen was cycled up to the maximum load of 1 Kg., three times before the final stress/strain characteristic was measured. (The maximum load of 1 Kg. resulting in a specimen extension well into the third phase of the tissue's stress/strain characteristic.)

Since the scar tissue micro-architecture was to be observed whilst it was in the strained condition, the Instron cross-heads were stopped after the fourth and final loading and the strain maintained. The scar tissue exhibited a degree of stress relaxation which was quite pronounced initially, but which decreased with time. Each specimen was allowed to relax until the stress reached a plateau, at which point the grips of jig I were re-attached to the lower end of the specimen, thus preventing it from contracting on removal from the Instron.

Jig II was then removed, and the specimen still maintained in its strained condition by jig I was immersed in a bath of fixative (10% formol saline). The jig was released when the tissue was sufficiently fixed not to contract on removal of the mechanical support (after a time period of approximately four hours.)

5.5. S.E.M. Observation

When the strained scar tissue strips were fully fixed sections were prepared for observation with an S.E.M. Three sections were prepared from each piece of scar tissue. The first section was cut transversely to expose the full thickness of the scar tissue in the direction parallel to the strain axis. The remaining two sections were cut horizontally to expose the mid-, and deep-dermis of the

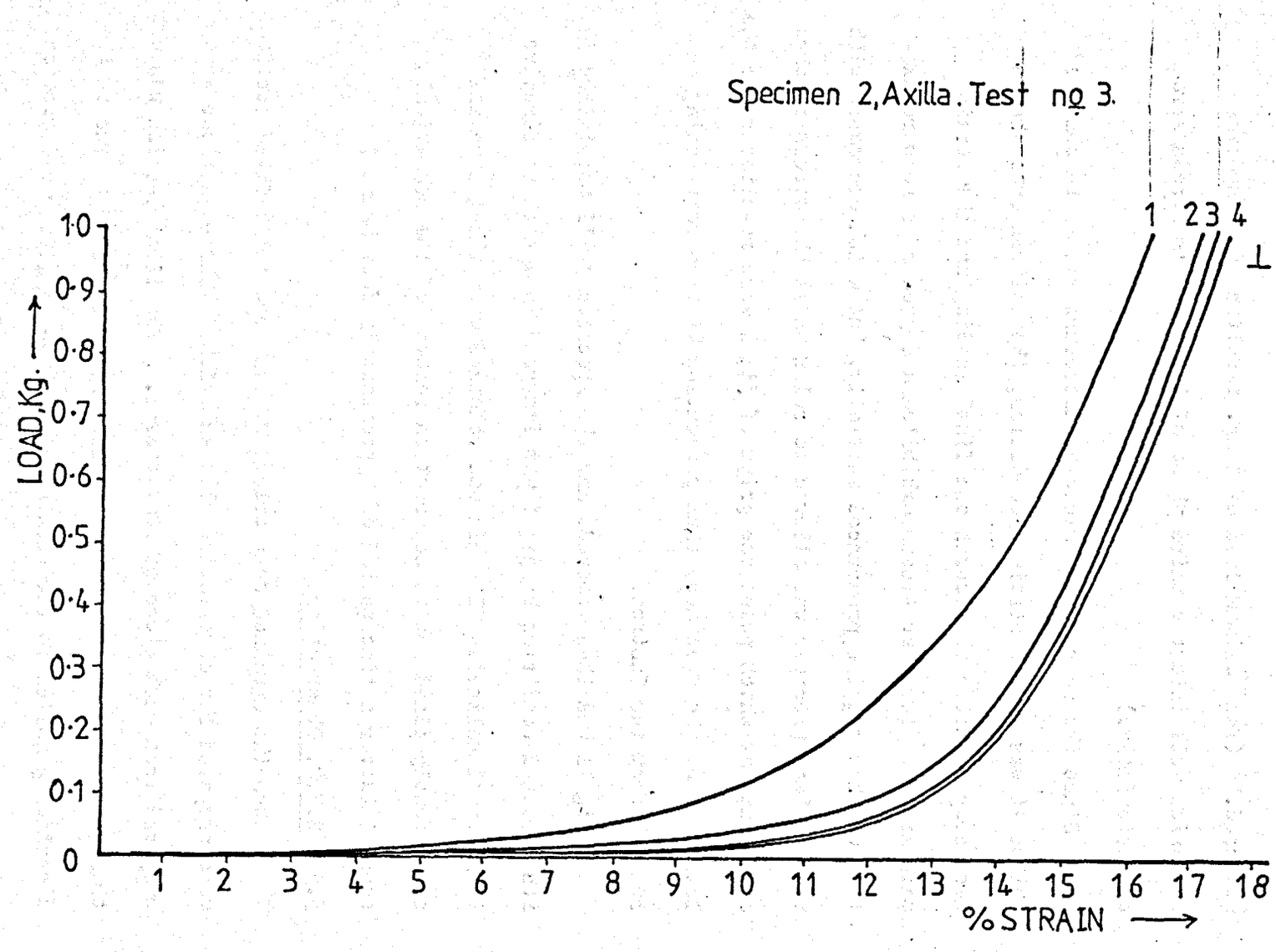


Fig. 5.5. Pre-conditioning of speciman prior to testing.

scar, respectively. All sections were cut using a hand held dermatome blade.

Un-strained sections of the scar tissue were also prepared for micro-structural analysis.

After cutting the specimens were prepared for observation with the S.E.M. using the tissue preparation protocol outlined in Chapter 3, sections 3.1.4 - 5.

5.6. Mechanical Results

Five specimens of hypertrophic scar tissue were obtained from patients undergoing surgery for the correction of a contracture deformity - three specimens were from the axilla and two from the front of the neck. (Fig. 5.2.)

Each piece of tissue was divided into two sections, and strips, of a constant width of 3m.m., were cut from each section. One group orientated parallel to the main contracture axis and the other group at right angles to this (Fig. 5.1.).

The specimens were then subjected to a programme of straining cycles, at a constant strain rate of 30% extension per minute on an Instron test machine (Instron Ltd., High Wycombe, England). On completion of the programme the specimens were maintained in their final maximum strained condition using a special jig, whilst they were being chemically fixed.

Sections of the strained scar tissue were then prepared for observation with the scanning electron microscope.

5.6.1. Pre-conditioning. The load-strain graph from a typical pre-conditioning loading cycle is shown in Fig. 5.5.

The load-strain characteristics of the first loading cycle display greater initial stiffness and a less distinct elbow than

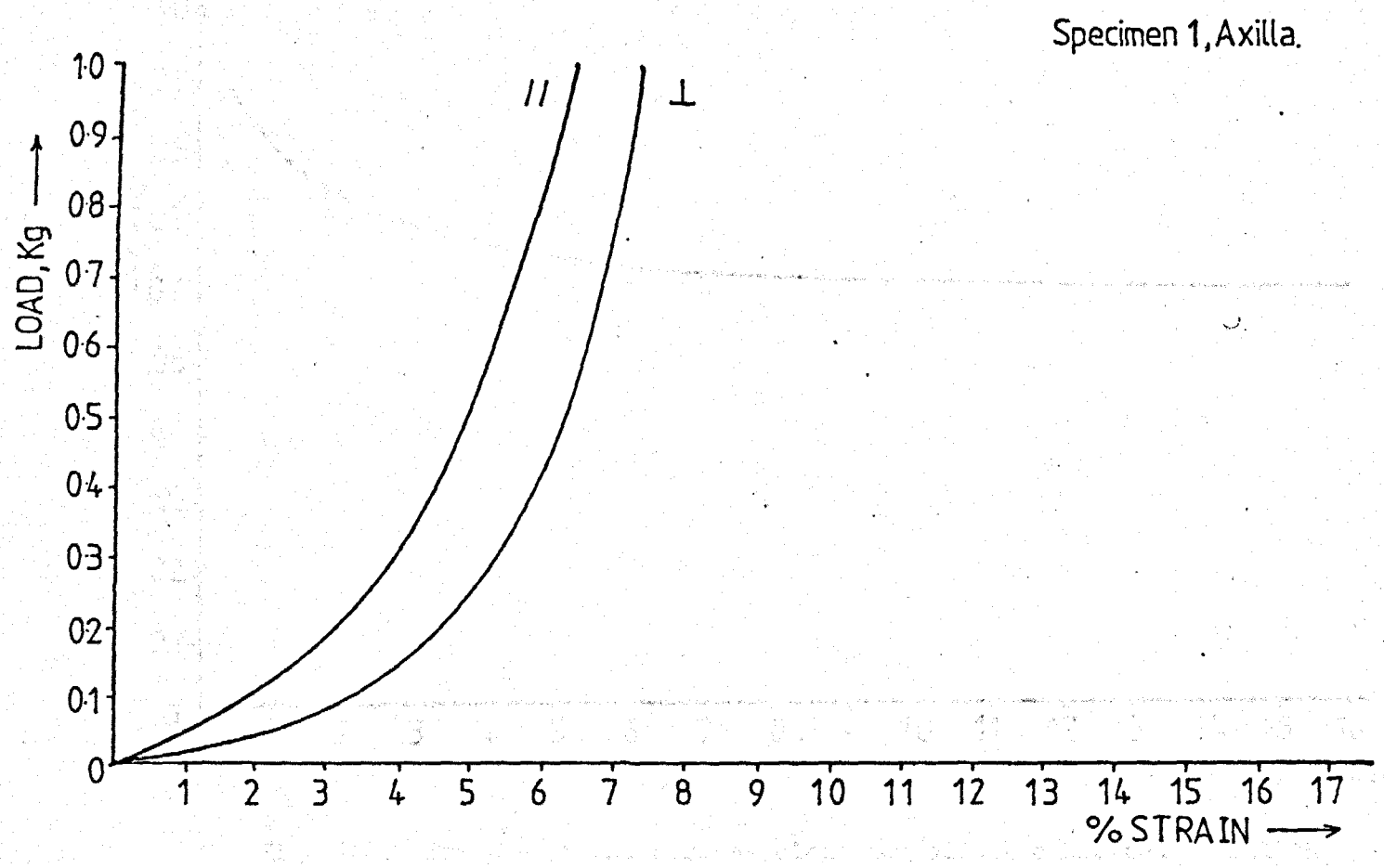


Fig. 5.7. In-vitro mechanical characteristics, 5 year old hypertrophic scar, parallel (//) and perpendicular (⊥) to the contracture axis.

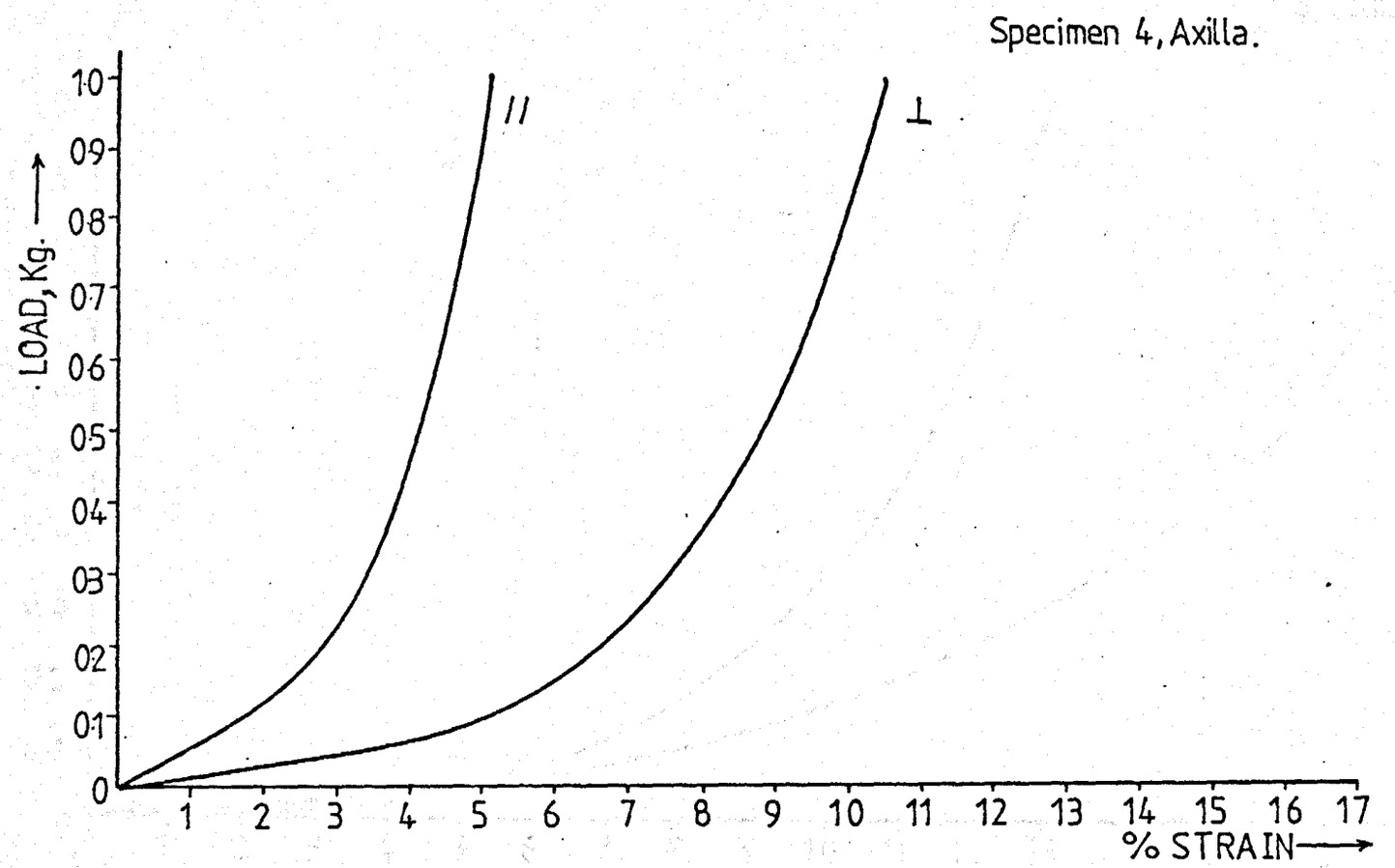


Fig. 5.9. In-vitro mechanical characteristics, 3 year old hypertrophic scar parallel (//) and perpendicular (⊥) to the contracture axis.

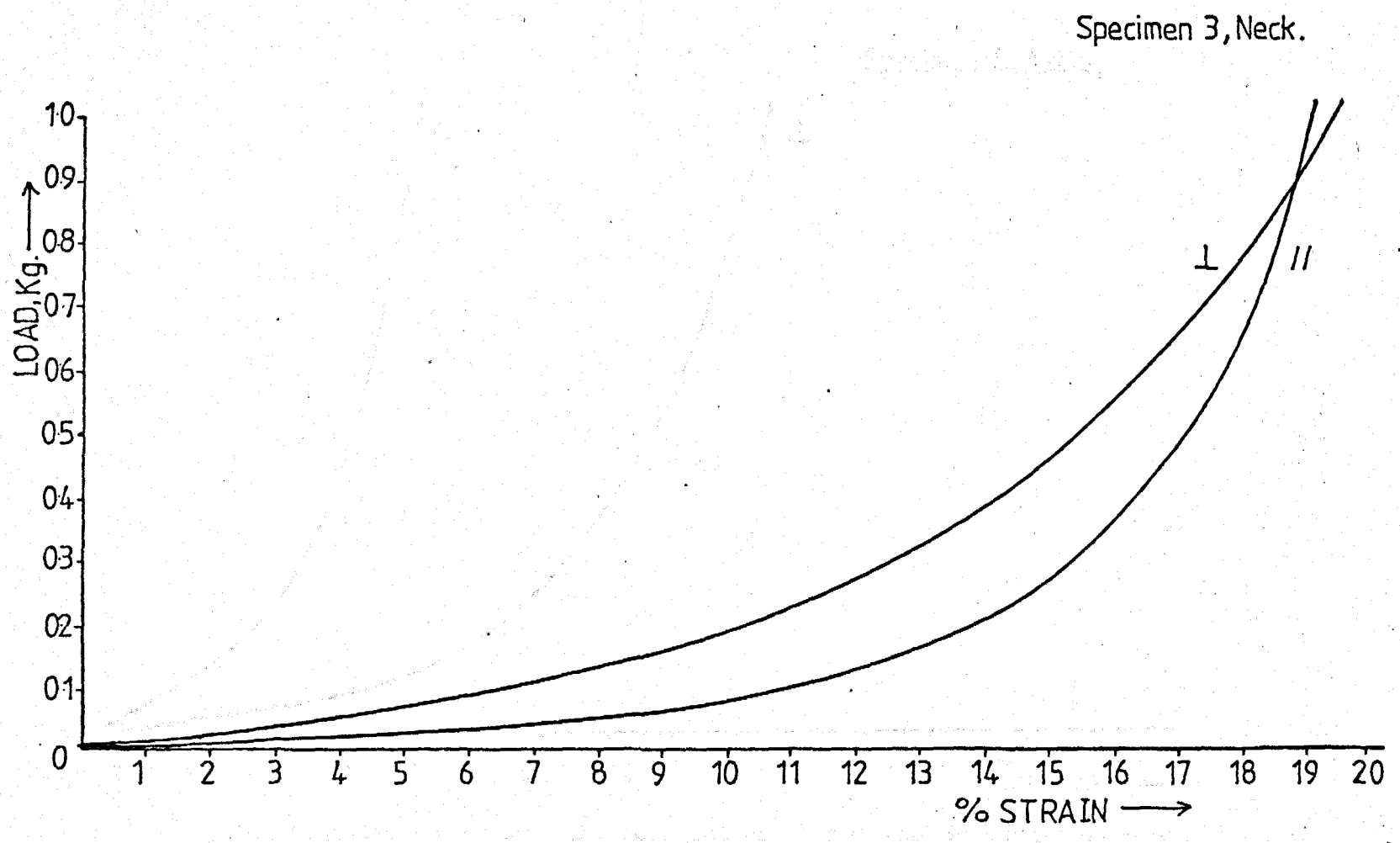


Fig. 5.10. In-vitro mechanical characteristics, 5 year old hypertrophic scar, parallel (//) and perpendicular (⊥) to the contracture axis.

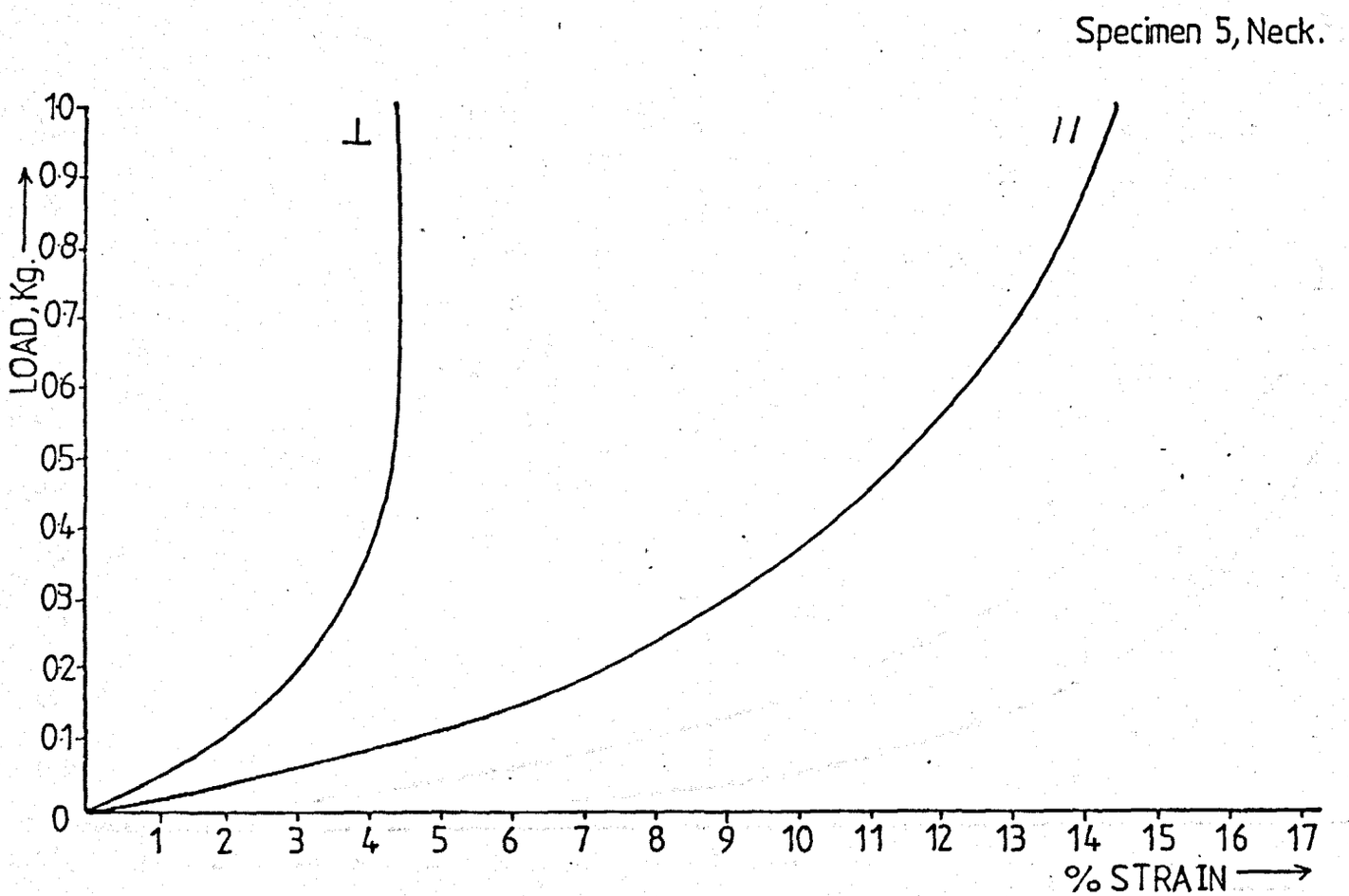


Fig. 5.11. In-vitro mechanical characteristics, 9 month old hypertrophic scar, parallel (//) and perpendicular (⊥) to the contracture axis.

subsequent cycles (see section 2.6.2.). Thereafter the increase in extension with repeated loading becomes less and by the fourth cycle stabilisation is apparent.

