Applied Surgical Pathology

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Preface

The surgeon, radiologist and pathologist nowadays interpret disease together. The development of the multi-disciplinary approach to so many surgical problems implies that communication between members of the investigating team must be of the highest order.

The purpose of this book is to develop the concept of the pathologist coming from the laboratory and the radiologist from the x-ray department to meet the surgeon on common ground at the patient's bedside or in the consultation clinic. It was with this goal in mind that a number of teams, each with specialized knowledge of the diseases of a major system, were assembled by the Editors.

The enormous expansion of functional pathology as shown by the use of biochemical tests and biopsy, together with the development of dynamic radiological procedures, has added new dimensions to the study of disease. We hope that a balance has been struck in describing the part that these procedures play in the investigation of disease and that the surgeon, radiologist and pathologist may appreciate better each other's point of view.

While this book is written for surgeons and those undergoing surgical training, it is hoped that it will interest pathologists and radiologists who work closely with them. The systemic approach to disease has been preserved with the exception of certain topics which are more appropriately dealt with in specialized texts. Advances in surgical biology are reflected in the chapters on wound healing, cancer and the cell, haemorrhagic diseases, thromboembolism, transplantation and the reaction to injury.

The illustrations have come from various sources and we acknowledge the help of many colleagues. We owe a special debt of gratitude to the Royal College of Surgeons of Edinburgh for free access to material in its museum. Various authors and publishers who have generously allowed use of illustrations are individually acknowledged in the text.

We are indebted to our publishers, Mr. Per Saugman and Mr. Nigel Palmer, for their assistance in the planning and execution of the book; Mrs. Linda Adler has given inestimable assistance in its compilation.

We would finally thank our wives and families for their patience and forbearance during the long hours of preparation of this text.

June 1975

Angus E. Stuart
Adam N. Smith
Eric Samuel

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CHAPTER 1

Wound Healing and Tissue Repair

The phenomenon of wound healing is fundamental to all surgery. The characteristic chain of events is found to some degree wherever there is tissue damage. The same general process is followed in the organizing blood clot, the bed of a chronic ulcer and in relation to an invading tumour. Unfortunately the non-specific connective tissue scar that results, is often responsible for more trouble than the original injury. Its weakness and brittleness are related to incisional hernias and its long-term contracting properties become manifest in such diverse

conditions as oesophageal stricture, mitral stenosis, hepatic cirrhosis, adhesions in peritoneum and tendon sheath, and the irritable post-traumatic cerebral scar. Recent interest has centred on the possibility of controlling these undesirable features by specific treatment of the wound during the early phases of healing.

Types of Healing

Although the elements of tissue repair are the same in each, open and closed wounds heal quite differently. When an incision is closed with sutures and heals without complication it is known as *healing by first intention*. Union takes place by a combination of epithelialization and connective tissue formation. When an open wound is allowed to close naturally, union is accomplished by a combination of wound contraction, connective tissue formation and epithelialization. This is known as *healing by second intention*.

Primary healing is quicker and easier than secondary healing and is, therefore, to be sought where tissue viability and healing potential is in doubt, e.g. in severe peripheral vascular disease primary healing may be accomplished where an open wound would persist, become infected and possibly progress to gangrene.

The closed wound is particularly susceptible to infection in the first 4 days and there is much to be said for leaving a contaminated wound open for this period. It may then be closed and will heal no less quickly than if it had been closed at first. This technique is called *delayed primary closure* (secondary suture), and the result is sometimes called *healing by third intention*.

Phases of Healing

Although healing is a continuous process it is customary to divide it into phases. In the first 3 or 4 days there is no recordable tensile strength or contraction but the period is one of intense biological and chemical activity. The characteristic changes are those of early inflammation, with hyperaemia of the wound margins and leucocytic infiltration. Enzymes are released and there is lysis and removal of devitalized tissue. Formerly this was called the 'lag' or 'latent' period of wound healing, but it is better known as the *substrate phase* in which the wound is made ready for healing to progress. This stage is essential. It cannot be accelerated and if it is prolonged, for example by steroids or infection, the completion of healing is that much delayed.

The second phase is one in which strength increases rapidly and contraction is prominent. This is the *phase of proliferation* or fibroplasia. It is dominated by the growth and activity of the fibroblast–capillary system. Collagen and ground substance are deposited in increasing amounts. Collagenolysis is also increased and as a result collagen is in a state of dynamic equilibrium in the wound. Collagen synthesis is in excess of lysis in normal situations but if, for example, ascorbic acid deficiency should supervene the balance is upset and the wound may break down.