5.6.2. Stress-relaxation. Fig. 5.6. shows an example of the stress-relaxation effect exhibited by all the specimens of hypertrophic scar tissue tested. The relaxation of the load required to maintain a constant extension, though initially quite rapid, soon reached a plateau. All test strips were left to relax for at least fifteen minutes, after which time the load-extension trace was well into the plateau phase, and chemical fixation could be commenced without the danger of further significant relaxation of the tissue.

5.6.3. Load-strain characteristics. An average of four tissue strips from each section of the scar samples were mechanically tested. The mean results for each section of hypertrophic scar tissue are shown in Figs. 5.7. - 5.11. The three distinct phases of the load-strain curve characteristic of normal skin are absent in the load-strain graphs of the hypertrophic scar tissue. Most noticeable is the absence of the initial lax phase where in normal skin large strains of the order of 25% to 60% are produced by only small increases in load. Instead large loads are required to produce even a minimal amount of extension.

The second phase of the normal skin characteristic is also less appreciable in the scar tissue characteristic. There is no distinct "elbow" in the curve where the graph changes from large extensions with small increases in load to a stiffer characteristic, but rather there is a much more gradual increase in stiffness, and it is difficult in some cases to ascertain where phase one finishes and phase three begins.

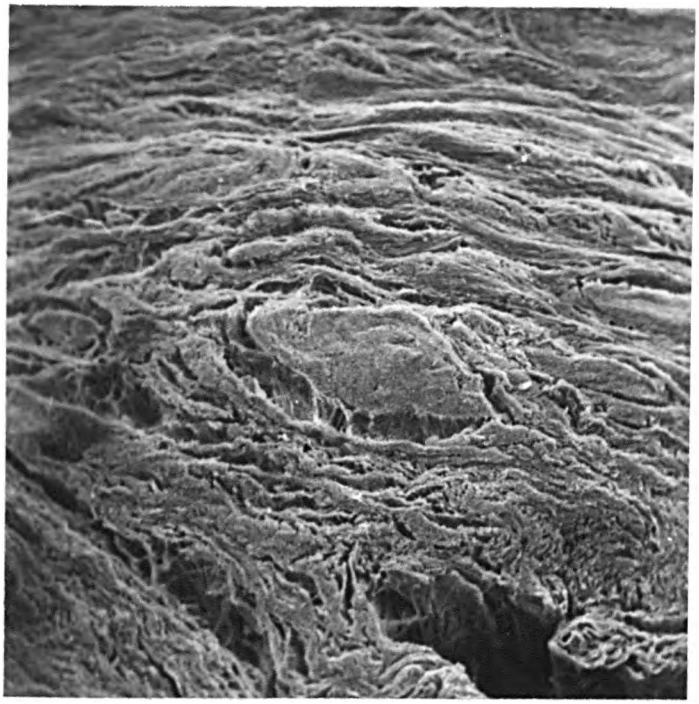


Fig.5.12. ——— 200μm. Transverse section (TS), unstrained scar with fibre bundles orientated out of the plane of the micrograph.

In general the scar tissue is much more inextensible than normal skin, with a maximum strain of the order of 5% to 20% being achieved with a load of 1 Kg.

All the pieces of scar tissue tested exhibited an anisotropy in their load-strain characteristics. For those samples of scar obtained from the axilla the tissue was more inextensible in a direction parallel to the visible contracture band than at right angles to it (Figs. 5.7. - 5.9.). However, for the two samples of tissue obtained from the front of the neck the scar was less extensible at right angles to the contracture axis (Figs. 5.10., 5.11.). This observation will be further discussed in Chapter 7, section 7.1.2.

5.7. S.E.M. Results

On completion of the mechanical testing and chemical fixing programmes, each tissue strip was divided into three, and prepared for viewing with the S.E.M. One full thickness transverse section, and two horizontal (one superficial and one deep) sections were prepared.

Full thickness transverse sections of the unstrained scar tissue were also prepared. These possessed a micro-architecture comparable to that previously described in section 3.3. as being characteristic of hypertrophic scar tissue. Fig. 5.12. shows the dermal region of a full thickness transverse section, with the large bundles of collagen fibres showing a preferential orientation normal to the plane of the micrograph.

After the completion of the mechanical tests it was found that this axis of "preferred orientation" of the collagen bundles was parallel to the direction in which the scar tissue was the least extensible.

Fig.5.13. ⟵⟶ 500µm. ⟷ LOAD AXIS, ⟷ CONTRACTURE AXIS. Strained section parallel to contracture axis. (TS).

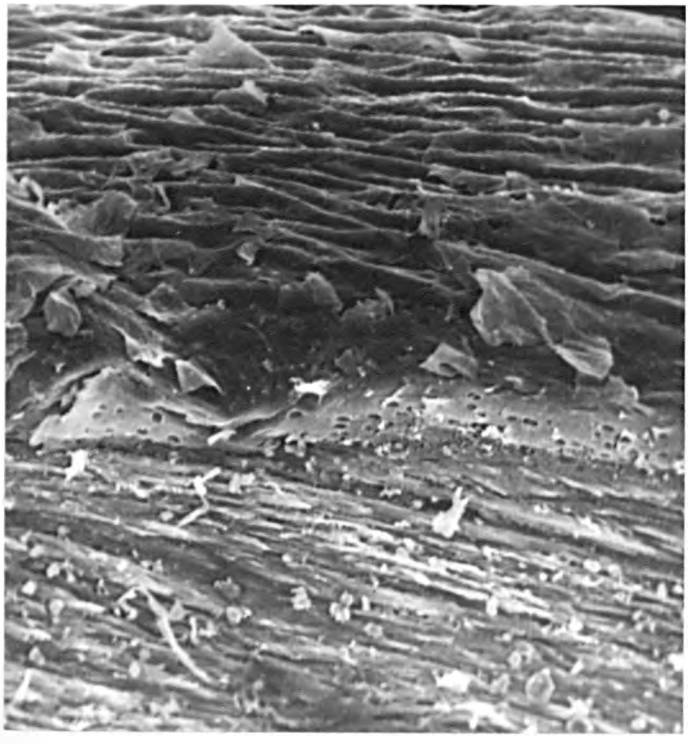


Fig.5.14. ⟵⟶ 50µm. ⟷ LOAD AXIS, ⟷ CONTRACTURE AXIS. Magnification of papillary layer. Alignment of fibres and orientation in scar surface pattern along the load axis. (TS).

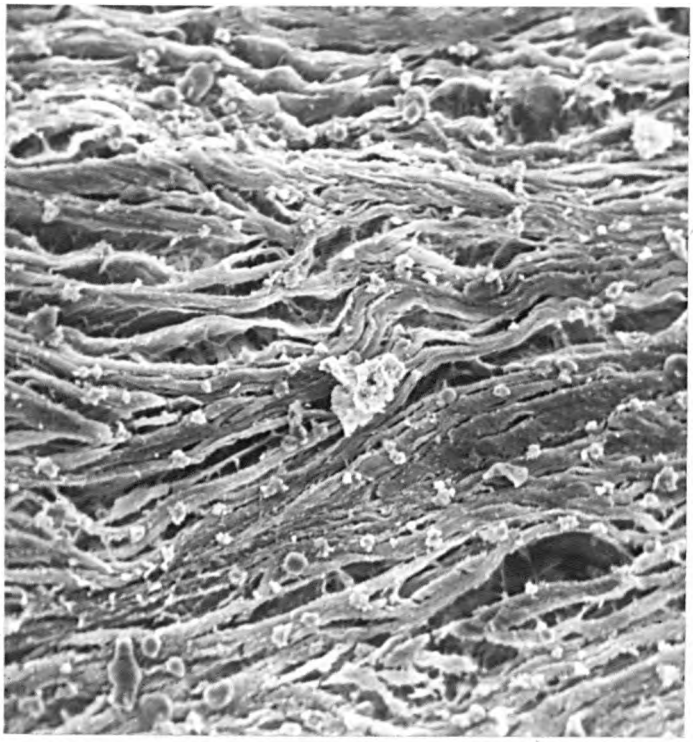


Fig.5.15. ——— 50μm. ⟷ LOAD AXIS, ⟷ CONTRACTURE AXIS.
(TS) Orientation of fibres of the mid-dermis.

Fig.5.16. ——— 50μm. ⟷ LOAD AXIS, ⟷ CONTRACTURE AXIS.
(HS) Alignment of fibres with load axis.

5.7.1. Micro-architecture parallel to direction of least extensibility. Fig. 5.13. shows a transverse section parallel to both the load axis and the axis of least extensibility. On magnification the collagen fibres of the papillary layer are almost straight and are aligned along the load axis. (Fig. 5.14.). The surface pattern of the scar tissue is also visible in this figure, and it too shows a marked orientation.

Figures 5.15. and 5.16. show the fibres of the mid-dermis of a strained specimen seen in the transverse and horizontal sections respectively. Again there is a marked degree of orientation.

Figure 5.17. is a magnification of the side of a specimen originally cut to show the horizontal section through the superficial dermis. Sheets of collagen fibres, arranged in lamellar like layers are clearly visible.

5.7.2. Micro-architecture perpendicular to direction of least extensibility. Fig. 5.18. shows a transverse section parallel to the load axis, but perpendicular to the axis of least extensibility. On comparison with Fig. 5.13., which is at the same magnification, the larger amount of extension in this specimen is evident, as is the associated "thinning" of the specimen.

To the right of the section shown in Fig. 5.18., in the mid-dermis, there is a cleft in the tissue. On magnification (Fig. 5.19.) this was found to consist of sheets of highly aligned collagen fibres arranged in a septal like formation similar to those found surrounding a collagen nodule (see section 3.3.3.).

Figure 5.20. shows the fibres of the papillary layer, and Fig. 5.21. the fibre bundles of the mid-dermis, seen in transverse section. Whilst the papillary layer fibres are orientated out of the plane of

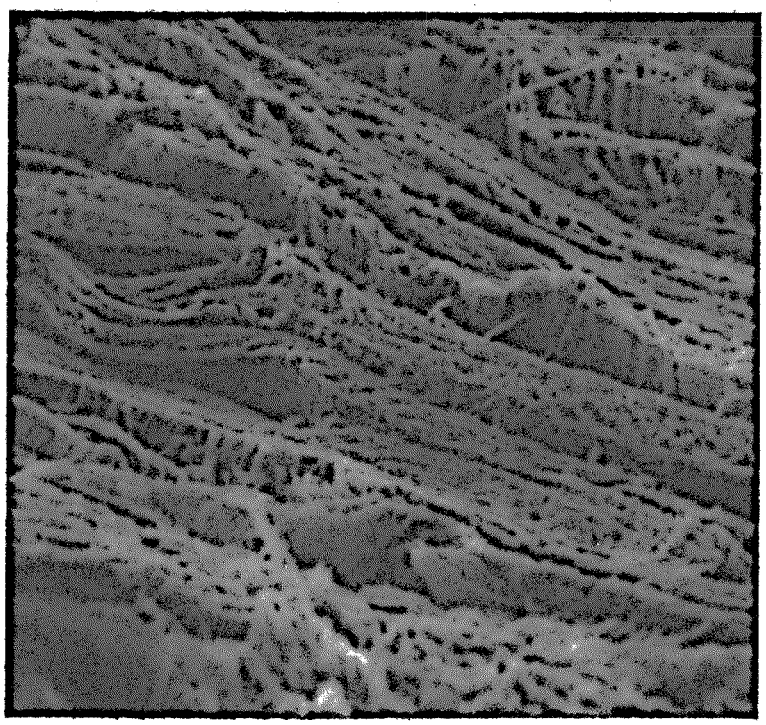


Fig.5.17. ——— 50μm. View of side of (HS) shown in Fig.5.16. showing lamellar-like layering of dermal fibre bundles.

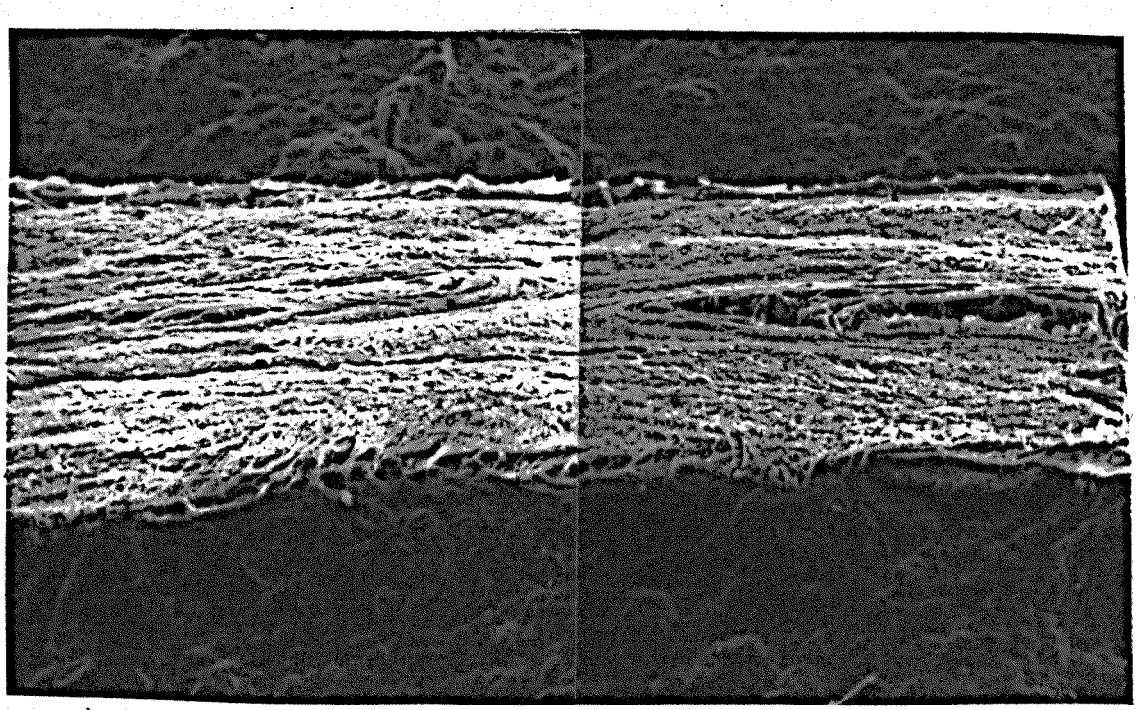


Fig.5.18. ——— 500μm. ⟷ LOAD AXIS, ⊙ CONTRACTURE AXIS. (TS) Section strained perpendicular to contracture axis. Compare degree of extension with that show in Fig.5.13.

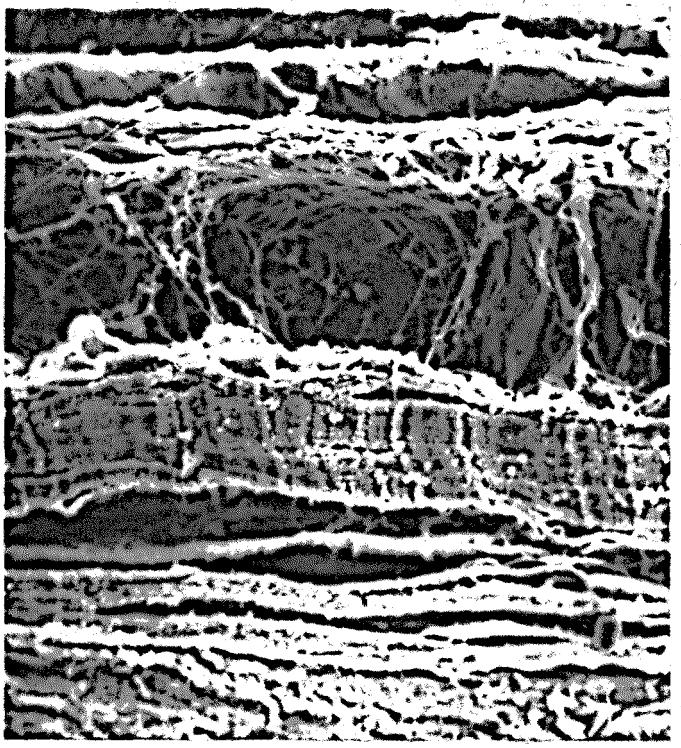


Fig.5.19. ——— 50μm. ⟷ LOAD AXIS, ⊙ CONTRACTURE AXIS.
(TS) Magnification of fibres around cleft in Fig.5.18. arranged in septal like formation.

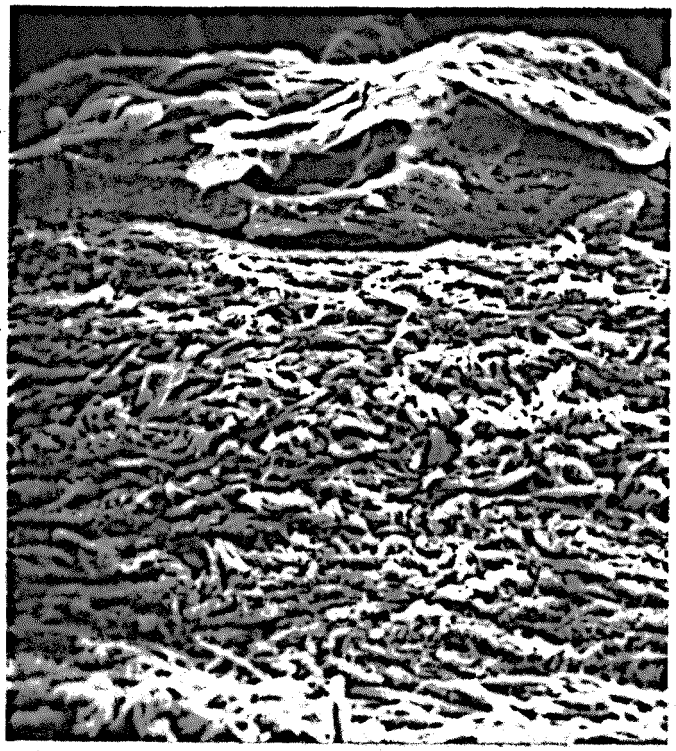


Fig.5.20. ——— 50μm. ⟷ LOAD AXIS, ⊙ CONTRACTURE AXIS.
(TS) Papillary layer fibres orientated out of the plain of the micrograph.

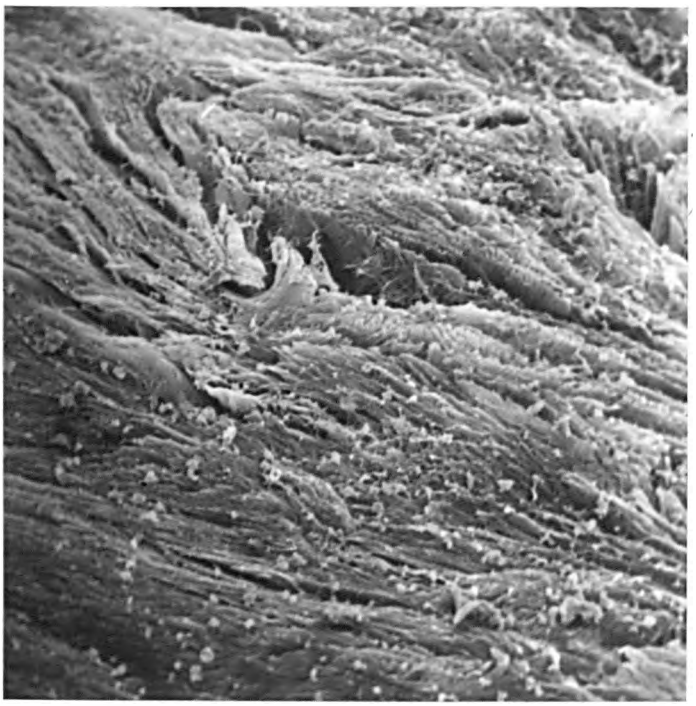


Fig.5.21._____ 50μm. ⟷ LOAD AXIS, ⊙ CONTRACTURE AXIS. (TS) Mid-dermis fibres orientated more obliquely than the papillary layer fibres.

the micrograph the mid-dermis fibres have been cut more obliquely, this point will be discussed in more detail in the following section.

The micro-architecture of the mid-dermis as seen in a horizontal section is shown in Fig. 5.22. There is a distinct diagonal pattern visible in the specimen demonstrating a re-orientation towards the load axis, and on magnification these are seen to be large bundles of orientated collagen fibres (Fig. 5.23.). Fig. 5.24. shows the hierarchical arrangement within one of these bundles of individual collagen fibres grouped to form smaller bundles, which in turn are grouped into a large bundle.