The second phase lasts on average 2–3 weeks and then gradually moderates to merge imperceptibly with the third phase or *phase of maturation*. Fibroblasts and macrophages disappear and the vascularity of the wound decreases. It becomes pale and more like normal tissue. Collagen turnover is still higher than normal but measurements of total collagen in the wound show a slight reduction. The continued synthetic and lytic activities suggest collagen remodelling may be occurring, but recent physical studies show that this is far from complete and the wound remains a somewhat weak and brittle tissue indefinitely.

Elements of Healing

Several distinct elements contribute to the process of repair. Although closely related they have individual behaviour characteristics and merit separate consideration.

The first element is *epithelialization*. This is the process by which surface covering of the wound is restored by a combination of cell migration and multiplication.

The second element is *contraction*. This is the physiological process by which the edges of an open wound gradually close together. The whole thickness of the skin and subcutaneous tissue surrounding the wound moves bodily inwards under the influence of the pull of fibroblasts at the wound edge. By and large it does not involve the synthesis of new tissue. Contraction must be distinguished from the pathological process of

scar contracture which causes distortion and limitation of movement.

The third element is *connective tissue formation*. This is the process by which the main body of the wound is united. It plays a fundamental role in all but the most superficial injuries and the strength of a wound following surgery is dependent on it. A high proportion of wound healing studies have been made solely on this element of repair, and it is often discussed as if it alone represented the entire process of wound healing.

The mechanism of connective tissue formation is similar in every situation. It is based on the development of *the fibroblast–capillary system*. This can be thought of as an 'organ of repair'. The fibroblast is the key cell synthesizing both collagen and intercellular ground substance. Adequate oxygenation demands that it be within 50 μm of the nearest capillary. As a result the fibroblast–capillary system develops as a unit and continues to grow until the wound is filled. This new vascular connective tissue is most obvious in open wounds where it is called *granulation tissue*, but it is also present in the narrow gap between the edges of the healing incised wound. As time passes this new tissue is transformed into the mature fibrous scar and the fibroblasts and capillaries disappear. Hence the fibroblast–capillary system is also known as 'the ephemeral organ of repair'.

A fourth element of repair is sometimes considered. This is the process of *reformation of tissue*. A familiar example is the process by which excessive blood loss is made good. The compensatory hypertrophy of one kidney following the removal of the other is also an expression of some sort of repair process.

These elements of repair are common to all types of healing but the relative contribution of each can be quite different. Healing in the incised wound is dominated by aspects of connective tissue formation whereas in the open wound, epithelialization and contraction are more obviously involved.

The Incised Wound

MECHANICAL ASPECTS

The rate of gain of strength has obvious clinical significance. It has been used as a measure of healing for over 100 years and is regarded by some as the single most useful indication of the progress of repair. Certainly its early use cast light on the mechanism of abdominal wound dehiscence [9]. The main problem here is a failure of fascial healing and tensiometric studies showed that strength recovery was much slower than previously believed. There is a rapid gain in strength during the first few weeks but thereafter strength increases more slowly. At the end of a year there is only 70 per cent recovery (Fig. 1.1). More recently the same strength

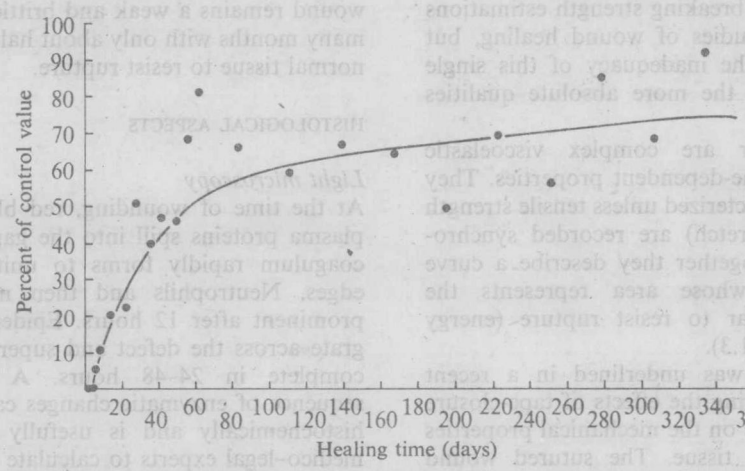

FIG. 1.1. Breaking strength of healing aponeurotic wounds. Strength increases rapidly for several weeks but then slows. There is only 70 per cent recovery by the end of a year. (Reproduced by permission from Douglas D. M. (1952) *Brit. J. Surg.* **40**, 79–84.)