5.8. Discussion

Clinically, hypertrophic scar tissue is hard to the touch and is stiffer than normal skin. If the scarred area is around a joint severe flexion deformities can be formed. These contractures are felt to be due partly to the contraction of the wound edges during the healing process, partly to the excessive fibrous tissue that is laid down in the open granulating space, and partly to the muscle pull around the joints (see section 2.8.4.).

Contractures which occur at the knees, elbows and axillae often take the form of webs of tissue which prevent extension/abduction of the limb. Three specimens of scar tissue from such contractures of the axilla were mechanically tested, and it was found that the scar tissue was more inextensible in a direction parallel to the visible contracture web. (Figs. 5.2., 5.7. - 5.9.).

A distinct web of scar tissue over a joint contracture is not always visible, particularly in such areas as the neck and hips. Instead, a solid plaque of scar prevents the full movement of the joint. In these instances the direction of least extensibility is

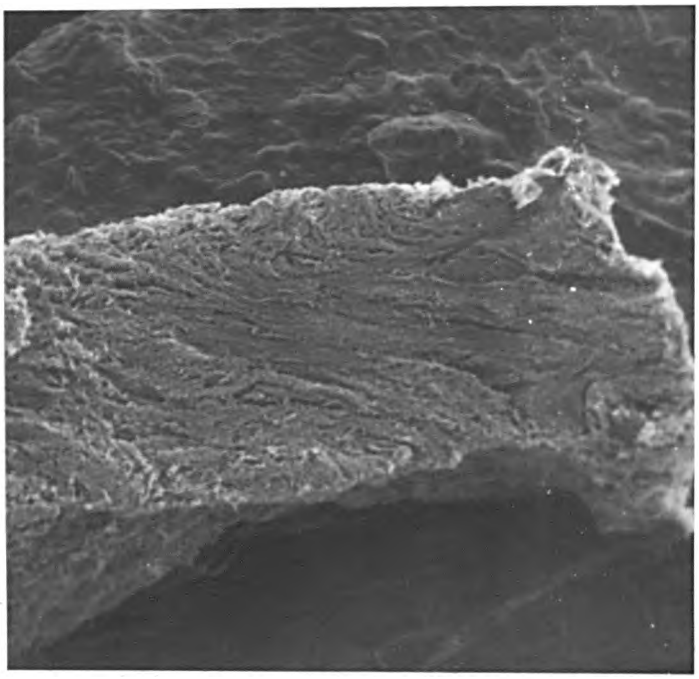


Fig.5.22. ——— 500μm. ⟷ LOAD AXIS, ↕ CONTRACTURE AXIS.
(HS) Mid-dermis diagonal pattern orientated towards the load axis.

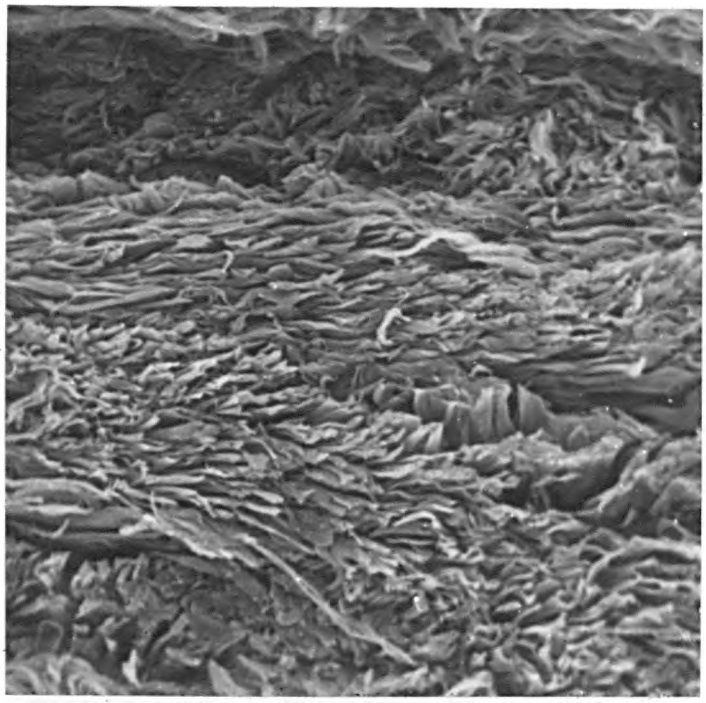


Fig.5.23. ——— 50μm. ⟷ LOAD AXIS, ↕ CONTRACTURE AXIS. (HS). Magnification of mid-dermis fibre bundles in Fig.5.22.

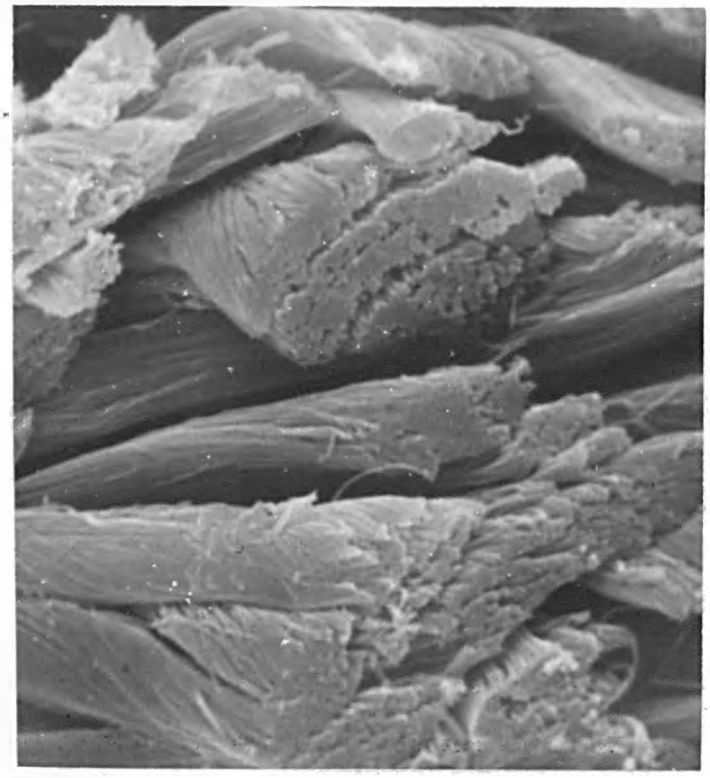


Fig.5.24. ——— 2μm. Hierarchical arrangement of fibre bundles.

not so apparent, but is usually assumed to be the direction in which the joint movement is most severely limited, i.e. in the neck the contracture is described as drawing the chin down to the chest, and it is therefore assumed that the caudio-cranal axis is the axis of least extensibility.

Two specimens of scar tissue from contractures of the neck were mechanically tested, and it was found that the direction of least extensibility was not in the caudio-cranal direction but rather in the circumferential direction.

It is recognised that the mechanical properties of normal skin are, in general, anisotropic (see section 2.6.). The "Lines of Skin Tension" have been mapped out for the body and these lines have been shown to be the directions of both greater tension and least extensibility within the skin. This "directionality" is also reflected in a "preferential orientation" of the micro-structure of the tissue (Flint, 1976).

Figure 5.25. shows the lines of skin tension or Langers lines for both the axilla and the neck. (Taken from Figs. 2.25., 2.26., section 2.6.1.). It can be seen that the directions of least extensibility measured in the mechanical tests on the pieces of scar tissue studied, lie parallel to the direction of Langers lines for those regions.

The load-strain curve characteristic for normal skin exhibits three distinct phases of extension (see section 2.6.2.). The initial phase of a fairly rapid increase in length for very small increments of force is reflected in the micro-architecture by a straightening of the undulating epidermis, and a re-orientation of the fibres in the dermis towards the direction of load. The second

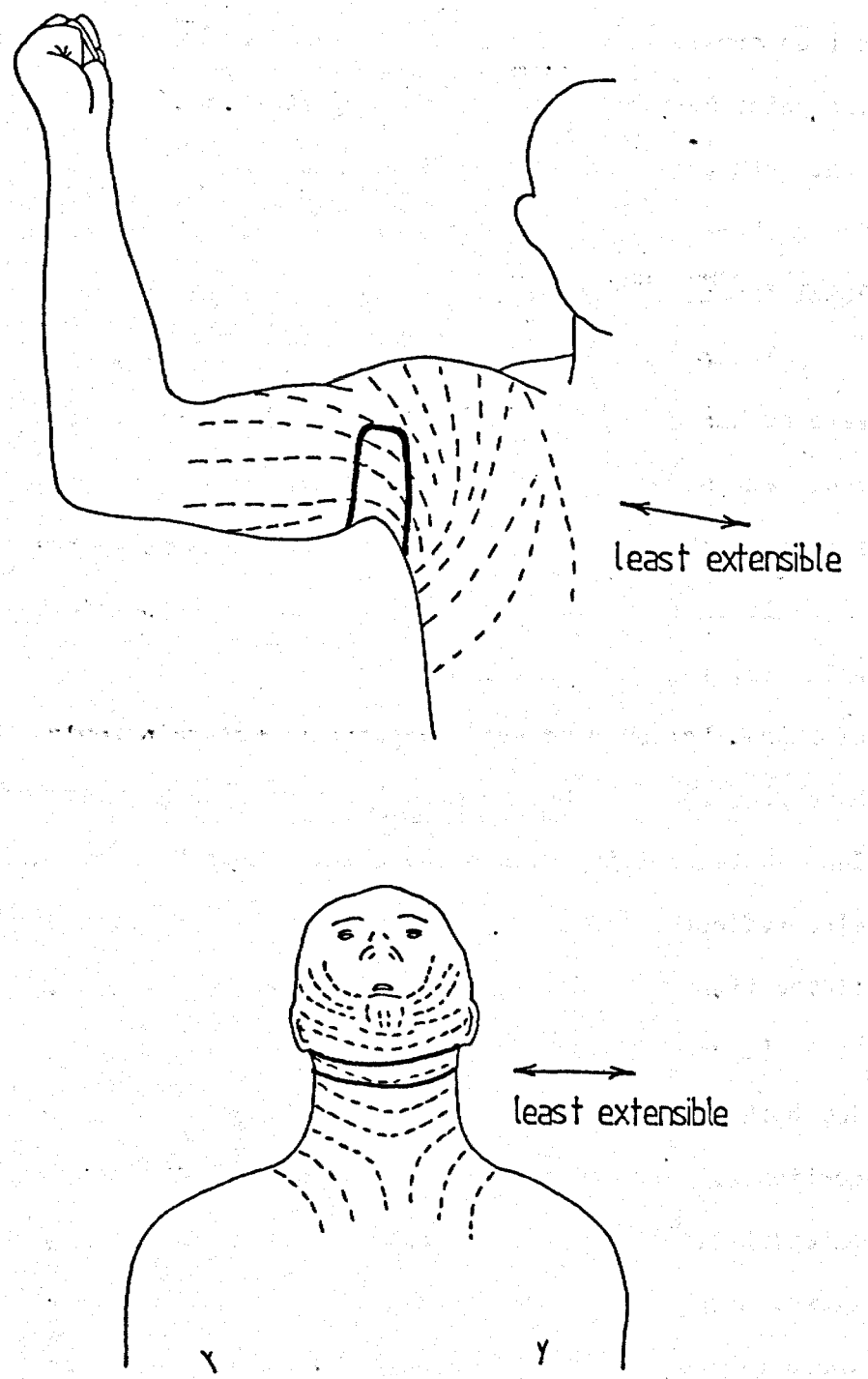


Fig. 5.25. Diagram of Langer's lines for the specimen areas mechanically tested.

phase constitutes the elbow of the graph, and at this stage the cells of the epidermis are becoming elongated; the papillary fibres are beginning to compact and the dermal fibres are straightening out.

When the scar tissue is loaded in the direction of minimal extensibility the majority of the fibre bundles are already aligned parallel to the load axis. During the initial increase in load there is an attempt at re-orientation by those fibre bundles in the dermis at a slight angle to the strain axis, but, since the regular undulations of normal skin epidermis are absent in the scar tissue the resultant extension is minimal and phase one of the characteristic is virtually missing.

Further increases in load for normal skin would result in a compacting and straightening of the dermal fibres. However, the dermal fibres of the scar tissue are already relatively straight and compacted so that increases in strain only result in a slight increase in extension depending upon the degree of fibre packing in the tissue (Figs. 5.13. - 5.17.), and the resultant load-strain characteristic lacks any distinctive elbow or second phase in its curve.

When the scar tissue is loaded in a direction perpendicular to the axis of minimum extensibility the majority of the fibre bundles are at right angles to the load axis. The regular epidermal undulations are again lacking, and a major re-orientation of the collagen fibre bundles through 90° is impossible and therefore phase one of a normal load-strain characteristic is missing. (See section 2.6.2.)

Due to the nature of in-vitro mechanical tests the sides of the tissue strips parallel to the load axis are by necessity unrestrained and therefore increases in load magnitude have a two-

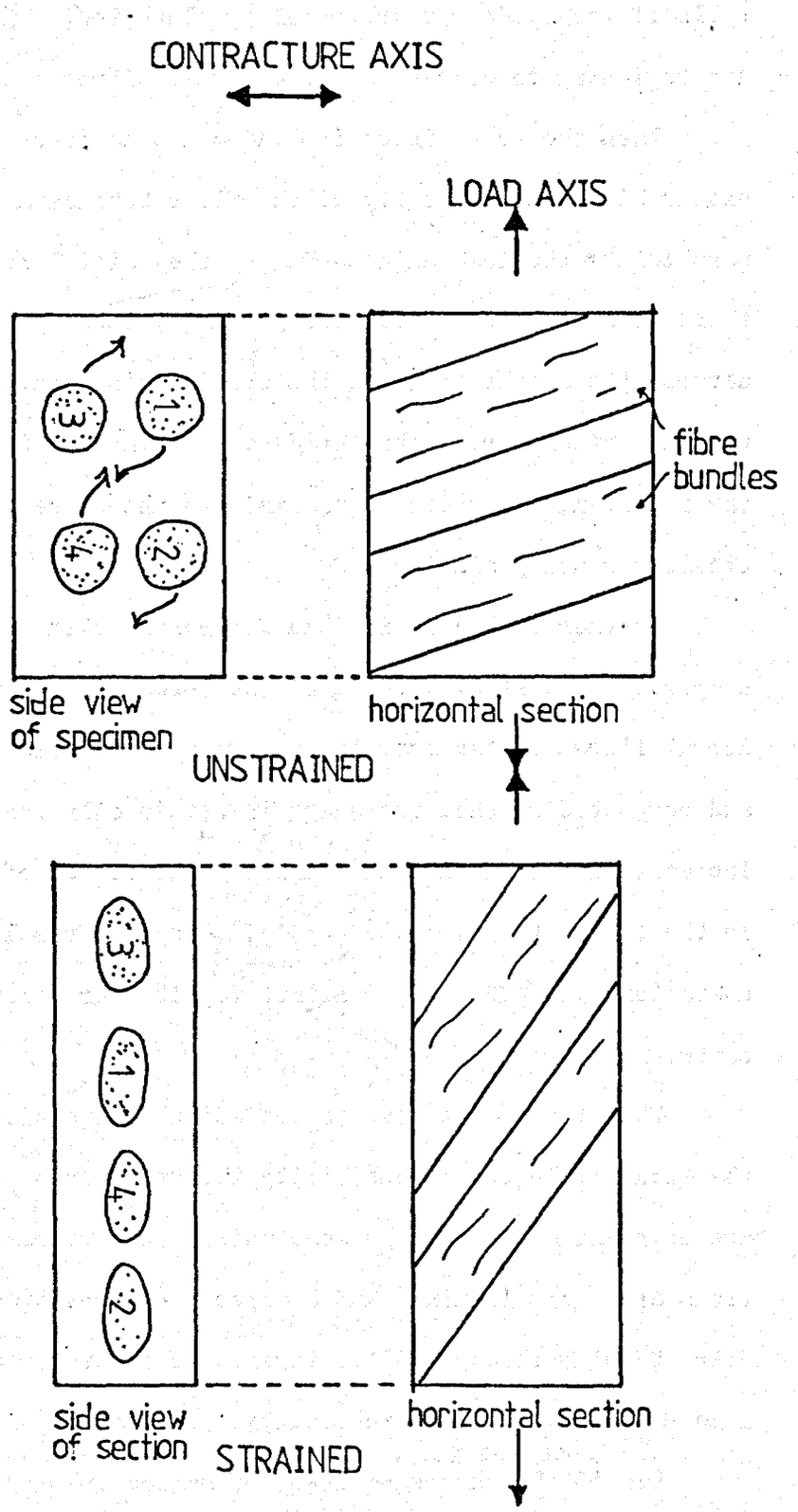


Fig. 5.26. Diagram of loading of a specimen at right angles to the contracture axis. Fibre bundles slip apart under load resulting in a micro-structural picture similar to that shown in Figs. 5.18. and 5.22.

fold effect on the dermal fibre bundles. Those fibre bundles which are truly at right angles to the load axis are flattened out by the forces so that their initially round cross-section becomes elliptical, presenting a micro-structural picture similar to that seen in Fig. 5.18.

However, for those fibre bundles at a slight angle away from the perpendicular, because their ends are unrestrained there is slipping of the fibre bundles (Fig. 5.26.). This results in an increase in extension with increasing load and a thinning of the tissue strip, giving a micro-structural picture similar to that shown in Figs. 5.18. and 5.22.

CHAPTER 6

IN-VIVO MECHANICAL PROPERTIES

6.1. Introduction

The overall effect of pressure therapy is an acceleration of the natural remodelling processes of the hypertrophic scar tissue. The consistency of an originally raised and rigid mass of tissue changes within days of commencing therapy, with a noticeable shrinking in tissue bulk and softening in the scar. (Bauer et al, 1976).

At the time of commencing this study no quantitative data was available in the literature on the changes in the mechanical properties of the tissue with pressure therapy. Considerable expertise had been acquired by researchers at the Bioengineering Unit, University of Strathclyde, in the mechanical testing of human skin 'in-vivo' (Gibson et al, 1969; Finlay, 1970; Stark, 1970; Finlay and Brown, 1971; Stark, 1977.). It was, therefore, decided to utilise this experience in an effort to monitor the changes in mechanical characteristics of hypertrophic scar tissue with pressure therapy.

6.2. Patient Selection

The patients selected for the study had to satisfy several requirements. In order that the load/strain characteristics of the scar tissue could be measured along the three chosen orientation axes the scar had to be reasonably level so that the load could be applied in a direction parallel to its surface, and it had to be large enough in area to permit the attachment of the extensometer tabs along the sides of the gauge area.

It was also decided to further limit the choice of suitable scars to those overlying bony regions of the body, since these areas were felt to obtain the maximum benefit from the pressure therapy

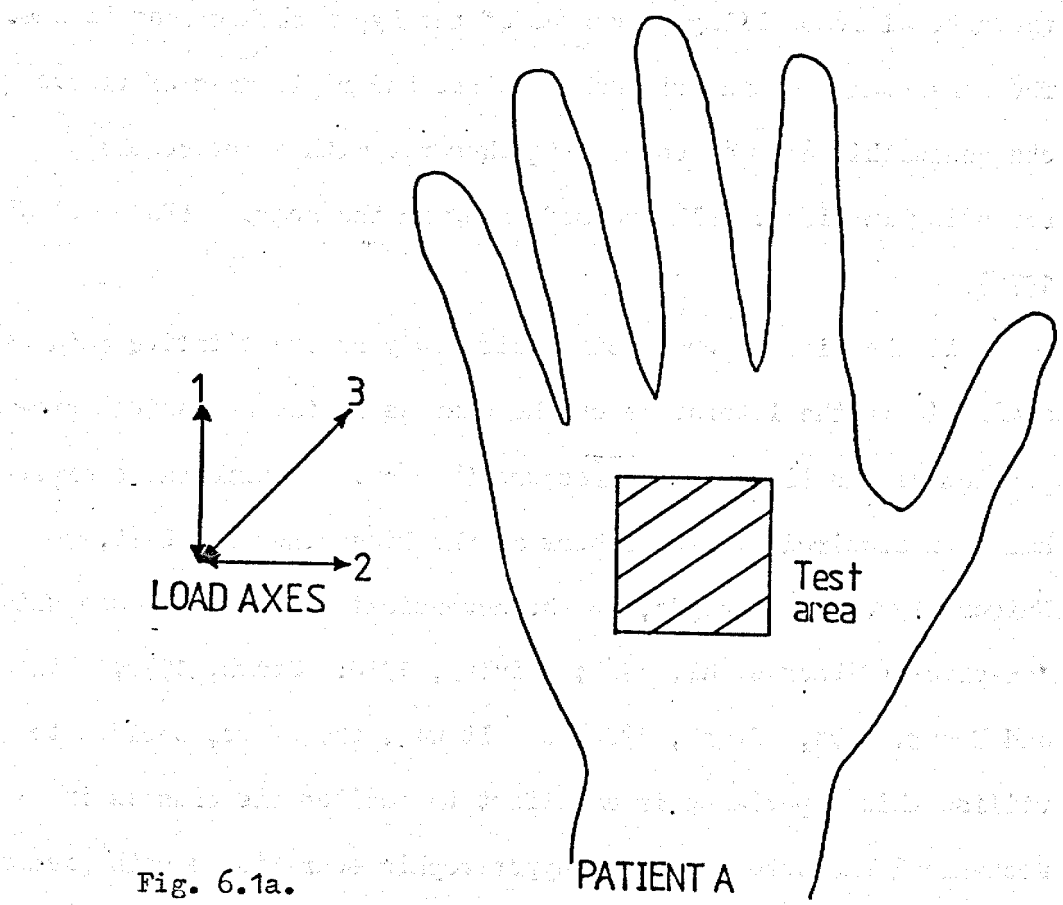


Fig. 6.1a.