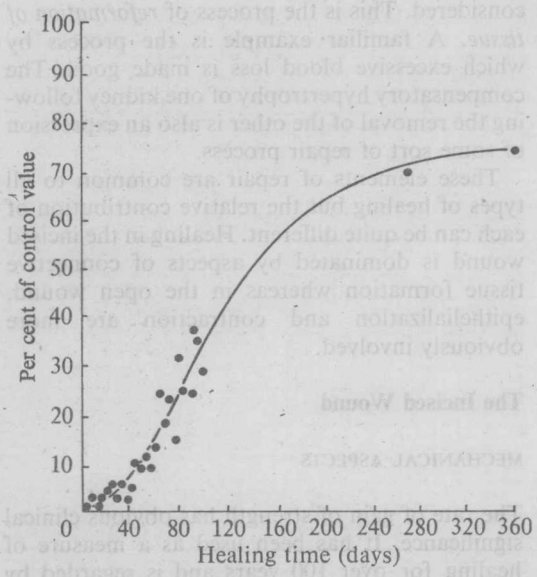

FIG. 1.2. Breaking strength of healing skin wounds. Strength recovers quickly to begin with but is still only 70 per cent of normal after a year.

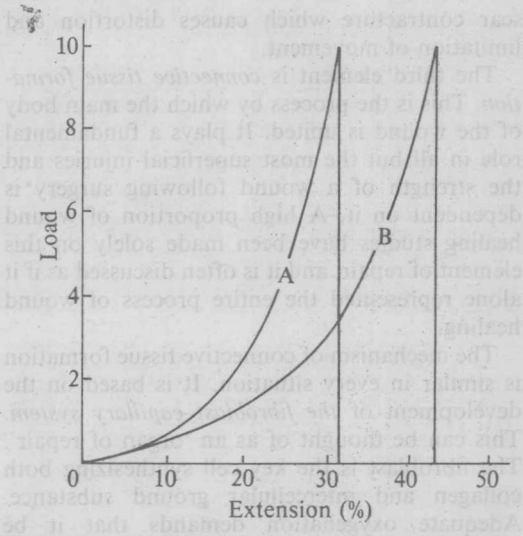

FIG. 1.3. Load-extension curves for wounds which break under the same load but differ in their extensions. Wound A is less pliable than wound B and is therefore more easily ruptured. The ability to resist rupture (energy absorption) is measured by the area under the curve.

changes have been recorded in healing skin wounds (Fig. 1.2). This is at first sight surprising for it is most unusual for the skin to break over an incisional hernia. The reason is simply that a skin scar, unlike its counterpart in fascia, is protected from direct physical forces by its elastic surrounds.

Recent bioengineering studies have confirmed the value of these breaking strength estimations in comparative studies of wound healing, but have underlined the inadequacy of this single test in describing the more absolute qualities of wound repair.

Skin and scar are complex viscoelastic materials with time-dependent properties. They are not fully characterized unless tensile strength and extension (stretch) are recorded synchronously. Plotted together they describe a curve (load-extension) whose area represents the ability of the scar to resist rupture (energy absorption) (Fig. 1.3).

This principle was underlined in a recent study [15] comparing the effects of tape-closure and suture-closure on the mechanical properties of maturing scar tissue. The sutured wound gained tensile strength slowly and by the end of 5 months had recovered 70 per cent of its strength. The tape-closed wound gained tensile strength more rapidly recovering 90 per cent of its strength in the same time period. Unfortunately the higher tensile strength was associated with a more rigid scar. This offset the tensile strength advantage and calculation of the energy absorption revealed no difference between the two wound types (Figs. 1.4, 1.5 & 1.6).

These mechanical studies emphasize that the wound remains a weak and brittle structure for many months with only about half the ability of normal tissue to resist rupture.

HISTOLOGICAL ASPECTS

Light microscopy

At the time of wounding, red blood cells and plasma proteins spill into the gap and a fibrin coagulum rapidly forms to unite the wound edges. Neutrophils and then monocytes are prominent after 12 hours. Epidermal cells migrate across the defect and superficial repair is complete in 24–48 hours. A characteristic sequence of enzymatic changes can be detected histochemically and is usefully employed by medico–legal experts to calculate the age of the wound in hours [30]. Fibroblasts can be recognized in the wound after 24 hours and collagen synthesis can be detected by the third day. At the

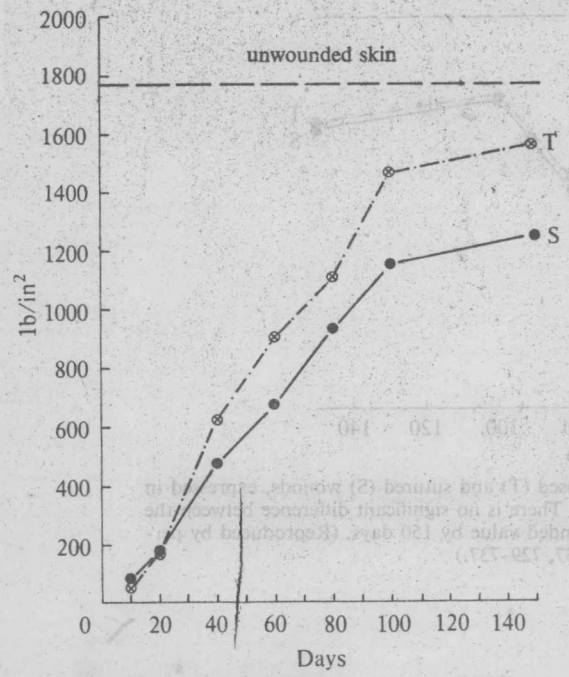

FIG. 1.4. Mean tensile strength of tape-closed (T) and sutured (S) wounds in rat skin expressed in pounds per square inch (lb/in²). Both gain strength with time but the tape-closed significantly more than the sutured. By 150 days it has recovered 90 per cent of the strength of unwounded skin whereas the sutured one has regained only 70 per cent. (Reproduced by permission from Forrester J.C. *et al.* (1969) New York: McGraw-Hill Book Company.)

end of a week fibroblasts dominate the cellular pattern. They are actively synthesizing collagen and mucopolysaccharides. New capillary formation is prominent.

Collagen is responsible for most of the strength of the wound. Unfortunately, wound collagen demonstrates physical abnormalities at several levels of examination. Its lack of birefringence indicates a failure of organization at the molecular or small fibril level (Figs. 1.7 & 1.8). Fibril aggregation is defective and the fibre diameters remain less than normal (Fig. 1.9). The large fibre inter-relationships are also abnormal and remodelling is incomplete.

Transmission electron microscopy

This instrument has played a vital role in elucidating cell and fibre morphology. Fibroblasts can now be clearly differentiated from macrophages and their local origin in the wound is finally established beyond doubt [34]. Their highly developed endoplasmic reticulum is characteristic and indicates their protein synthesizing ability (Fig. 1.10). Radioisotope studies have confirmed the intracellular synthesis of collagen. The collagen is not stored in the fibroblast but is secreted to the exterior through small channels or vesicles derived from the endoplasmic reticulum. The other proteins synthesized in the endoplasmic reticulum are transferred to the exterior via the smooth-chambered Golgi apparatus.

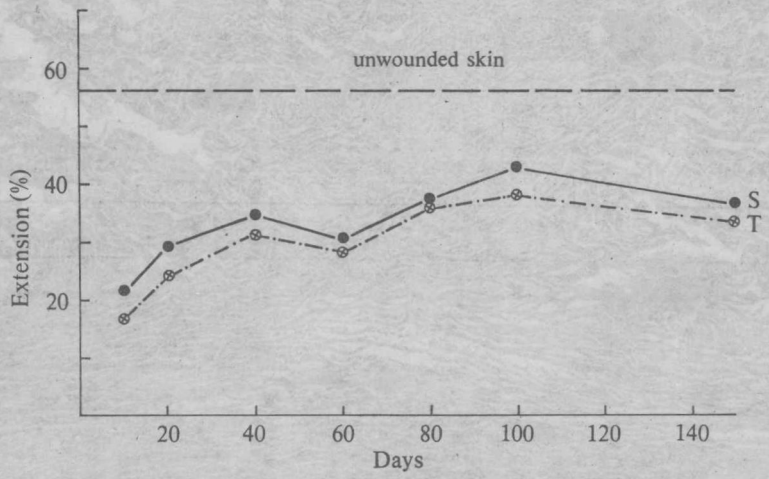

FIG. 1.5. Mean extension at break of tape-closed (T) and sutured (S) wounds, expressed as a percentage of original length. The sutured wound is more extensible. There is little recovery after 40 days. (Reproduced by permission from Forrester J.C. *et al.* (1970) *Brit. J. Surg.* **57**, 729–737.)