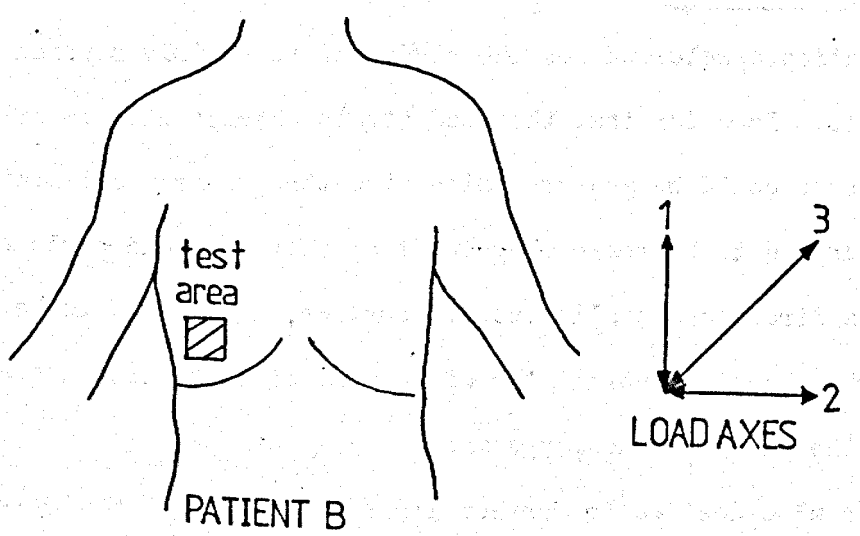


Fig. 6.1b.

Fig. 6.1. Diagram of test areas and load axes for in-vitro mechanical tests.

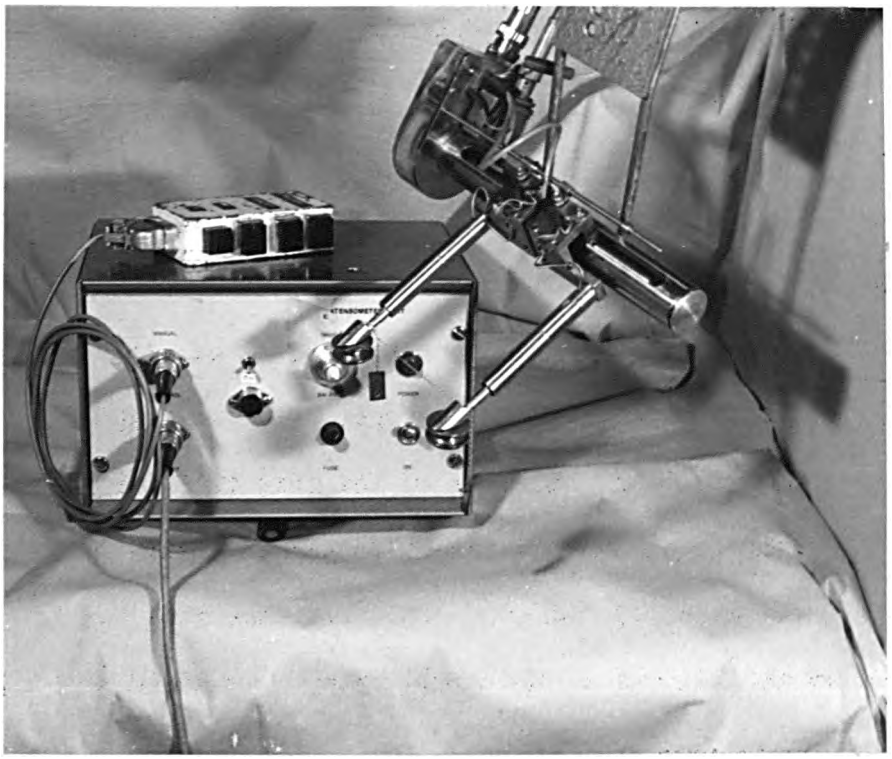


Fig.6.2. 'Quasi Static Extensometer.' - used to load skin uniaxially at constant strain rate.

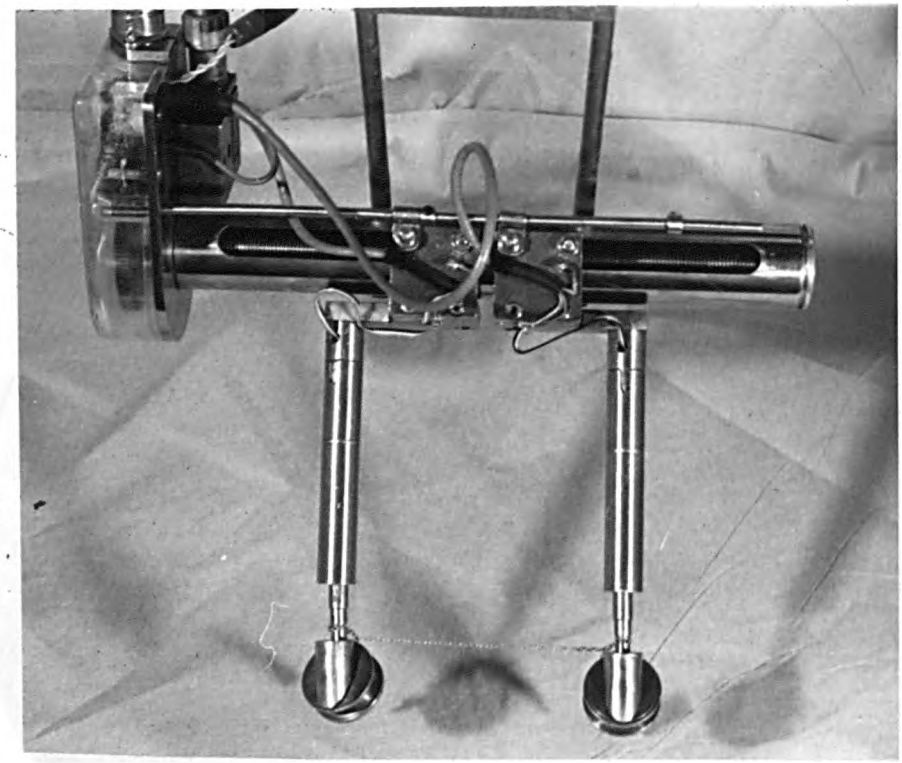


Fig.6.3. Operating head of 'Quasi Static Extensometer' showing stain gauged cantilever arms of load cells.

because of the more constant pressure which could be maintained by the garment acting upon this relatively firm support.

Two patients were chosen, who satisfied both the above conditions and the further requirements that the scar tissue was still in its active phase and that a course of pressure therapy had not yet commenced.

6.2.1. Patient records. Patient A, was male, early twenties with active hypertrophic scars of both hands following a burn injury. The area monitored was the back of the left hand (Fig. 6.1a.). Patient B was again male, in his mid-thirties, with substantial scarring of the chest following a burn. The area monitored was the right anterior aspect of the chest over the lower portion of the rib cage (Fig. 6.1b.).

6.3. Instrumentation

The instrumentation used had been in use for some time within the Bioengineering Unit, but had originally been designed and built by Dr. J.H. Evans (1967).

This system, described as a "Quasi Static Extensometer," (Q.S.E.) had been modified over the years and was again modified by the author. The system is shown in Fig. 6.2. and 6.3. and the wiring diagram in Fig. 6.4.

The device applies a known load in a uniaxial direction to the skin via double-sided adhesive tape from metal tabs (Fig. 6.3.). This load is transmitted to load cells within the strain gauged cantilever beams by jeweller's chain, which encircles the pulley wheels at the ends of the beams. A constant speed a.c. reversing motor drives the two load cells apart and hence applies extension to the skin via a flexible drive, a gear train and the counter cut

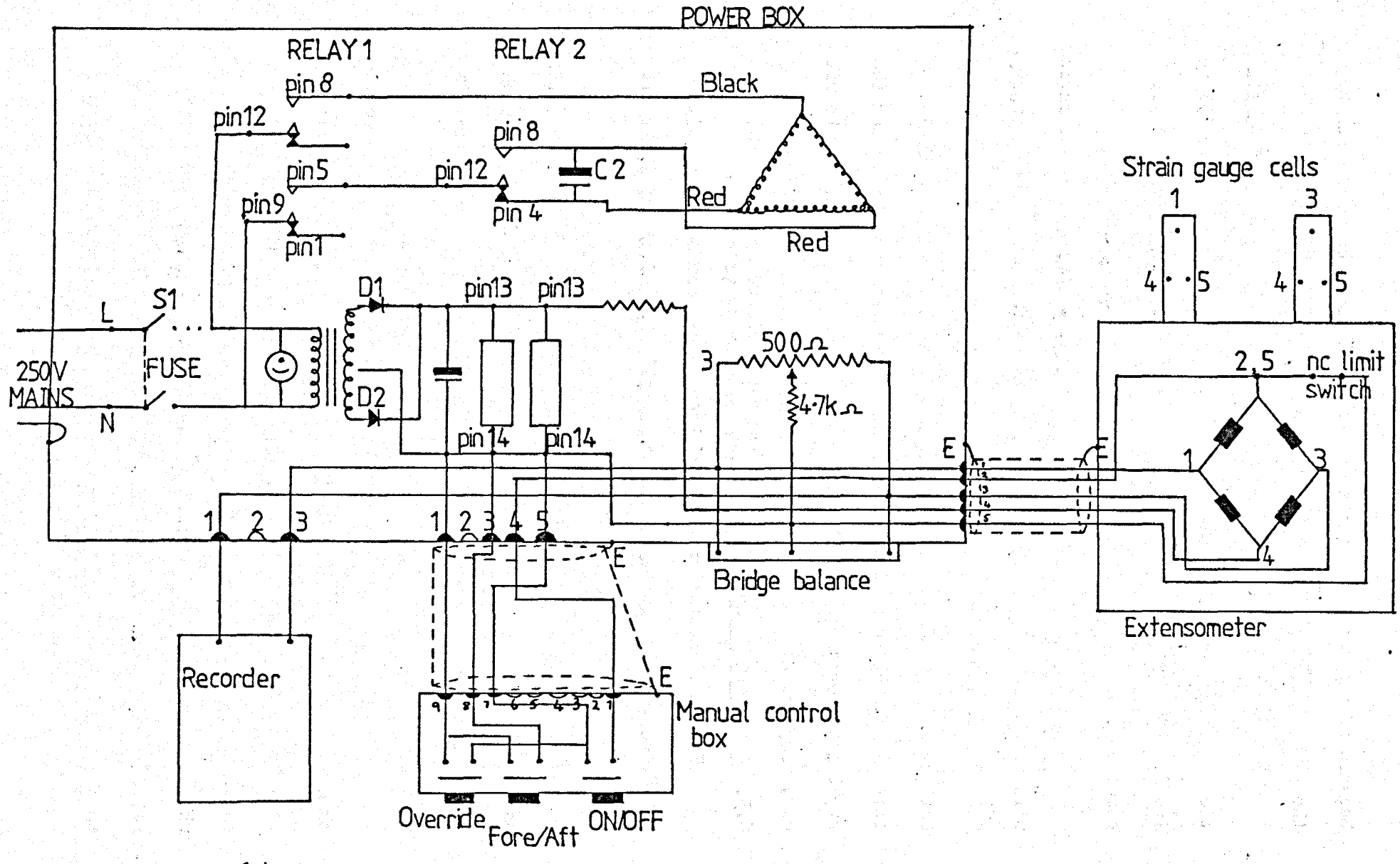


Fig. 6.4. Wiring diagram for 'Quasi Static Extensometer'.

lead screw to which the cells are attached.

The two load cells embody a full bridge, strain gauge load measuring system, the output from which can be recorded on a standard chart recorder.

6.3.1. Instrument calibration. To determine the load cell deflection with load the system was set up as shown diagrammatically in Fig. 6.5. On application of given loads W, the length L was measured using 'inside calipers' and a micrometer. Fig. 6.6. shows the relationship obtained between the deflection Δ L cms and the load W gms, from which the relationship,

$$L \text{ mm} = 0.04773 \text{ W gms.}$$

was obtained.

The Q.S.E. was calibrated before use by applying a succession of loads, W (as in Fig. 6.5.) and measuring the deflection recorded by the graph recorder. As can be seen from Fig. 6.7. the response of the load measuring system was linear. A variable resistor is included in the excitation line to the strain gauge bridge which allows the sensitivity of the instrument to be adjusted, this necessitated that the device be calibrated before each set of tests. However, since the response was linear this only required the deflection measurement for one known load to be recorded prior to use.

6.4. Measurement of Extension

Initially it was decided to use a photographic technique to measure the extension of the scar tissue with load, similar to that used in the 'in-vitro' tests (Chapter 5). For this purpose a grid was printed on the scars' surface prior to testing (using a non-waterproof ink). (Fig. 6.8.). A camera, positioned above the patient, was then used to take photographs of the grid at known

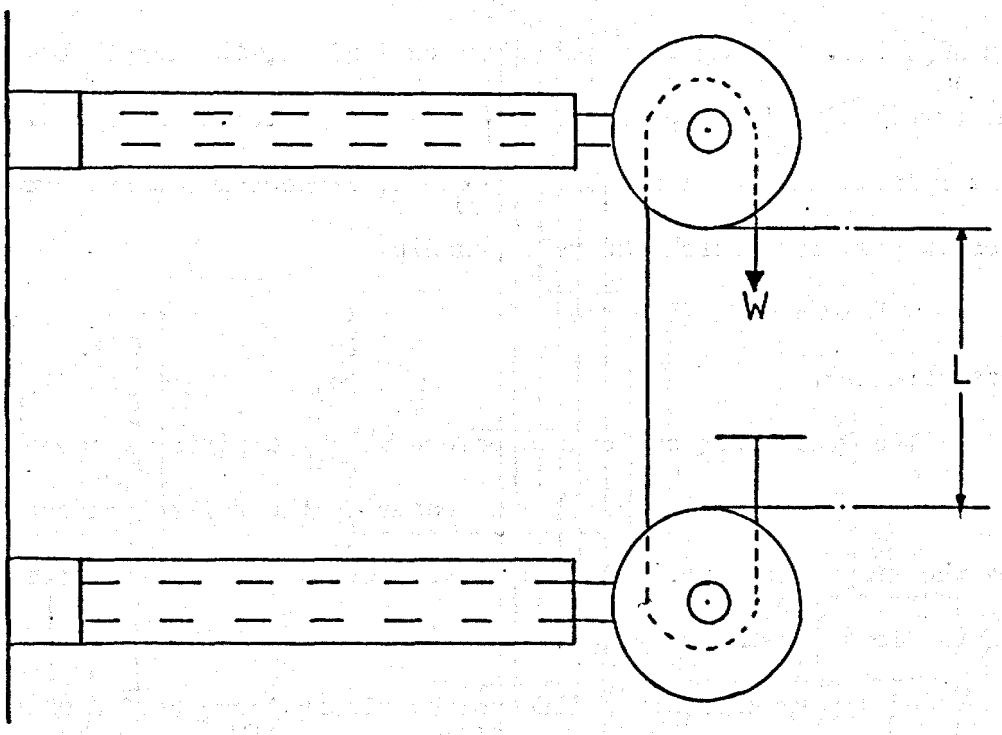


Fig. 6.5. Arrangement of extensometer operating head for determination of load cell deflection.

(After Stark, 1970)

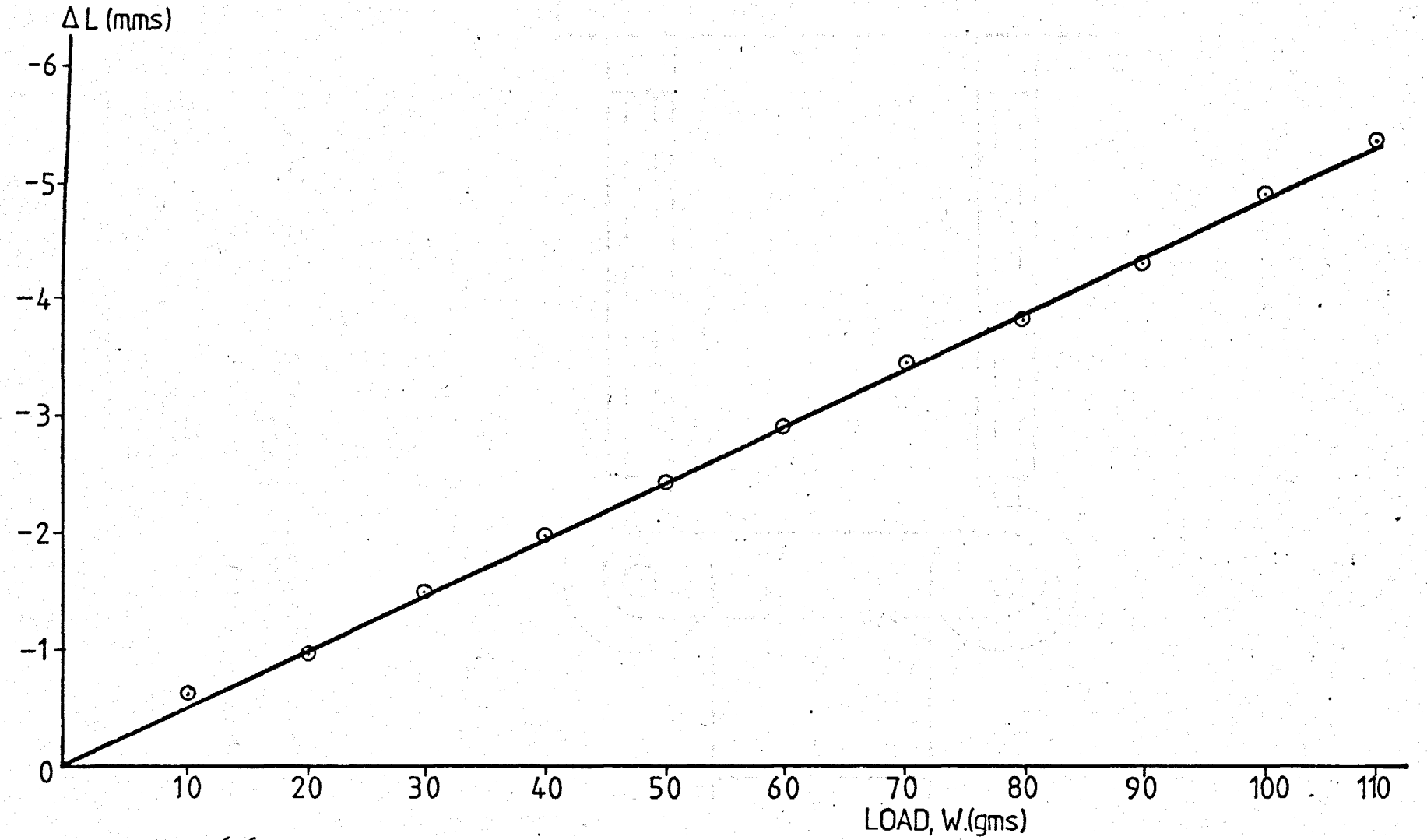


Fig. 6.6. Deflection of cantilever load cells under load.

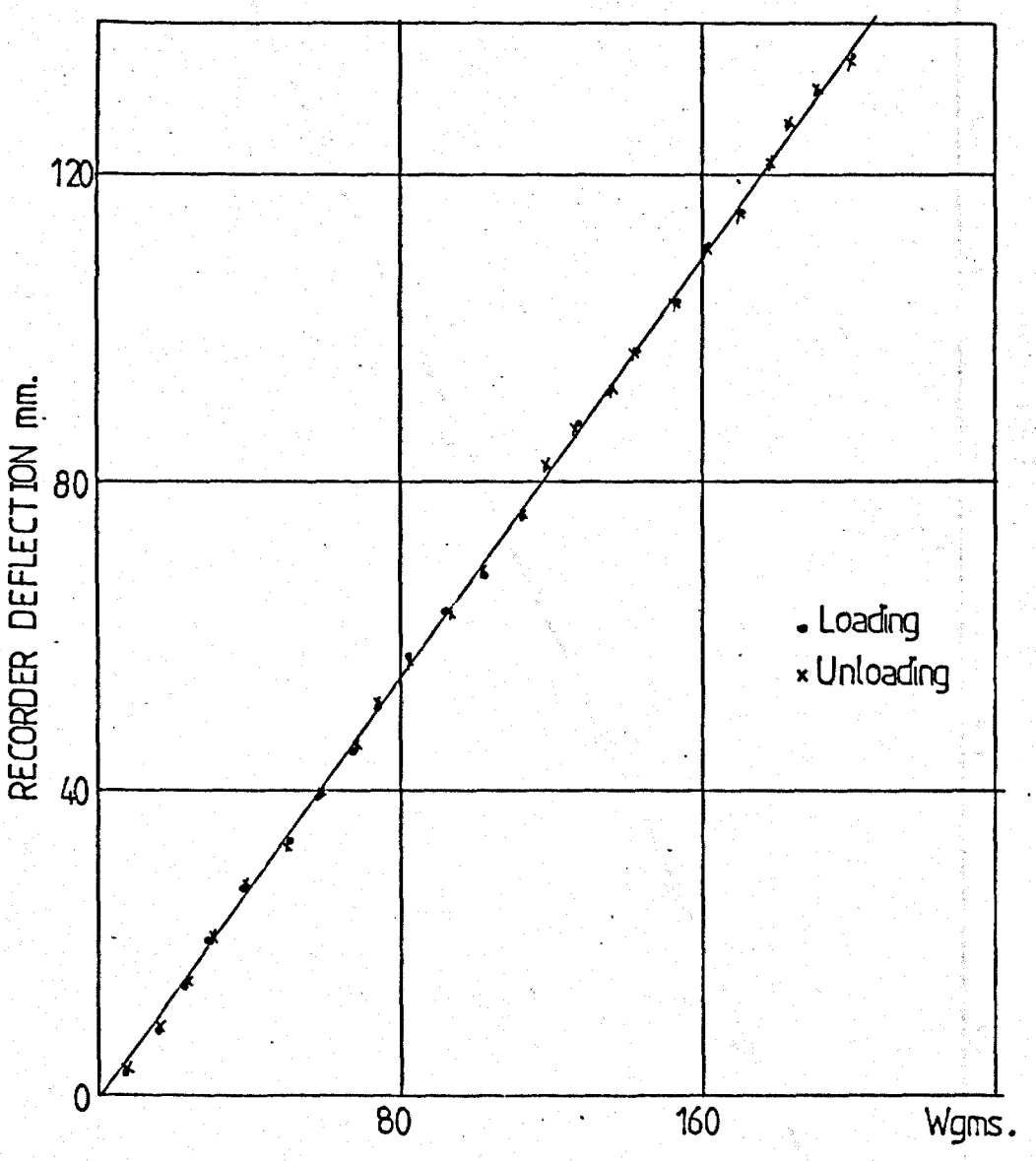


Fig. 6.7. Output of complete load measuring system when extensometer loaded incrementally.