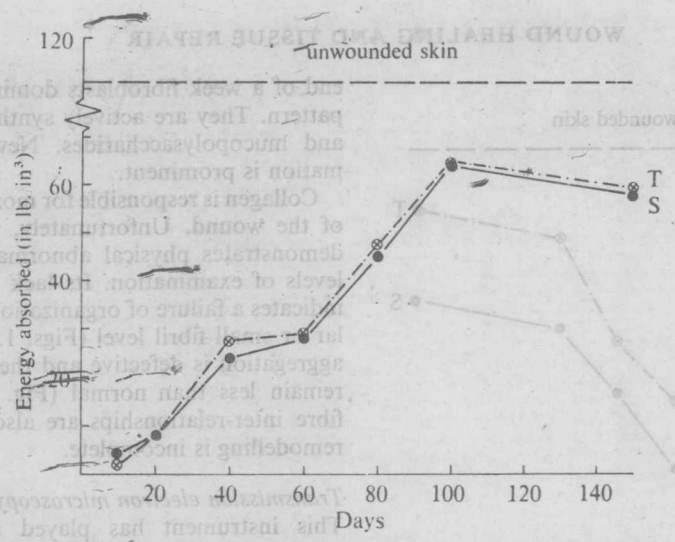

FIG. 1.6. Mean values of energy absorption of tape-closed (T) and sutured (S) wounds, expressed in inch-pounds per cubic inch of tissue tested (in. lb./in³). There is no significant difference between the wounds and only a 50 per cent recovery of the unwounded value by 150 days. (Reproduced by permission from Forrester J.C. *et al.* (1970) *Brit. J. Surg.* **57**, 729–737.)

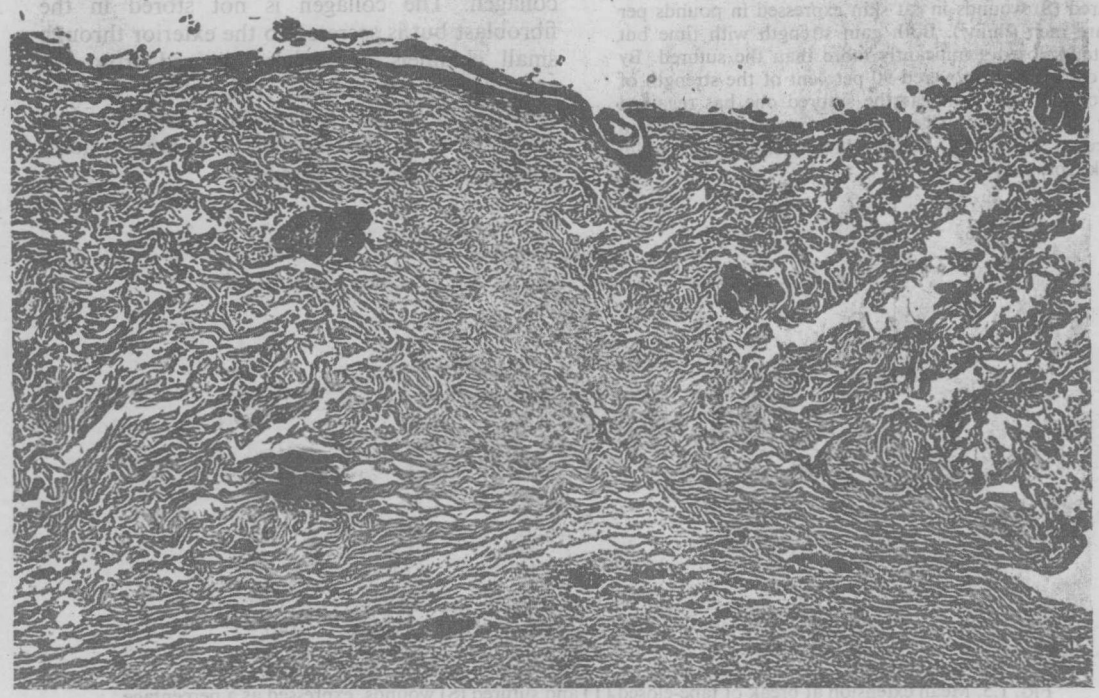

FIG. 1.7. Photomicrograph of a 100-day tape-closed wound in rat skin. The panniculus muscle has retracted off the picture on either side and the resulting scar is triangular in shape. The collagen in the scar is more compact than in normal skin but it is difficult to assess fibre alinement and organization at this magnification (Light Microscope × 60). (Reproduced by permission from Forrester J.C. *et al.* (1969). In *Repair and Regeneration. The Scientific Basis for Surgical Practice*, eds. Dunphy J.E. & Van Winkle W. Jr., pp. 71–85. New York: McGraw-Hill Book Company.)

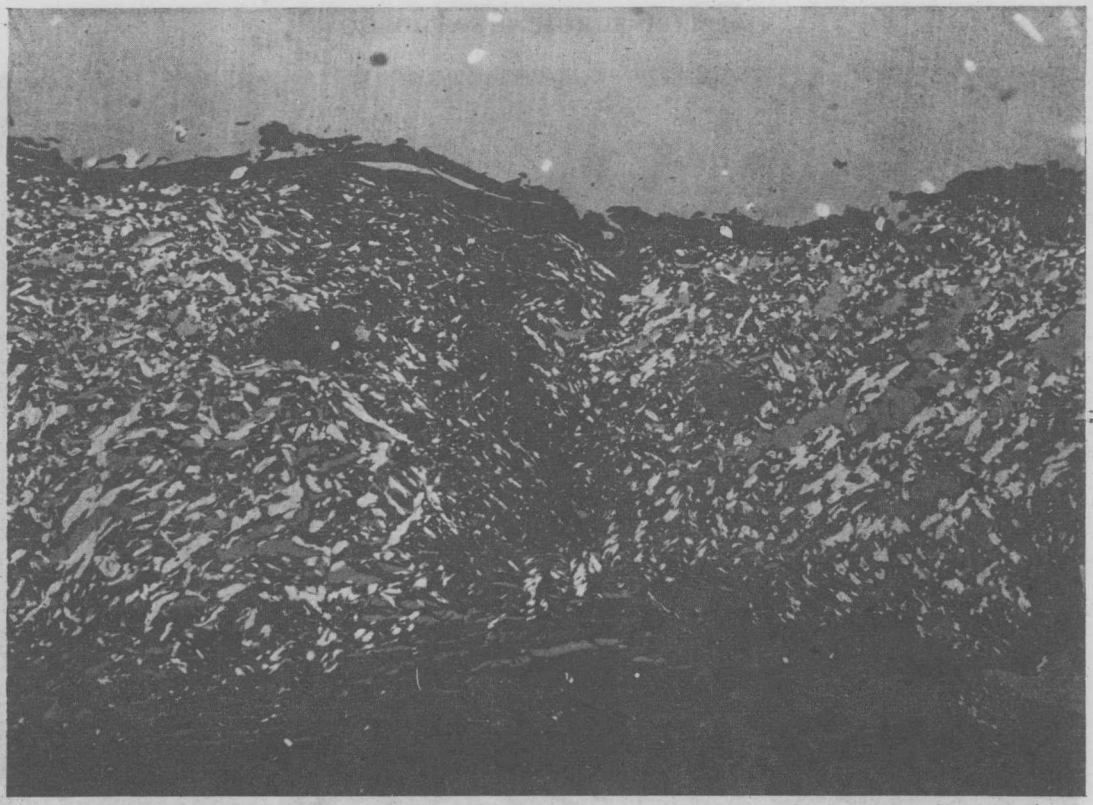

FIG. 1.8. Photomicrograph of the 100-day wound in Figure 7 viewed in polarized light. The normal dermal collagen on either side of the wound is birefringent. The almost complete absence of birefringence in the wound indicates a failure of organization of the collagen fibre subunits (Light Microscope ×60). (Reproduced by permission from Forrester J.C. *et al.* (1969). In *Repair and Regeneration. The Scientific Basis for Surgical Practice*, eds. Dunphy J.E. & Van Winkle W. Jr., pp. 71–85. New York: McGraw-Hill Book Company.)

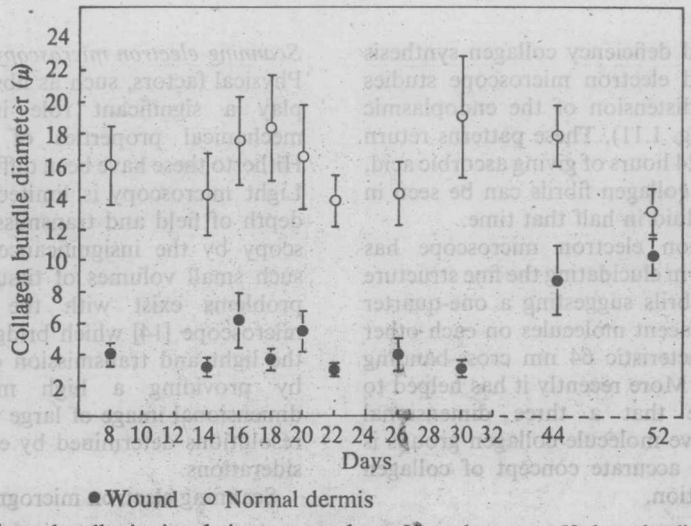

FIG. 1.9. Collagen bundle size in relation to wound age. Note that up to 50 days the diameter of the wound-collagen bundles is significantly smaller than those of normal dermal collagen. (Reproduced by permission from Douglas D.M. *et al.* (1969) *Brit. J. Surg.* **56**, 219–222.)