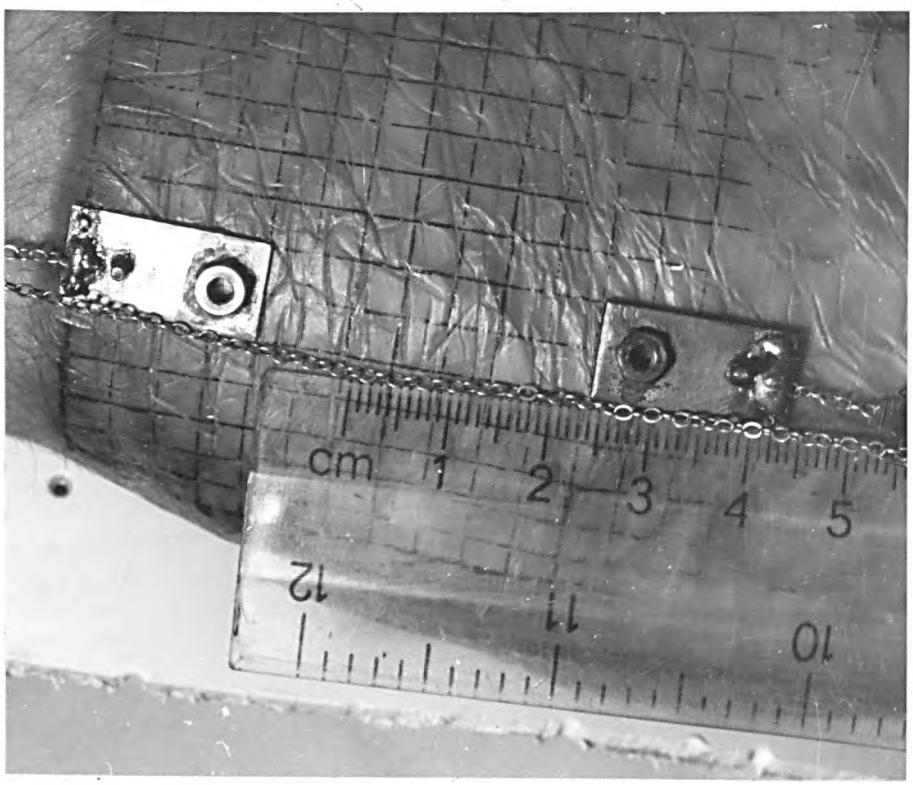


Fig.6.8. Extensometer tabs in-situ on boundaries of test grid printed on scar surface.

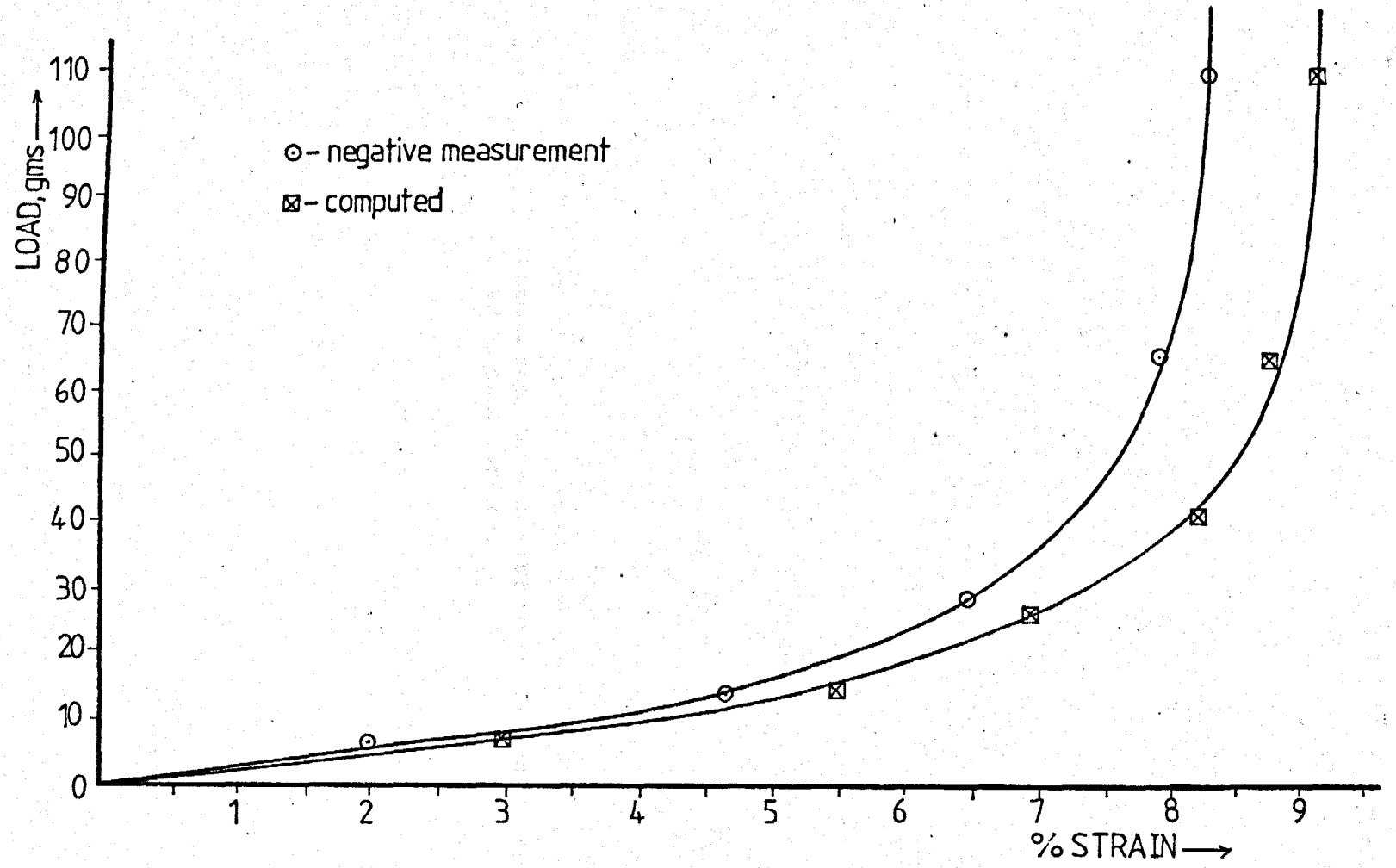


Fig. 6.9. A comparison of the load/strain results obtained by negative measurement and by computation.

loads, and the corresponding extension of the tissue could then be measured directly from the negatives.

However, due to technical and operator difficulties, a high proportion of the films obtained were unusable (non-synchronisation of the flash and camera resulting in under-exposed negatives) and therefore the author had to resort to the technique used by Stark (1970) in which extension was computed from the rate of separation, and the deflection with load, of the load cells.

A comparison of the results obtained by negative measurement, and by computation was made using one of the few films that was correctly exposed. (Fig. 6.9.). It was found that the results obtained by computation were slightly higher than those obtained by direct measurement (thought to be due to slight slippage of the tabs at higher loads), but since the object of the tests was to monitor the changes in mechanical properties rather than to definitively characterise them the error was considered by the author to be acceptable.

6.4.1. Extension computation. The following section is taken from Stark (1970). From the load/time recording it is possible to estimate the extension between tabs as follows:

With reference to Figs. 6.10. and 6.11.

gauge length = D mm

distance between cantilever bases = L mm

Extension between tabs at T_1 = $\frac{D_1 - Do}{Do} = e_1$

$$\frac{(D_1 - Do)}{Do} = \frac{1}{Do} (2(L_1 - Lo) - 2 L_1)$$

where L_1 is the cantilever deflection

$L_1 = 0.04773$ Wgms. ..... (6.1.) from previous calibration

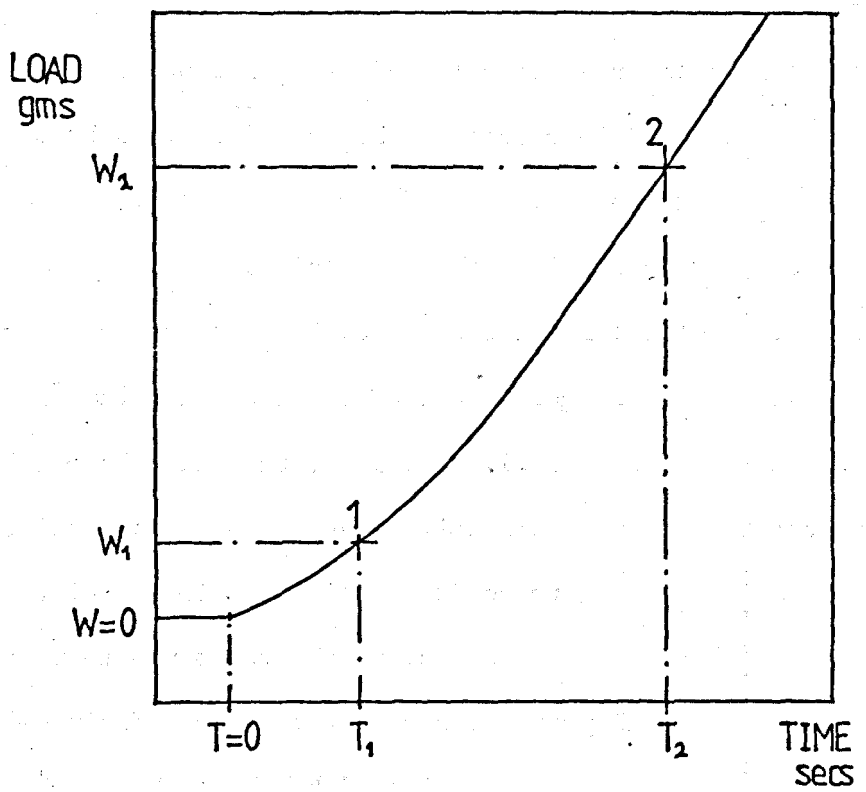


Fig. 6.10. Using the load/time recording it is possible to estimate the extension between the extensometer tabs.

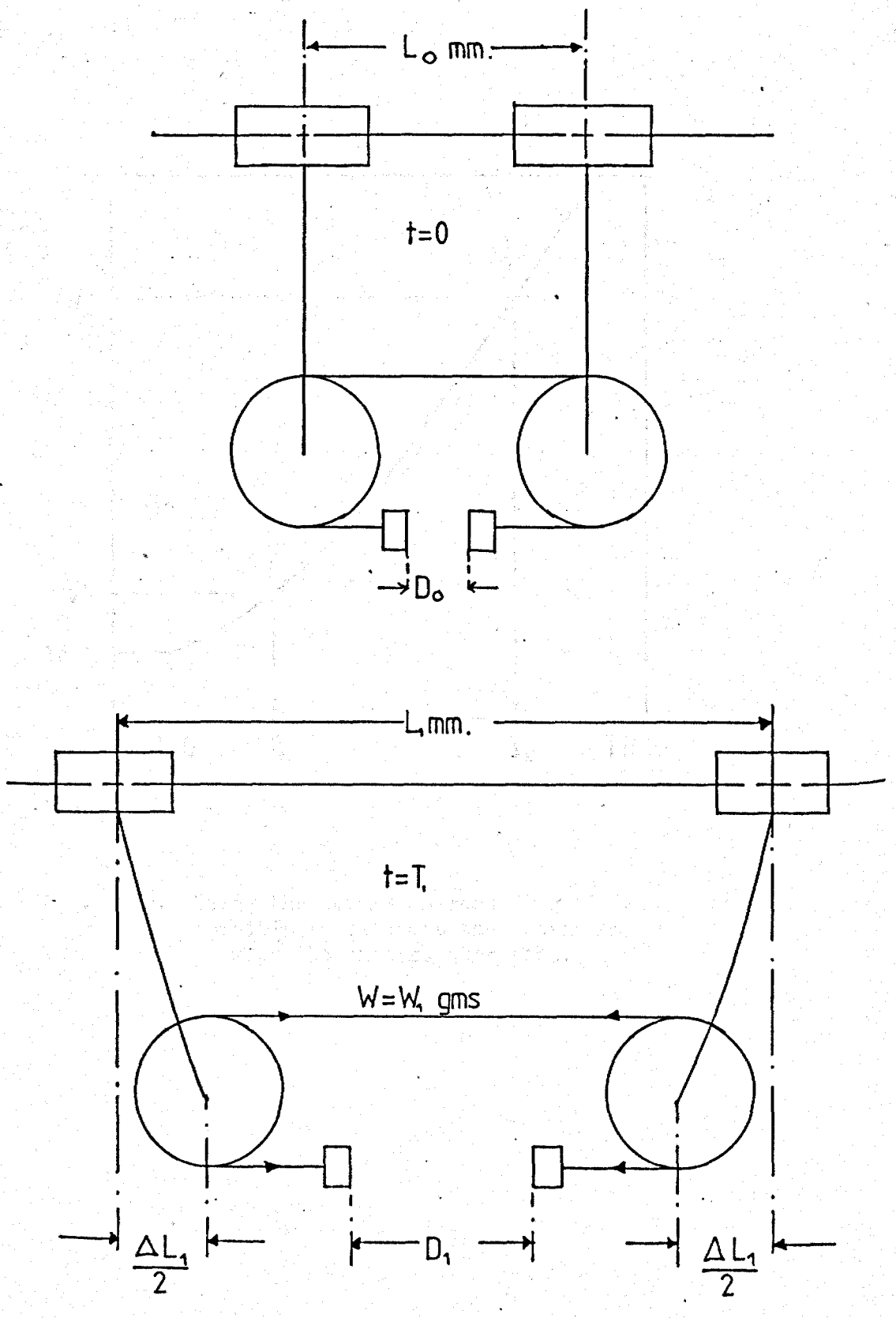


Fig. 6.11. Measurements used in computation of extension.
(After Stark, 1970)

also $(L_1 - L_o)$ = $(0.762 \times T_1)$ mm, the bases of the cantilevers being driven apart at 0.762 mm/sec.

$$\therefore \quad e_1 = \frac{1}{Do} \quad (2 \times 0.762.T_1 - 2 \times 0.04773\ W_1) \ldots\ldots (6.2.)$$

6.5. Experimental Protocol

As already stated, skin is a highly viscoelastic material, and as such possesses a memory of previous deformations. It was not possible to subject the test area of scar tissue to a "pre-conditioning" cycle as specified in section 5.4.1., since the epidermis of the scars monitored was extremely friable and the repeated application of loads to the same area would have given rise to an unacceptable degree of patient discomfort.

Instead of a "pre-conditioning" programme the patients were allowed to relax for twenty minutes before starting tests: this involved first removing the pressure garment and then the patient taking up the test position (Patient A, sitting with the left hand resting palm down on the bench, fingers extended but relaxed; Patient B, supine with arms by his sides.)

After this period the test area was swabbed down with solvent ether to remove any oils or grease and to ensure satisfactory adhesion of the tabs, and the test grid was printed onto the scars surface using a rubber pad and washable ink.

A load/strain characteristic was obtained for each of the three axes shown in Fig. 6.1. A gauge length of 30 m.m. was used for axes 1 and 2, and of $30\sqrt{2}$ m.m. for axis 3.

Both patients were initially tested before being fitted with pressure garments, and then again at their next attendance at the outpatients clinic. Thereafter the mechanical characteristics of

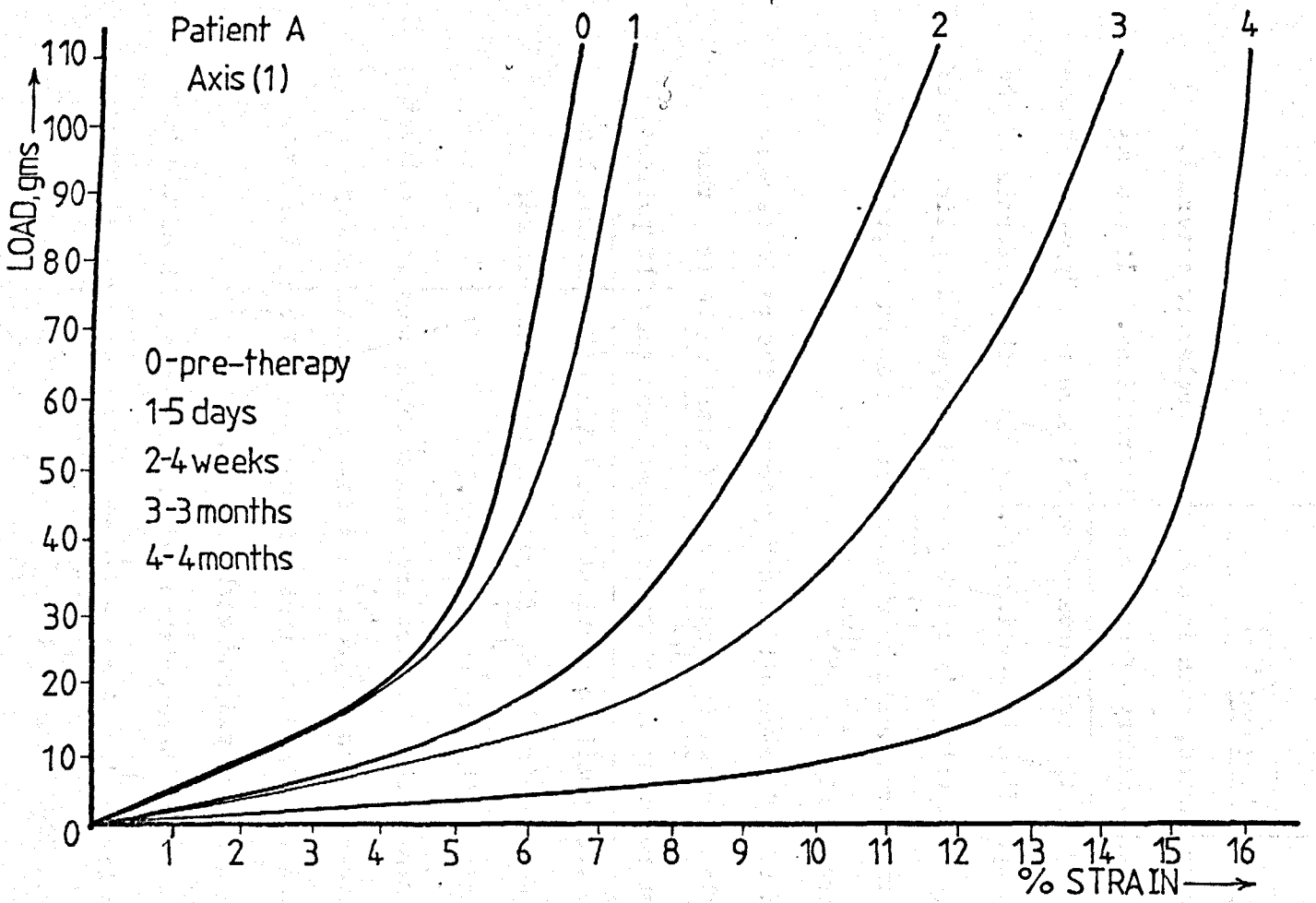


Fig. 6.12. In-vivo mechanical characteristics, showing gradual increase in extensibility with pressure therapy.