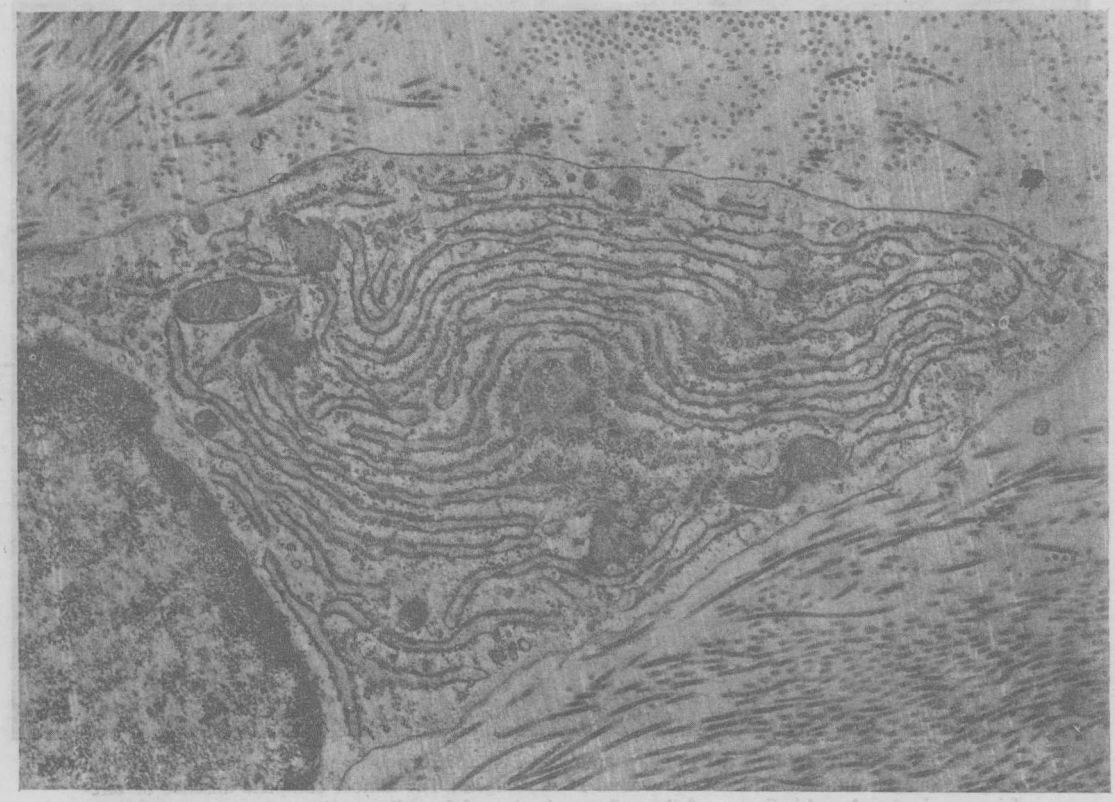

FIG. 1.10. Electron micrograph of part of a normal fibroblast. Note the characteristic well developed endoplasmic reticulum. The lining ribosomes, which are responsible for its 'rough' appearance, are the active site of collagen synthesis. New collagen fibrils are rapidly excreted and are seen here surrounding the cell (Electron micrograph × 13,500). (Courtesy of Professor Russell Ross, Seattle.)

In ascorbic acid deficiency collagen synthesis is incomplete and electron microscope studies show a striking distension of the endoplasmic reticulum [33] (Fig. 1.11). These patterns return to normal within 24 hours of giving ascorbic acid, and recognizable collagen fibrils can be seen in the extracellular fluid in half that time.

The transmission electron microscope has proved invaluable in elucidating the fine structure of the collagen fibrils suggesting a one-quarter stagger of the adjacent molecules on each other to give the characteristic 64 nm cross-banding that is so typical. More recently it has helped to provide evidence that a three dimensional arrangement of five molecule collagen groups is probably a more accurate concept of collagen molecule aggregation.

Scanning electron microscopy

Physical factors, such as fibre shape and weave, play a significant role in determining the mechanical properties of skin and wound. Hitherto these have been difficult to demonstrate. Light microscopy is limited by resolution and depth of field and transmission electron microscopy by the insignificance of sampling from such small volumes of tissue. Neither of these problems exist with the scanning electron microscope [14] which bridges the gap between the light and transmission electron microscope by providing a high magnification three-dimensional image of large volumes of tissue at resolutions determined by electron optical considerations.

Scanning electron micrographs of normal skin

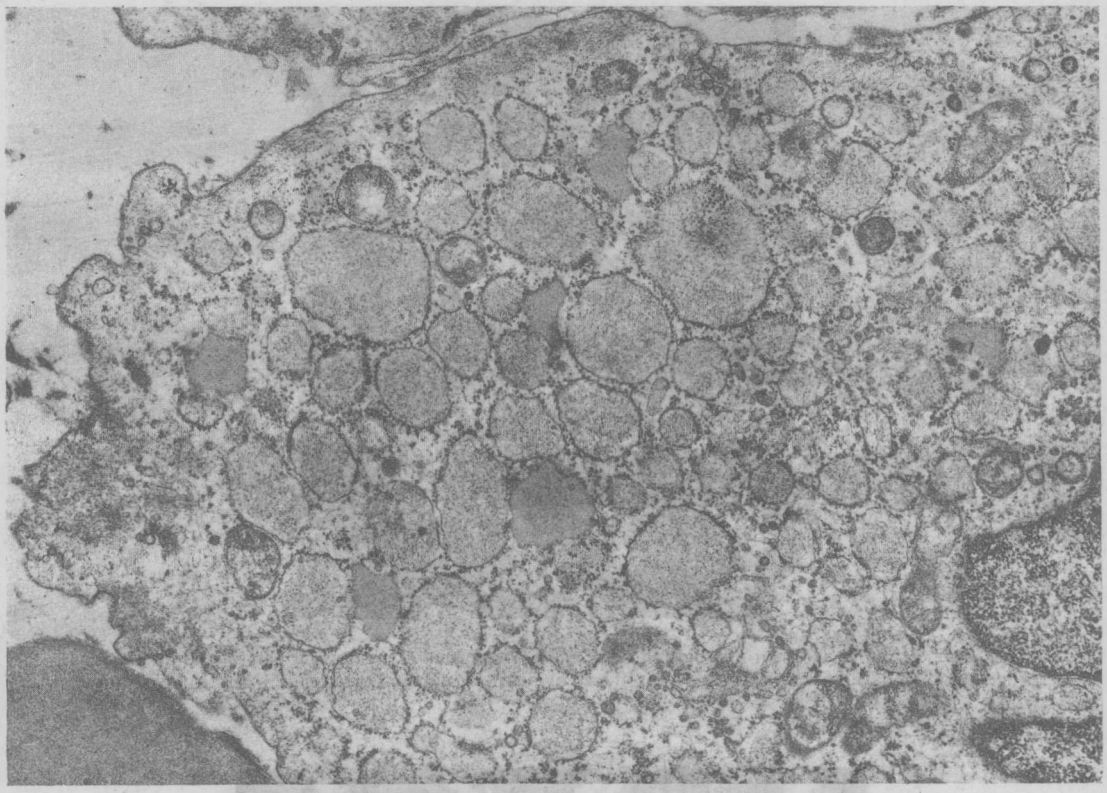

FIG. 1.11. Electron micrograph of part of a scorbutic fibroblast. Note the typical distended endoplasmic reticulum. There is no sign of collagen but it will appear within 24 hours of providing ascorbic acid (Electron micrograph ×13,500). (Courtesy of Professor Russell Ross, Seattle.)

collagen reveal a well-organized network of large collagen fibres (Fig. 1.12). At high magnification each large fibre can be seen to be made up of a bundle of fine cross-banded fibrils (Fig. 1.13).

The fibre patterns in healing wounds, however, are quite different. In the sutured wound, collagen fibrils lie in a relatively haphazard arrangement (Fig. 1.14). Whereas in the tape-closed wound the fibrils are more oriented and they show signs of aggregating together into small bundles across the wound (Fig. 1.15). This increased orientation could explain both the increased tensile strength and the decreased extensibility of the tape-closed wounds. Orientation is used in industry to increase tensile strength. The highly oriented taped wound specimen would have little natural extensibility since this is normally dependent on the network architecture of the collagen fibre mat, and is simply a manifestation of the fibres taking up the line of an applied force.

As time passes the collagen fibrils in both sutured and tape-closed wounds come together to form large irregular masses without evidence of fibril substructure (Fig. 1.16). Although the collagen fibre patterns clearly change as time passes, remodelling is minimal and there is little to suggest that the normal network architecture is ever restored.

BIOCHEMICAL ASPECTS

Studies of collagen content

Early histologists were impressed with the fibroblast proliferation in wounds but it soon became clear that the collagen content gave the best correlation with the development of tensile strength. Subsequent studies of collagen accumu-