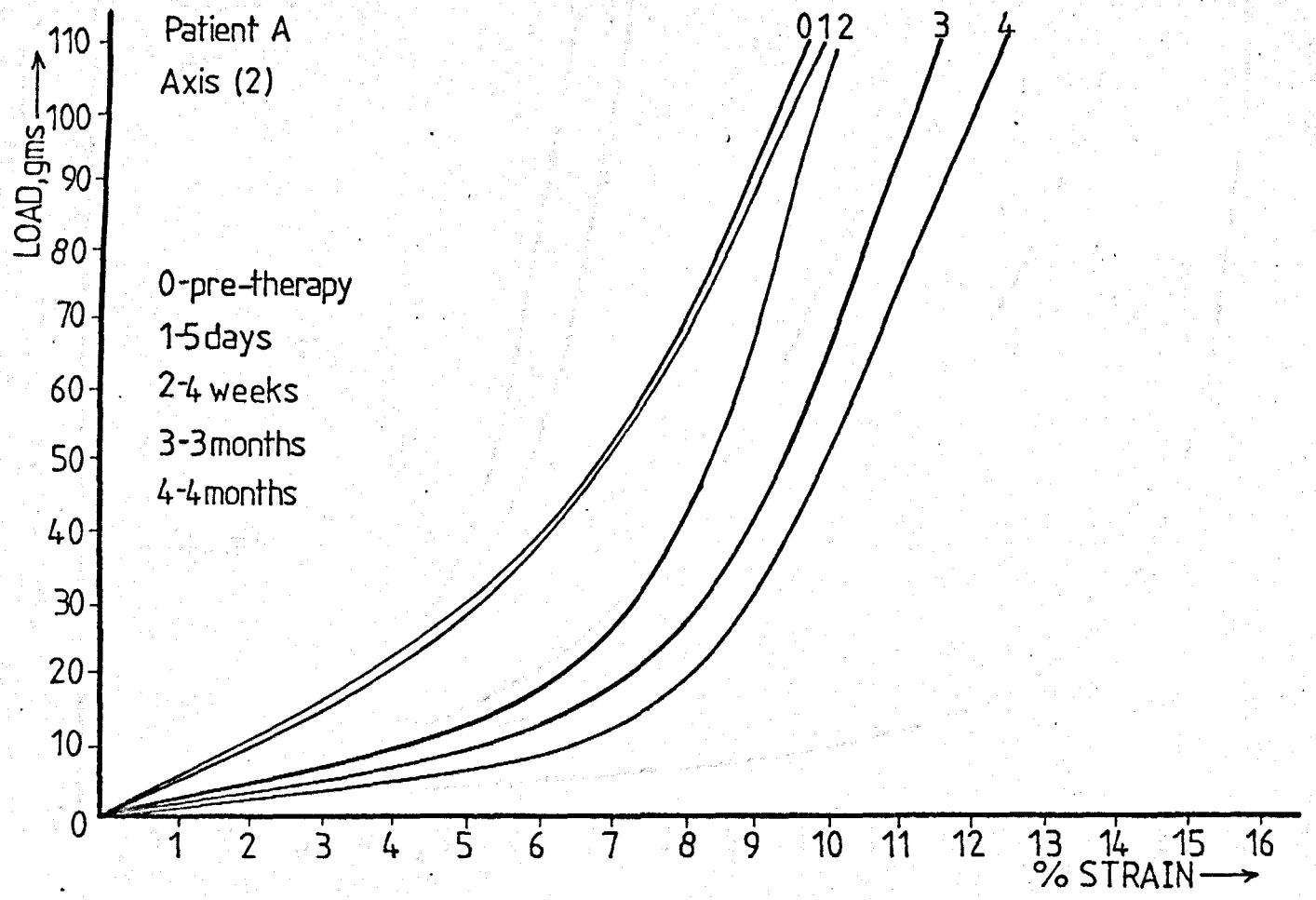


Fig. 6.13. In-vivo mechanical characteristics, showing gradual increase in extensibility with pressure therapy.

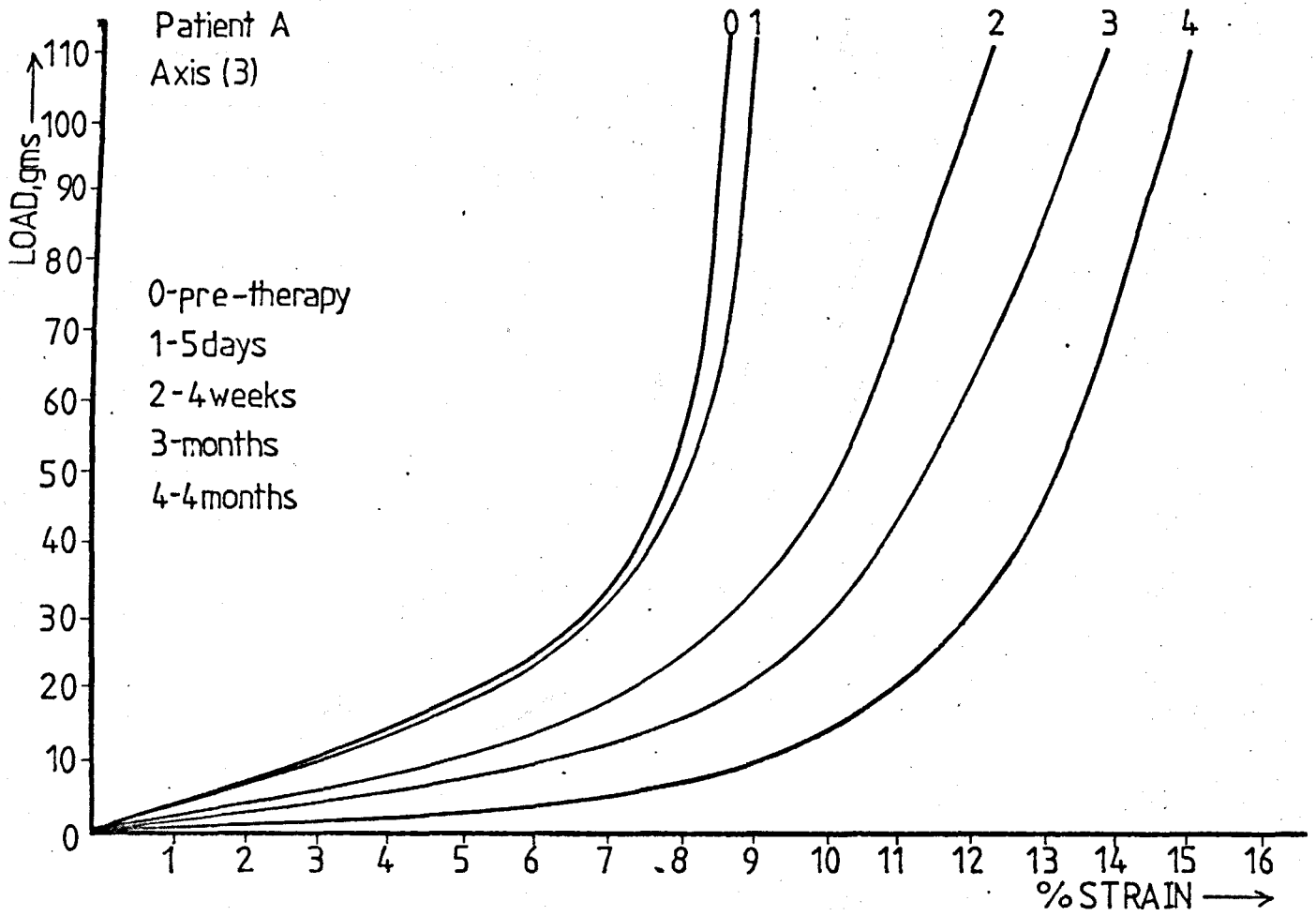


Fig. 6.14. In-vivo mechanical characteristics, showing gradual increase in extensibility with pressure therapy.

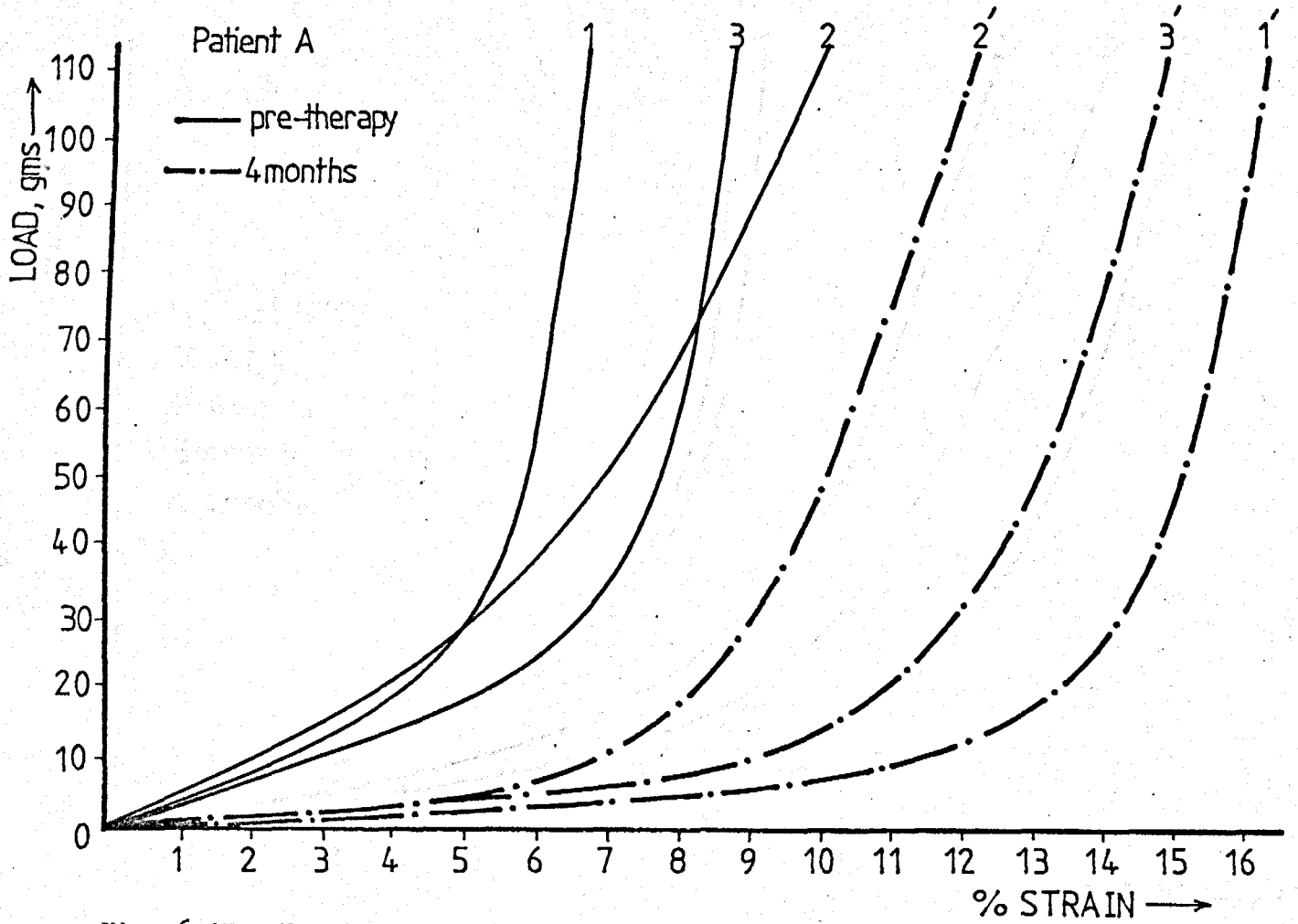


Fig. 6.15. In-vivo mechanical characteristics, showing overall increase in extensibility in the three test axes after 4 months pressure therapy.

the tissue were measured at intervals of up to two months.

6.6. Results

The changes in the mechanical characteristics of hypertrophic scar tissue with pressure therapy were monitored for two patients: Patient A, the back of the left hand and Patient B, the lower right anterior aspect of the chest. Each test area was measured before the commencement of pressure therapy and thereafter at intervals of up to two months.

Load/strain characteristics were obtained for each of three axes (Fig. 6.1.) using a standard gauge length for each test. The maximum load to which the scar tissue was subjected varied depending upon the condition of the scar epidermis and the stage of the therapy.

Patient A was tested at intervals of 3 days, 5 days, 4 weeks, 12 weeks and 4 months after being fitted with a pressure glove.

Patient B was tested at intervals of 3 weeks, 2 months and 4 months after being fitted with a pressure vest.

The results of the mechanical tests are summarised in Figs. 6.12. to 6.19.

Clinically both patients commented on the relief of the symptoms of itchyness and tenderness within days of starting the therapy, and there was a measurable increase in extensibility in the scar of Patient A after five days of wearing the pressure glove.

After 4 months of wearing the pressure garments the scars of both patients were noticeably more extensible to the touch. The tissue had lost its angry red appearance and was much more pliable.

6.7. Discussion

Pressure therapy does result in a remodelling of the mechanical characteristics of hypertrophic scar tissue, such that they tend more

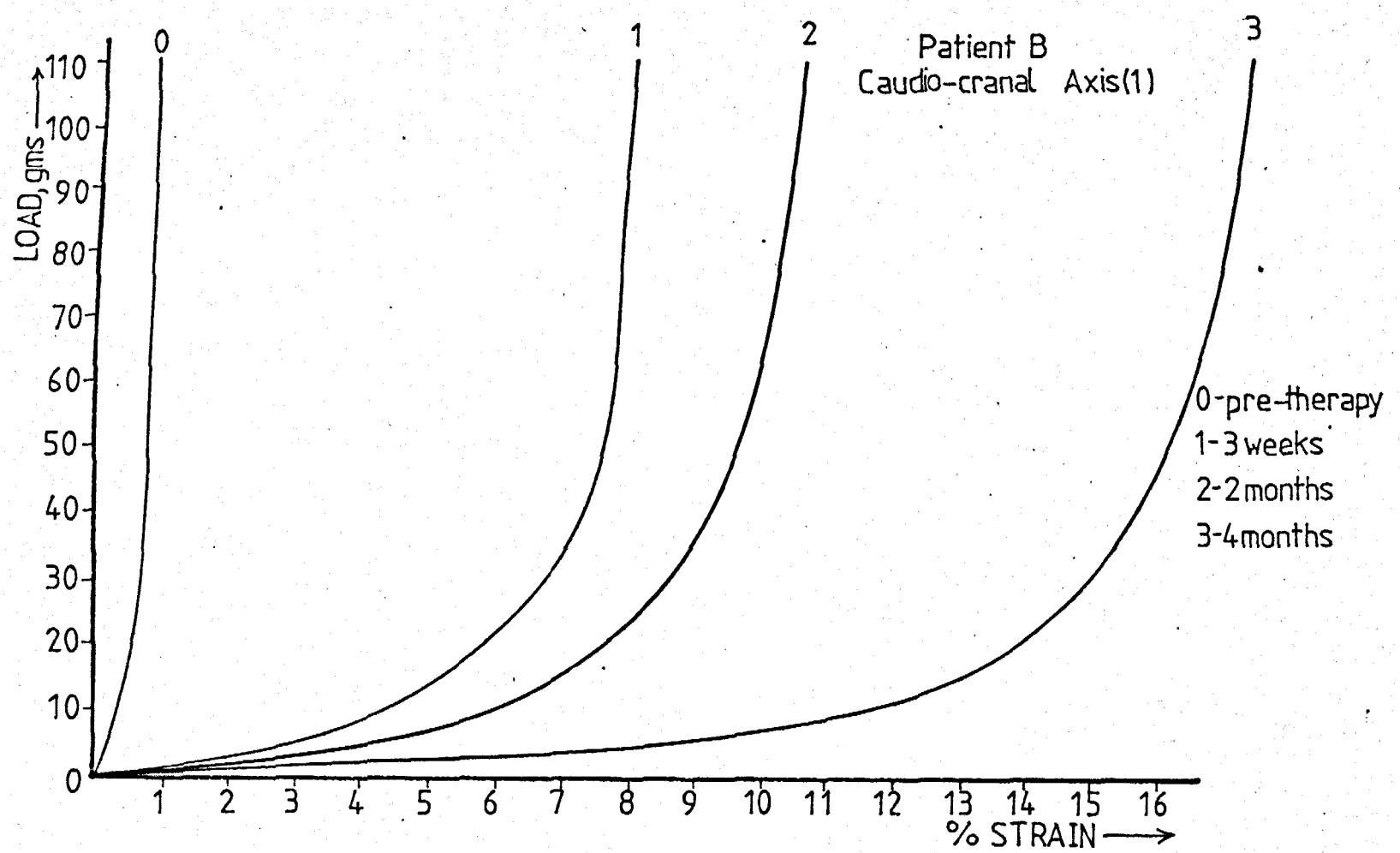


Fig. 6.16. In-vivo mechanical characteristics, showing gradual increase in extensibility with pressure therapy.

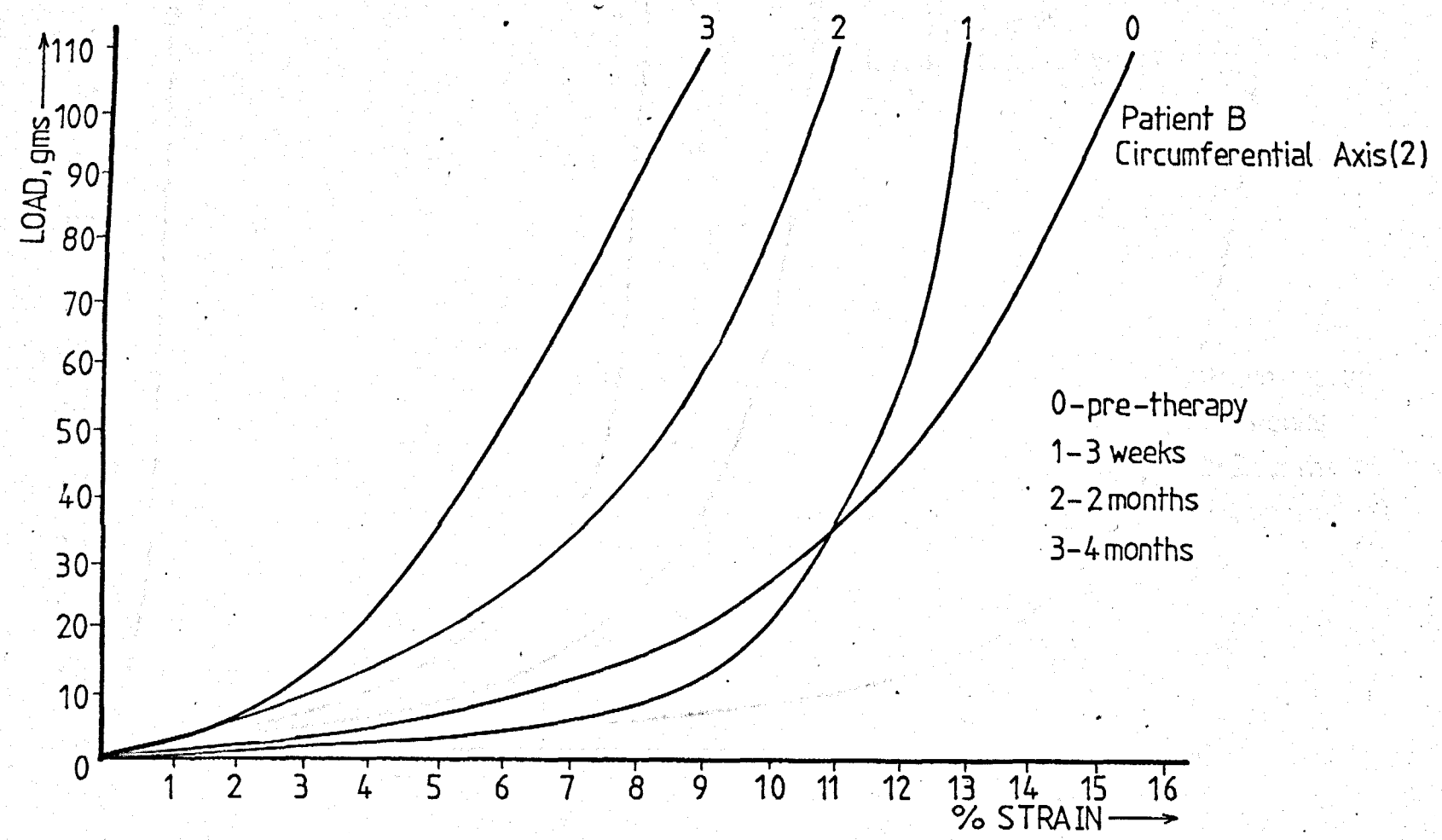


Fig. 6.17. In-vivo mechanical characteristics, showing decrease in extensibility with pressure therapy.

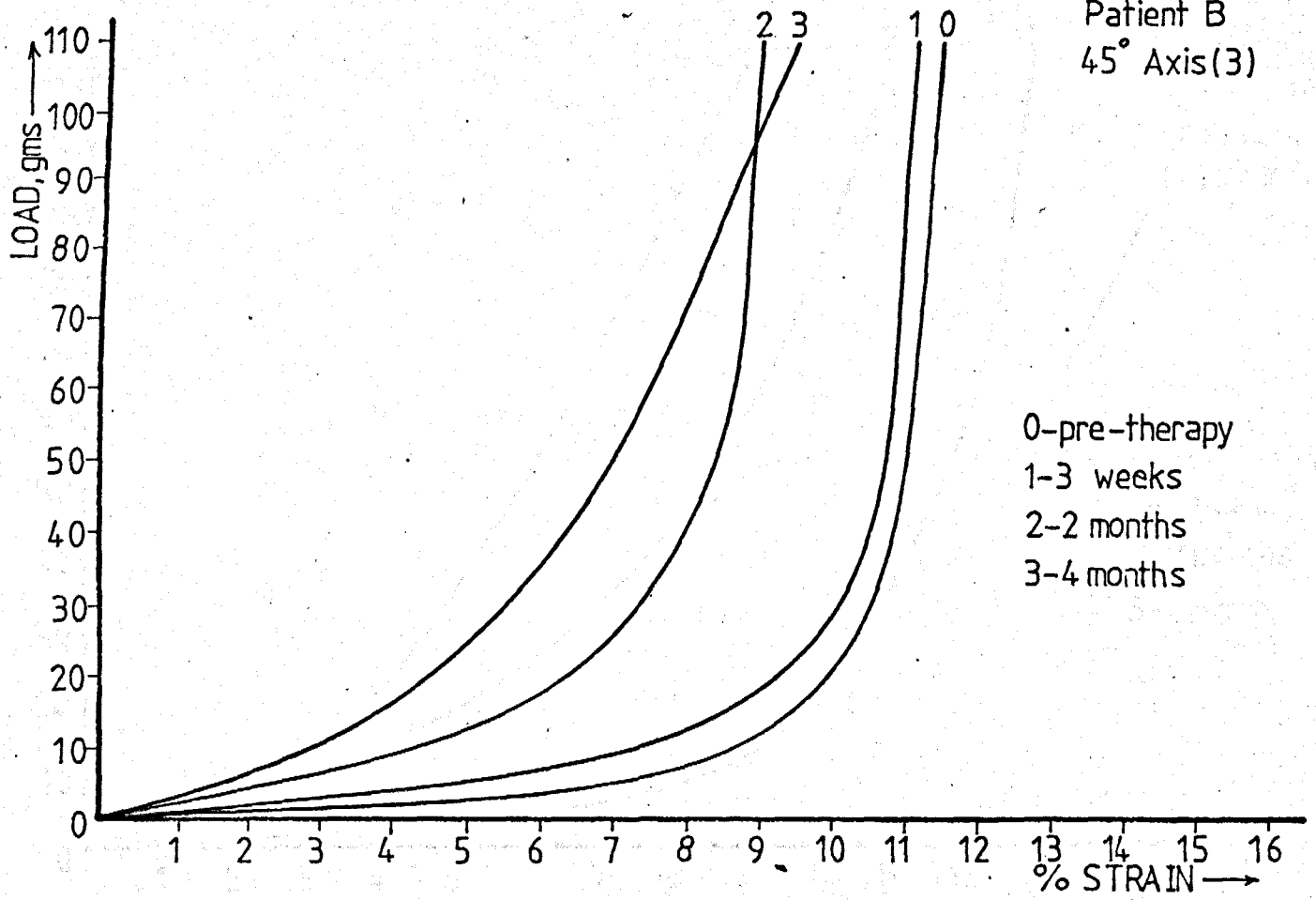


Fig. 6.18. In-vivo mechanical characteristics, showing decrease in extensibility with pressure therapy.

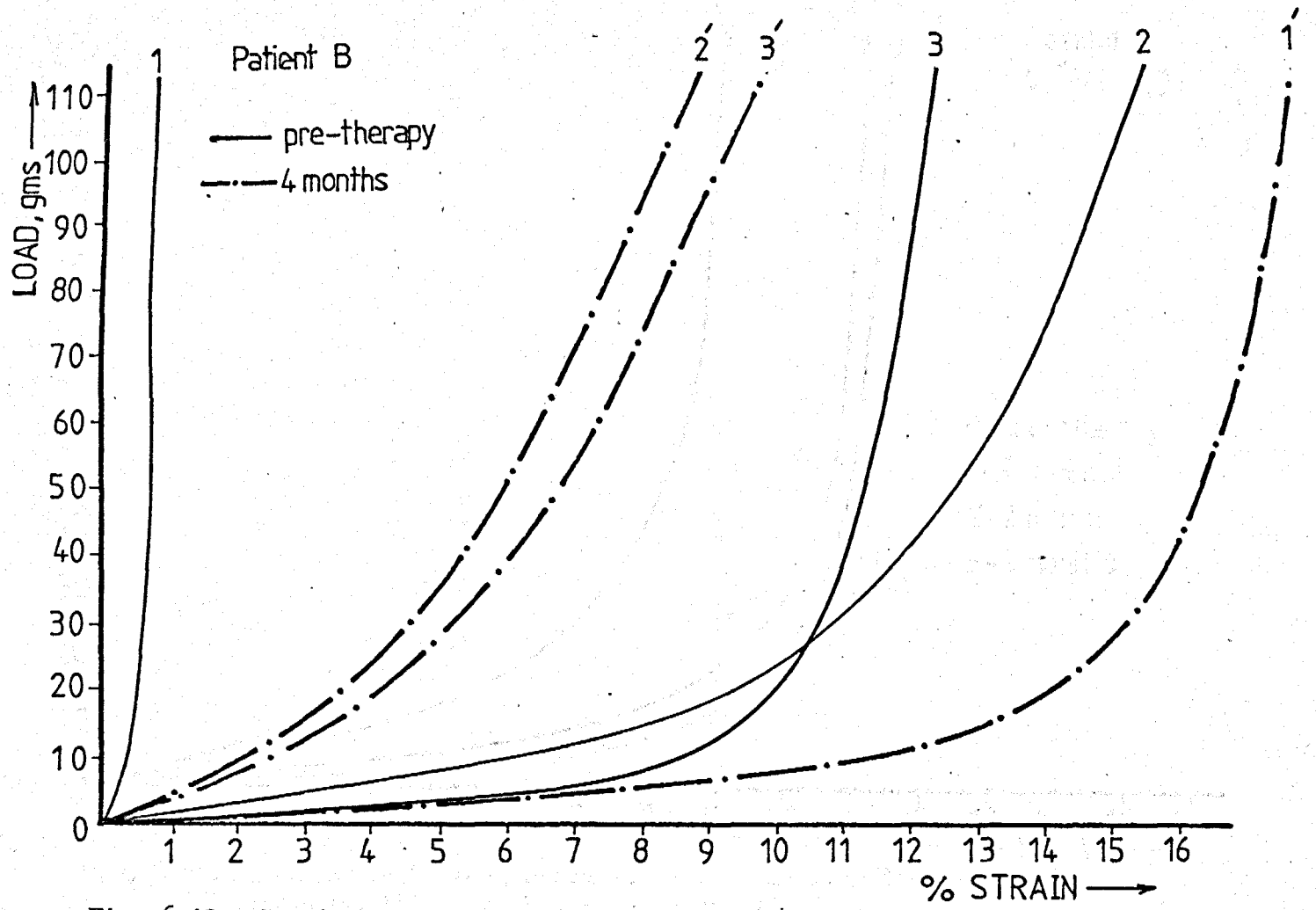


Fig. 6.19. In-vivo mechanical characteristics, showing increase in extensibility in caudio-cranal axis (at the expense of the other two axes) with pressure therapy.

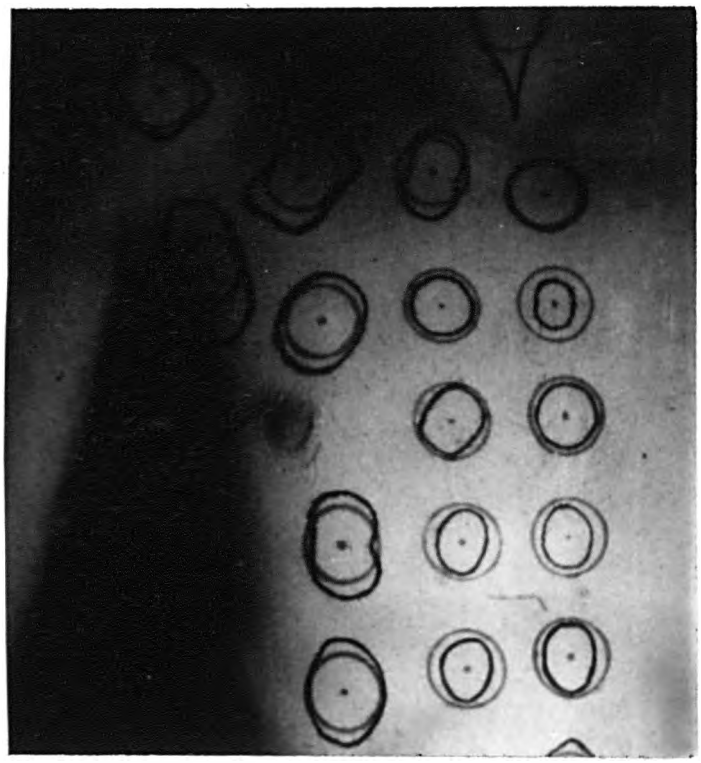


Fig.6.20. Polar plots of extension obtained with constant load. Normal skin, chest, right anterior aspect.
(After Stark, 1970).

towards those of normal skin.

With Patient A the increase in the amount of extension produced by a given load was measurable after only five days (Figs. 6.12 - 6.14) and all three test axes showed a continued increase in extensibility with maintained pressure.

However, with Patient B, although there was a large increase in extensibility in the direction which had originally been the least extensible (the caudio-cranal axis), there was a decrease in extensibility for the circumferential and the 45° axes (Figs. 6.17. and 6.18.).

Fig. 6.19. shows the load/strain characteristics for Patient B for the three test directions both before commencing pressure therapy, and four months later. Initially there was gross anisotropy in the characteristics; the tissue was virtually inextensible in the caudio-cranal axis. After four months of constant pressure the extensibility in this direction had vastly improved, whilst there had been a gradual stiffening in the other two directions. Fig. 6.20. shows the polar plots obtained by Stark (1970) for normal skin, of the % extension achieved with a constant load (14.2 g/m.m. which was sufficient to achieve an extension marginally in excess of that associated with the elbow in the load/extension relationship (see section 2.6.2.)), the true circles representing an extension of 20%. It can be seen that the mechanical characteristics of the scar of Patient B after four months of therapy are much more like those of normal skin. The tissue, though still less extensible than normal skin, does not now exhibit the gross anisotropy in its characteristics.

Fig. 6.15. shows a similar set of "before and after" mechanical characteristics for Patient A. However, in this instance there is

no marked anisotropy in extensibility before treatment; and there is a general increase in extensibility with therapy in all three directions tested, although again the direction that was initially the most inextensible shows the greatest improvement.

Both of these sets of data imply that the tissue has undergone a remodelling of its mechanical characteristics with the application of pressure therapy. The effect of this remodelling is to alter the characteristics towards those of normal skin, and, if there is initially gross anisotropy in the extensibility this will result in a "swings and roundabouts" situation where some axes have to "give up" some of their extension capabilities so that the abnormality can be rectified.

CHAPTER 7

DISCUSSION

"This is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning."

Winston Churchill. 1874-1965.

7.1. Introduction

The medical literature abounds with case reports on the disfiguring and disabling sequelae of excessive scar formation. Although much effort has been put into detailing the early stages of wound healing the analysis, by comparison, of the later stages of scar formation and scar tissue remodelling has been relatively neglected.

In Chapter 3 the results of a detailed analysis of the micro-structure of hypertrophic scar tissue are presented. These results have been used as a base upon which to investigate both the inter-relationship between the mechanical properties of the scar tissue and its micro-structure, and the effect of pressure therapy upon these properties.

The changes observed in hypertrophic scar tissue micro-structure with pressure therapy are presented in Chapter 4. The inter-relationship between micro-structure and mechanical properties, and the changes in mechanical properties with sustained pressure are discussed in Chapters 5 and 6 respectively.

7.1.1. Micro-architecture. The overall micro-architectural picture of hypertrophic scar tissue presented in Chapter 3, is that of tightly packed, smaller than normal diameter collagen fibres, grouped into large bundles or sheets. This observation differed from that presented by Larson, Linares, Kischer and colleagues, (Larson et al, 1968, 1970, 1974a,b, 1976a,b; Linares et al, 1972, 1973, 1976, 1977, 1978; Kischer et al, 1971, 1972, 1974a,b, 1975, 1978) who described a whorled pattern of collagen fibres orientated around a central mass or nodule. These nodules were also observed by the author, but whereas Larson and colleagues described the centre

of the nodules as being formed by a complete fusion of the fibres into a homogenous mat of collagen, the micrographs in section 3.3.3. show that the nodules are in fact the cut ends of extremely large (up to 1.5 m.m. in diameter) bundles of collagen fibres. Similar observations of criss-crossing bands of fibres enclosing other transversely cut bands of collagen fibres have been shown by Brown and Gibson (1974) and Hunter and Finlay (1976).

Most of the descriptions of the micro-architecture of hypertrophic scars has been confined to the dermis of the tissue. Although the results presented in Chapter 3 show some abnormalities in the epidermis, papillary and deep-dermal layers of the scar tissue the major area of involvement in the excessive fibrous growth was found to be the mid-dermis of the tissue. An observation which supports the general view in the literature.

The results obtained when observing tissue sections with a light microscope are in effect only two dimensional views of a three dimensional structure. With the greater depth of focus available with the advent of the scanning electron microscope a closer approximation to a three dimensional picture is obtainable. Unfortunately a high proportion of researchers still interpret their results as though they were dealing with a planar structure, so that they describe the development of a hypertrophic scar as the collagen fibres progressing from a curvilinear pattern into whorl-like arrangements and finally into nodules surrounded by septal-like capsules, without once describing what is happening in the third dimension.

The micrographs shown in Chapters 3, 4 and 5 show that the collagen fibres of hypertrophic scar tissue are arranged in a distinctly anisotropic three-dimensional pattern, which has a direct

bearing on the mechanical properties of the tissue.

The pattern of blood vessels observed in the scar tissue studied is described in Chapter 4, section 4.4.5. Large diameter vessels (up to 20μm) arranged in loops, were observed in the papillary and superficial layers of the hypertrophic scars. Longacre and colleagues (1976) described similar large calibre, engorged blood vessels. These vessels were still in evidence after four months of continuous pressure therapy, although they were not visible in the mature, successfully remodelled hypertrophic scar studied.

7.1.2. Mechanical characteristics of hypertrophic scar tissue.

In Chapter 5 the results obtained from a study of the mechanical characteristics of five specimens of hypertrophic scar tissue obtained from contracture deformities of the neck and axilla are presented. In general the scar tissue is much less extensible than normal skin. The initial lax phase in the characteristic of normal skin, where large strains of the order of 25% to 60% are produced by only small increases in load, is absent in the load/strain characteristic of the scar tissue. Instead quite high loads are needed to produce even small strains.

All the specimens of hypertrophic scar tissue exhibited anisotropy in their mechanical characteristics, this anisotropy was also reflected in the fibrous micro-architecture of the tissue. The less mature, more 'active' scars showing the highest degree of anisotropy. Fig. 5.11. shows the mechanical characteristics of a nine month old contracture of the neck as compared to the characteristics of a five year old neck contracture shown in Fig. 5.10.

Tension has been cited as an aggravating factor in the aetiology of hypertrophic scar tissue (Trusler and Bauer, 1948; Howlem, 1951;

Longacre et al 1968). There are two types of tension acting in the skin; these are the static or inherent tensions as described by Langer (1861) (Fig. 2.25.), and the dynamic tensions produced by muscle and joint movement (Thacker et al, 1976). The latter forces having a direct influence on the magnitude and direction of the static tensions.

The results presented in Chapter 5 showed that the scar tissue obtained from contractures of the neck and axilla was much more inextensible in a direction parallel to Langers lines, and that the collagen fibres in the dermis were orientated along this axis.

On examining the distribution of static and dynamic tensions for those other areas of the body particularly prone to the formation of contracture deformities i.e., the backs of the knees and the insides of the elbows, it can be seen that the contracture bands of the scar tissue lie in a direction parallel to both Langers lines and the dynamic tensions that would be produced by the flexing of these joints.

The observation that the collagen fibres within a contracture band are orientated in the direction of maximum tension refutes the hypothesis put forward by Finlay and Hunter (1976) that the large amount of fibrous tissue in these scars was due not to excessive forces acting on the wound, but to the inability of the fibroblasts to lay down the collagen in the direction of stress.

In Chapter 6 the results obtained from the monitoring of changes in mechanical properties with pressure therapy are presented. The overall effect of the therapy was an improvement in extensibility of the tissue and a remodelling of the characteristics towards those of normal skin (Figs. 6.15. and 6.19.).

Both of the areas of scar tissue monitored consisted of large plaques of hypertrophic scar tissue lying over relatively flat, bony regions of the body, (back of the hand, and lower right anterior aspect of the chest, respectively) as opposed to the contracture webs lying across joints, studied and discussed in Chapter 5. In these regions of the body the distribution of the static and dynamic tensions is somewhat different and the distortional effects of joint movement are now minimal.

From Fig. 6.19. it can be seen that the scar tissue exhibits marked anisotropy in its mechanical characteristics, being much less extensible in the caudio-cranal axis, that is at right angles to Langers lines for this region (Fig. 2.25.). This would appear to contradict the results presented in Chapter 5. However, as stated above, the relative distribution of the static and dynamic tensions in these and similar body sites is different.

A wound heals and restores the skin's continuity by the triple processes of epithelisation, wound contraction and scar formation. Wound contraction is the centripetal movement of the whole thickness of the skin surrounding the wound and this results in the surrounding skin being stretched, thinned and placed under tension (Peacock and Van Winkle, 1968). The size of the wound does not affect the rate of contraction, but rather it is limited by the amount of mobile uninjured skin available to contract. Therefore the greatest amount of contraction of the wound edges will occur in those directions in which the skin is most extensible, i.e. at right angles to Langers lines (Fig. 2.20.).

In those areas of the body in which there is a high degree of dynamic tension produced by muscle and joint movement, the tension

produced by the contraction of the wound edges is insufficient to influence the orientation of the collagen fibres. However, in those areas where the dynamic tensions are minimal the collagen fibres will be layed down in the direction of maximum wound edge contraction in an effort to relieve the increased tension in the surrounding skin produced by this contraction.

Other researchers have reported on the effect that tension has on the process of wound healing. Sussman (1969) and Myers and colleagues (1969) showed that increased tissue traction across a wound can result in an increase in the tensile strength of the wound tissue. This increase in tensile strength was observed by Forrester and co-workers (1969) to be reflected in the fibre micro-architecture. The resultant scar, though of greater tensile strength, was less extensible than the scar of a wound that had not healed under tension, and thus the final result was a brittle scar, with no greater ability to absorb energy than the supposedly weaker scar.

The general view of the maturation of scar tissue is that the process involves both new collagen being laid down and existing collagen being remodelled (Arem and Madden, 1976). Since collagen is a non-living material the remodelling that occurs must ultimately depend upon the ability of the living cells to sense and transduce the mechanical forces into biomechanical action. Therefore the degree of force, the rate, frequency and duration of its application, and the loading velocity will all be important in producing the remodelling of the hypertrophic scar tissue.

7.1.3. <u>The effect of pressure therapy on hypertrophic scar tissue</u>. The results of the observation of the effect of pressure therapy on the micro-architecture, and the mechanical properties of

hypertrophic scar tissue are presented in Chapters 3 and 6 respectively.

The tissue micro-structure progressed from the densely packed orientated arrangement of collagen fibres with an abnormally high number of fibroblasts, and large diameter blood vessels indicative of active hypertrophic scar tissue; to the less cellular and less tightly packed, parallel aligned fibre pattern similar to that observed in specimens of mature non-hypertrophic scars (Brown and Gibson, 1974).

The effect of the pressure therapy on the mechanical properties of the hypertrophic scar tissue, was a progressive remodelling of the characteristics to give a generally more extensible tissue. In the scar tissue which was originally grossly anisotropic this necessitated the remodelling of the tissue in such a way that the final result was an increase in extensibility in the direction where it was most needed at the expense of a stiffening in the other two directions.

7.2. Summary and Suggestions for Future Work

The work presented in this thesis can really only be considered as a pilot study of the characteristics of hypertrophic scar tissue. A detailed micro-architectural picture of the tissue as viewed with the scanning electron microscope has been established, and this picture has been used as a basis upon which to study both the inter-relationship between micro-structural and mechanical properties, and the effects of pressure therapy in the treatment of these disfiguring scars.

Although the S.E.M. has a large depth of focus which enables the 3-dimensional arrangement of the tissue structure to be better

observed, individual tissue components cannot easily be labelled. The selective staining which is possible when using the light microscope is required to label the individual cells and fibres that make up the tissue.

Some light micrographs of hypertrophic scar tissue are presented in Chapter 4. These demonstrate the increase in information that can be achieved by using a combined S.E.M. and light microscopy protocol. The excessive number of fibroblasts visible in the light micrograph were not visible in the S.E.M. micrograph, whilst the 3-dimensional arrangement of the collagen fibres visible with the S.E.M. could not be seen using the light microscope.

The Transmission Electron Microscope (T.E.M.), with its high magnification, offers the means to observe the structure of the individual cells and the ultrastructure of the other constituents of the tissue.

By using these three types of microscopy a comprehensive analysis of hypertrophic scar tissue is possible. The differences already observed between the scar tissue and normal skin in cell type, cell distribution, fibre formation and packing and other parameters (see Chapter 2) by different researchers need to be combined, using these techniques, to explain the prime requisite of why some wounds heal with the formation of hypertrophic scar tissue.

Tension has been shown to be one of the key factors in the government of the orientation of the fibres of the dermis and their corresponding mechanical properties (see Chapter 5). Observing the mechanical properties of the tissue, in-vitro, gives useful information on the inter-relationship between micro-structure and mechanical characteristics, but the analysis of the in-vivo characteristics,

and the way in which they change with time and therapy is required to fully understand this relationship; since the very nature of in-vitro tests introduces discrepancies in the results obtained.

The changes in mechanical properties with pressure therapy, in-vivo, for two patients are discussed in Chapter 6. More work needs to be done on monitoring these properties throughout the development of the scar tissue; from its earliest stages of development right through to its mature, fully remodelled state. Since the mechanical characteristics of a tissue are dependent upon the micro-structure of that tissue, the effects produced by the abnormalities in micro-structure could then be observed and used in the investigation of the aetiology of the scars.

Pressure therapy accelerates the natural remodelling process of hypertrophic scar tissue. The tissue biopsies used by the author to observe the changes in micro-structure with pressure therapy (Chapter 4) were really too small to allow the detailed observation of changes in fibrous micro-architecture. The size of the tissue biopsy available is obviously limited, but in order to observe the fibre orientation and packing properly as large a tissue biopsy as possible is required.

The way in which pressure therapy remodels the mechanical characteristics of the hypertrophic scar tissue is still not clear. It perhaps achieves the remodelling by relieving the aggravating tensional forces acting on the scar by supplying a mechanical support to the tissue. To further investigate the way in which the therapy works, the mechanical properties of the scar tissue need to be measured in-vivo, whilst the pressure is being applied to the tissue.

There are other forms of pressure therapy which have also been

reported as being successful in helping to prevent, and accelerate the remodelling of, hypertrophic scar tissue and contracture deformities. (See section 2.9.5.). These take the form of various types of splinting, used predominantly over joints where contractures are formed. Both static; in which the joint is held immobile, and dynamic; in which a system of springs allows, and in some forms encourages, movement of the joint; splints have been used successfully (Larson et al, 1971, 1974; Huang et al, 1978). These types of treatment also need to be fully investigated, so that an optimised therapy regimen can eventually be achieved which will give the maximum benefit to the patient.

In conclusion, pressure therapy accelerates the natural remodelling processes of hypertrophic scar tissue, and with further developments of combinations of static and dynamic pressure and splinting therapy the techniques can be optimised to give the greatest benefit to the patient. These forms of therapy have basically been used in the correction of already formed hypertrophic scars. The prime requirement of future work must be in the prevention of the formation of these disfiguring and disabling scars. Further study of both the micro-structure and the mechanical properties of hypertrophic scars will eventually explain the aetiology of this tissue, and in doing so will show the way in which its formation, and the associated debilitating consequences, can be prevented.

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APPENDIX A

Patient Case Histories (Chapter 3)

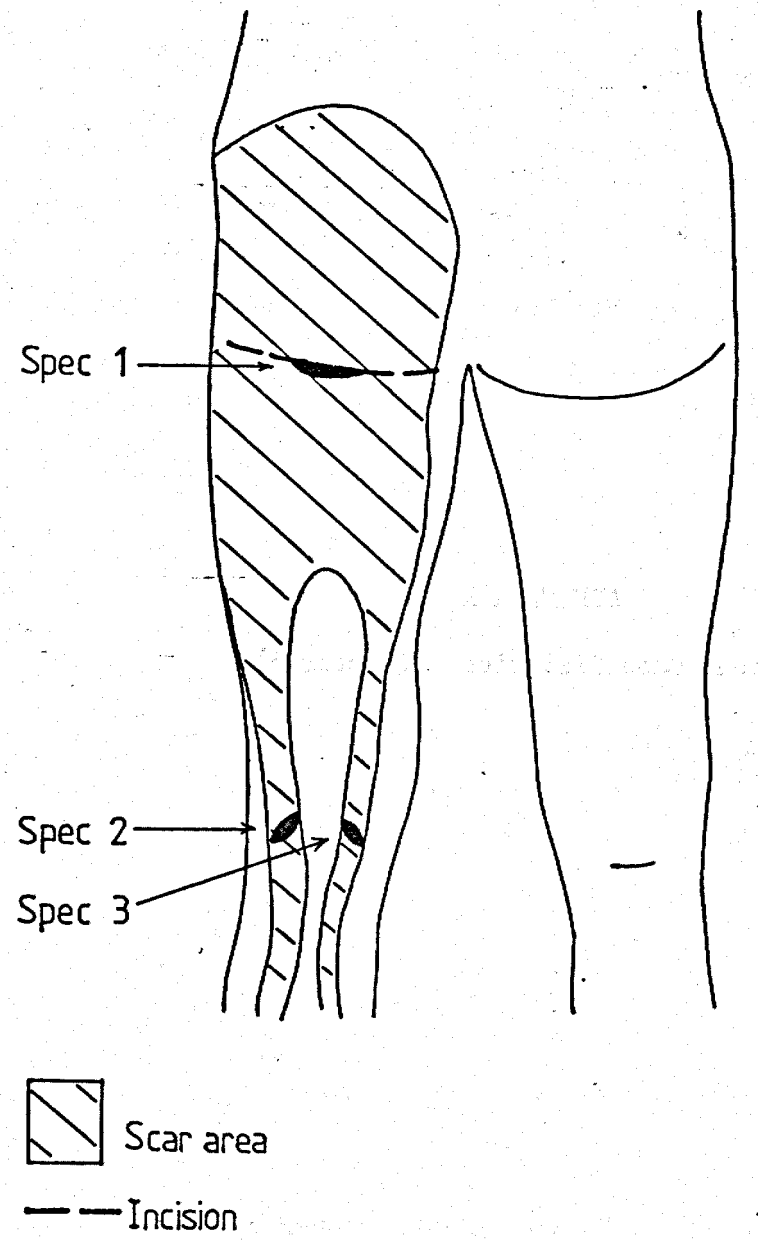

Fig. A1. Case no. 1. Specimen sites.

Case No. 1

Sex	Male
Race	Caucasian
Date of Birth	4.8.64
Date of Injury	?.9.75
Date of Specimen Excision	21.1.76
Cause of Injury	Scald

Extent/Severity Range of severities from superficial, through partial thickness to full thickness. Scarring of left buttock and back of left leg. Complete absence of buttock crease. Scar over this region, red, shiny and hard to the touch. Two fairly tight bands of scar tissue traversing the posterior knee (Fig. A1.) These were not greatly restricting knee extension but patient complained of stiffness on exercising.

Initial Treatment Grafted?

Previous Treatment Two previous attendances?

Surgical Procedure Incision made to relieve scar tension over the buttock (Fig. A1.). SSG taken from right buttock to resurface resulting defect. SSG applied one day post-operatively.

Z-plasties performed on the two contracture bands traversing the back of the knee.

The site and orientation of the specimens obtained are shown in Fig. A1.

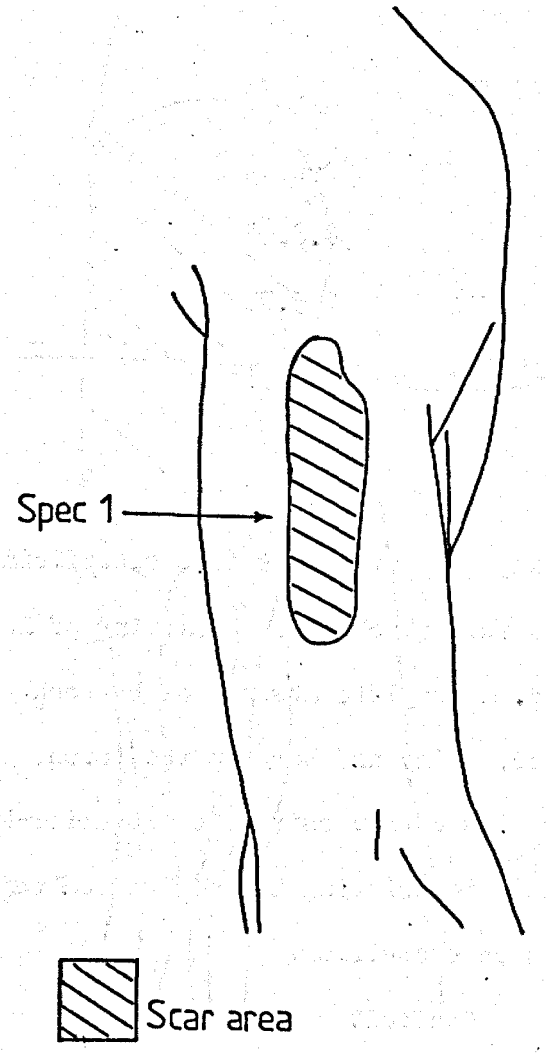

Fig. A2. Case no. 2. Specimen site.

Case No. 2

Sex	Male
Race	Caucasian
Date of Birth	28.8.61
Date of Injury	?.12.74
Date of Specimen Excision	21.6.76
Cause of Injury	Scald

Extent/Severity Partial thickness injury to right deltoid region. Healed spontaneously. Original wound area approximately 6 cm long and 2 cm wide. Final scar area 10 cm long, 3.5 cm wide and in places 1.5 cm thick. Diagnosed as keloid. No functional disability, but cosmetically unacceptable.

Initial Treatment Healed spontaneously

Previous Treatment None

Surgical Procedure Entire scar excised down to the fat layer. SSG taken from left anterior thigh to resurface defect one day post-operatively.

Scar/Specimen site shown in Fig. A2.

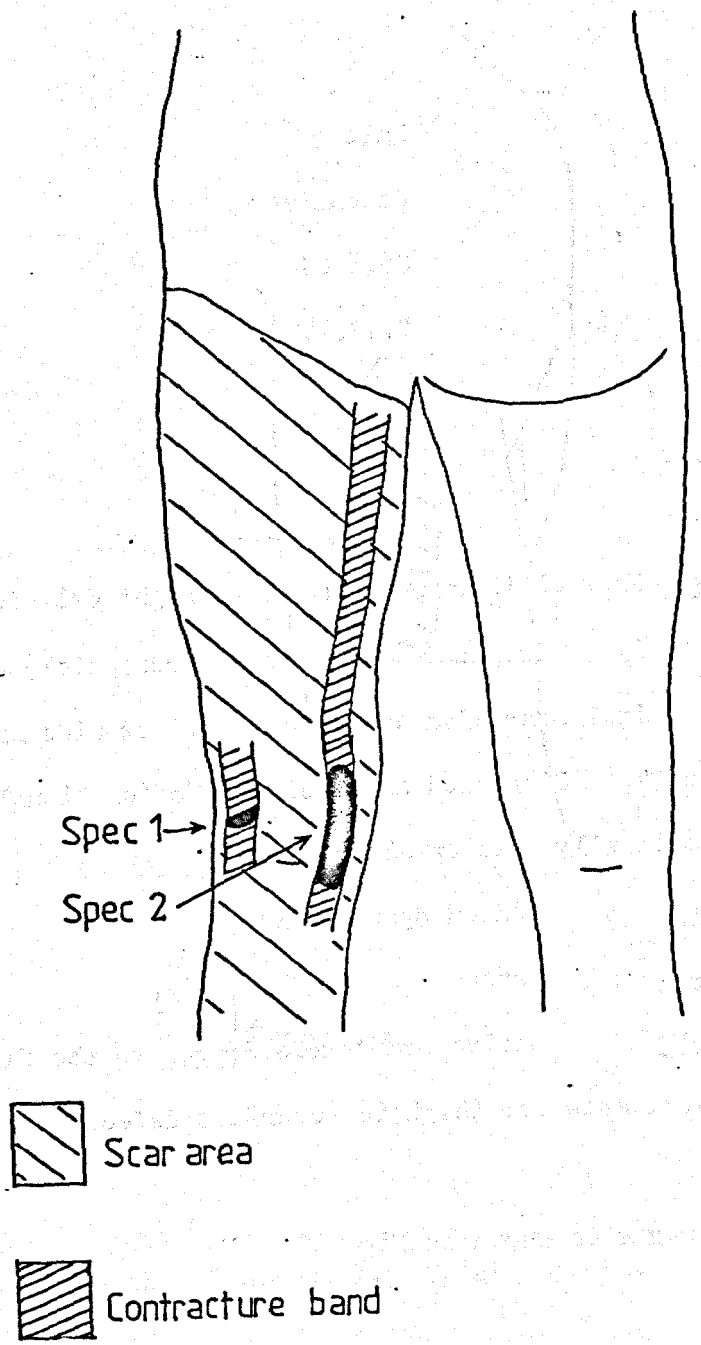

Fig. A3. Case no. 3. Specimen sites.

Case No. 3

Sex	Female
Race	Caucasian
Date of Birth	21.6.69
Date of Injury	1.5.74
Date of Specimen Excision	23.8.76
Cause of Injury	Degloving of left leg in road traffic accident

Extent/Severity Degloving of left leg from top of leg to $^{2}/_{3}$ down between knee and ankle. General appearance very poor. Large areas of hypertrophic scar tissue interspersed with areas of meshed and SSG. Contracture bands over back of knee. Areas where original graft had taken quite pliant.

Initial Treatment SSG obtained from right buttock and thigh. Graft meshed before application due to large wound area. Donor site healed well. Incomplete 'take' of graft.

Previous Treatment None

Surgical Procedure Most pronounced contracture band ran down the medial aspect of the leg from crutch to just below the knee. Lower region of this band was excised. Although the bulk of the scar was removed there remained thin bands of scar tissue deep within the wound. These bands were cut in an effort to break up the scar fibre orientation and ease the tension across the joint.

A smaller excision was made in the lateral contracture band. SSG taken from buttock to resurface defects one day post-operatively.

Scar/Specimen site shown in Fig. A3.

APPENDIX B

Scale diagram of rotary biopsy punch locking collar

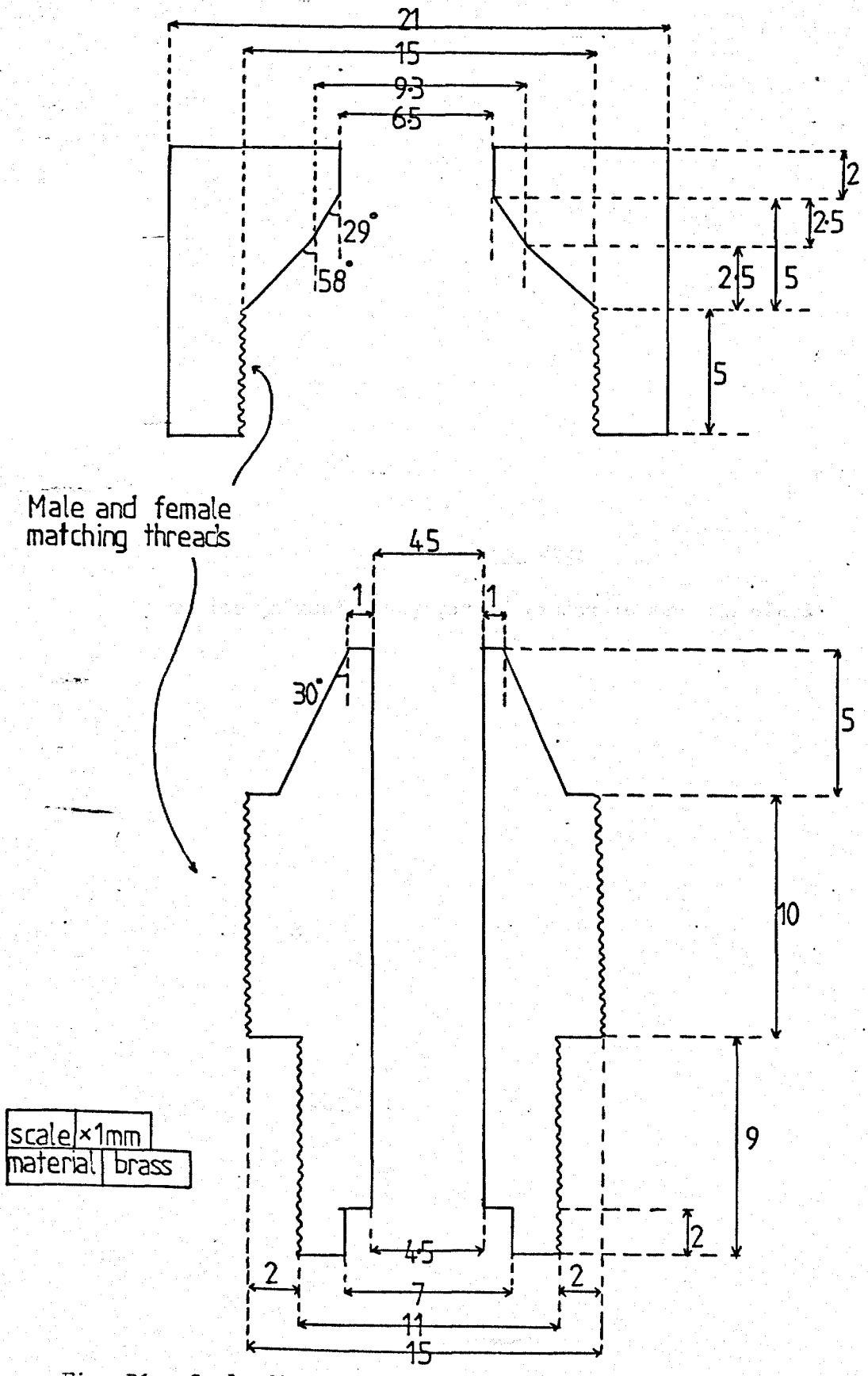

Fig. B1. Scale diagram of rotary biopsy punch collar to which quick release socket and cutting bit are attached.

APPENDIX C

T.E.M. Tissue Processing Schedule and L.M. Staining Procedures

C.1. T.E.M. Tissue Processing Schedule

C.1.1. Fixatives

Fixative A. 5% Glutaraldehyde.

0.2M Sodium cacodylate buffer. pH = 7.5	80 ml
25% Glutaraldehyde.	20 ml
	100 ml

Fixative B. 1% Osmium tetroxide.

O_sO_4	1 gm
Distilled water/ 0.1M sodium cacodylate	100 ml

C.1.2. Epon Resin

Resin A

Epon 812	62 ml
DDSA	100 ml

Resin B

Epon 812	50 ml
MNA	44.5 ml

Final Embedding Mixture, 10 ml/specimen

Resin A	7 ml
Resin B	3 ml
DMP 30	0.25 ml

C.1.3. Tissue processing schedule

1.	Fixative A	not less than 2 hrs.
2.	Mince specimen	
3.	0.1M sodium cacodylate + 0.1M sucrose	overnight or store
4.	Fixative B	30 min.- 60 min.
5.	0.1M sodium cacodylate	15 min.
6.	" " "	"
7.	" " "	"
8.	" " "	1 - 2 hrs.
9.	30% ethanol	30 min. - 60 min.
10.	50% "	"
11.	70% "	"
12.	90% "	"
13.	100% "	"
14.	100% Propylene oxide	"
15.	" " "	"
16.	Propylene oxide/complete resin (1:1)	30 min.
17.	" " / " " (1:3)	"
18.	Complete resin	overnight in dessicator
19.	Embed in complete resin and polymerize at 60°C overnight	

C.2. L.M. Staining Protocol

C.2.1. Hematoxylin and Eosin stain (H & E)

1. Harris' Hematoxylin

Hematoxylin crystals	5 gm
Alcohol 95%	50 ml
Ammonium or potassium alum	100 gm
Distilled water	1,000 ml
Mercuric oxide	2.5 gm

Dissolve the hematoxylin in the alcohol, and the alum in the water. Mix the two solutions. Bring mixture quickly to the boil, remove from heat, add mercuric oxide. Reheat until mixture becomes dark purple. Remove from heat, and plunge container in cold water.

2. Acid Alcohol

70% alcohol	1,000 ml
Hydrochloric acid, conc.	10 ml

3. Alcoholic Eosin

Eosin Y, water soluble	2 gm
Distilled water	160 ml
Alcohol 95%	640 ml

4. Staining procedure

1.	Xylol, absolute alcohol, 95%, 75% alcohol, distilled water	
2.	Harris' Hematoxylin	5 min.
3.	Rinse in tap water	
4.	Differentiate in acid alcohol. (Check differentiation of nucleii, should be distinct in very pale background).	
5.	Wash in running water	10 - 20 min.
6.	Stain with Eosin	15 sec - 2 min. (depending on degree of counter-stain required)
7.	95% alcohol	

8. Absolute alcohol 2 changes

9. Xylol 2 changes

10. Mount in Permount

C.2.2. Acid Orcein and Giemsa Stain (AO & G)

1. Acid Orcein

Orcein Synthetic Harleco	0.2 gm
70% ethyl alcohol	100 ml
Hydrochloric acid, conc.	0.6 ml

Dissolve orcein in alcohol and add HCl. (Solution improves on standing and is stable for many months.)

2. Giemsa Solution

One drop of Giemsa stock solution for each 20 ml of distilled water.

3. 1% alcoholic solution of eosin yellowish

A few drops of this solution are added to 95% alcohol for decolorization of excessively blue sections.

4. Staining procedure

1. Xylol, absolute alcohol, 95% and 70% alcohol.

2. Immerse in Acid Orcein 30 - 60 min.

3. Wash in running water 10 min.

4. Stain in dilute Giemsa solution 12 - 15 hrs.

5. Wipe excess fluid off slides. Excessive blue staining can now be rectified using 1% alcoholic solution of eosin yellowish.

6. Remove sections when collagen begins to turn pink from blue.

7. Dehydrate in two changes of absolute alcohol.

8. Xylol. 2 changes.

9. Mount in Permount.