Factors influencing wound healing

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I. WOUND HEALING

Until comparatively recently it was considered to be self-evident that wound healing and infection were both parts of the same process. The words 'laudable pus' speak for themselves.

De Chauliac and Paré were amongst the first generation of surgeons who were dissatisfied with this concept, and showed that healing could be achieved without infection if better treatment was applied to the wound. Semmelweis and Lister clearly showed that the two processes were distinct, and thus laid the basis for modern surgery.

Nowadays the roles are reversed, and there appears to be a real danger of wound healing without infection being taken for granted. Many are unaware that most complications can be prevented, because they do not have satisfactory knowledge of the healing process. A surgeon-to-be must therefore become conversant with the basic principles of this process. He must accurately assess the damage caused by the wound and the body's capacity for repairing this damage. He must learn to allow nature to take its course, and only to interfere when nature threatens to 'go astray'. But, first of all, he must begin at the beginning and realize that three important phases in the healing process can be differentiated: a reaction phase, a regeneration phase, and a remodelling phase.

1. Reaction phase

The reactions of the body to a wound are the same as those occurring with inflammation, the symptoms being *rubor, calor, tumor* and *dolor*. The redness and warmth are caused by dilatation of the vessels, the swelling by exudate, and the pain by a number of factors, including pressure on nerves, action of chemical products (histamine, serotonin and bradykinin), and ischaemia.

Following trauma, this classical syndrome is preceded by a short period (of approximately 10 minutes) of vasoconstriction. This is when the intrinsic and extrinsic coagulation mechanisms are brought into action, the primary purpose of which is to close off the damaged vessels and then to activate the inflammatory mechanism by means of chemical signals. The vessels in the immediate neighbourhood of the wound, particularly the venules, dilate and the capillary permeability is increased. Exudation of plasma occurs, accompanied by haemoconcentration; the blood flow slows down, and there is margination of leukocytes. The leukocytes

adhere to the wall and then migrate between the endothelial cells, so that within a few hours already the damaged area is saturated with granulocytes and macrophages, which are then guided by chemotaxis to the point where they are most needed. They are used in the clearing away of dead tissue (blood platelets and cells), and of foreign particles and bacteria, both by ingestion (phagocytes) and digestion (lysosomes).

What are the chemical mediators which set the stage for enacting the complicated scenario of the processes of vascular dilatation and chemotaxis, and how are they activated? It appears that not only are humoral factors at work in the effector systems, but also cellular factors, involving the action of the products of a number of cells.

Humoral factors

The effector systems (Fig. 1) at present known to be involved in the healing process are all characterized by the 'cascade effect': each successive factor is activated by its predecessor. Four systems are important for an understanding of the healing process: the kinin system, the fibrinolytic system, the intrinsic coagulation system, and the complement system.

The kinin system, the fibrinolytic system and the intrinsic coagulation system have in common the fact that they are activated by the Hageman factor (XII). This factor is in turn activated by contact with a negatively charged surface (such as collagen), the rough intima of a vessel, or a foreign body (such as glass). The complement system is activated by antigen-antibody interactions.

Kinin system: In the kinin system, kallikreinogen (a non-active proteolytic enzyme) is first converted into kallikrein. This enzyme releases bradykinin from α_2-
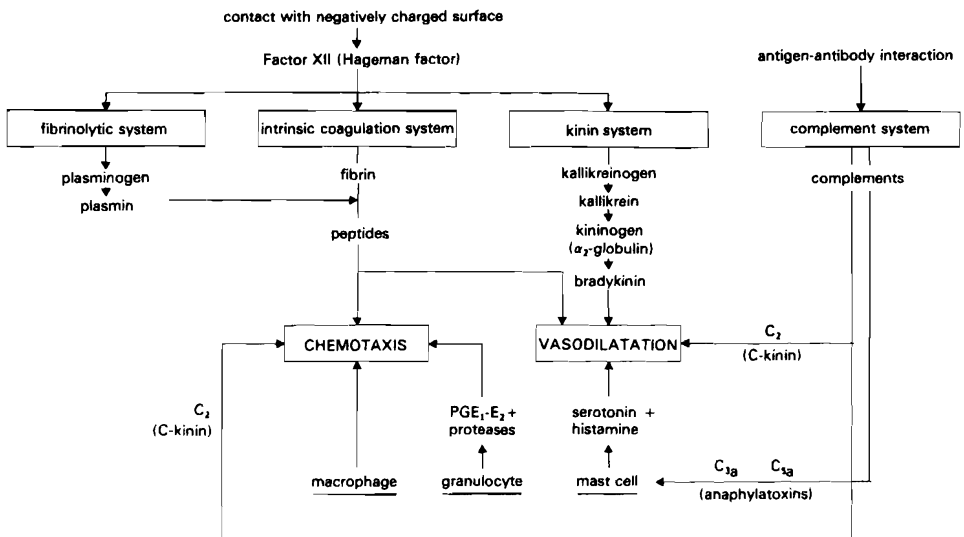

Fig. 1. Effector systems.

globulin, a kininogen. Release of bradykinin is accompanied by vasodilatation and an increase of capillary permeability.

Intrinsic coagulation system: Here, three important phases can be differentiated: an initial phase, in which thromboplastin is formed; a second phase, in which prothrombin is converted into thrombin under the influence of thromboplastin and Ca^{++}; and a third phase, in which the trombin converts soluble fibrinogen into insoluble fibrin. Once it has been formed, fibrin appears to eliminate thrombin and thus to prevent the formation of further superfluous coagulation.

Fibrinolytic system: In this system, breakdown of fibrin occurs under the influence of plasmin, which is activated by plasmogen. The resulting peptides also cause an increase in capillary permeability and chemotaxis.

Complement system: Of the nine components of the complement system (C_1-C_9), C_2 or C-kinin, and C_{3a} and C_{5a} (two anaphylatoxins) are important. C-kinin has a direct vasodilator effect; C_{3a} and C_{5a} have an indirect vasodilator effect via the mast cells, and also cause chemotaxis.

Cellular factors

Important mediators in the healing process (Fig. 1) are produced by the following cells:

Mast cells: Histamine and serotonin are formed in the granules of these cells. Both these products have important vasodilator effects. They are released into the circulation by degranulation, a process which is aided by the complement components C_{3a} and C_{5a}.

Granulocytes: The prostaglandins E_1 and E_2 are formed particularly in these cells, and these two substances appear to have vasodilator and chemotactic properties. Proteases are also activated in the lysosomes found in these cells, and they in turn also have a function to fulfil when the cell membrane is lysed.

Macrophages: One of the most important functions of these monocytes, and one to which more attention will be paid when we discuss wound repair, appears to be the production of chemotactic substances whose purpose is to attract other macrophages.

2. Regeneration phase

Elimination of debris

Deprivation of oxygen leads to cell death. This statement illustrates in a simple fashion the essence of trauma. Tissues are injured and the blood vessels which supply them are torn. The function of any cells which are still viable is threatened. Before the body can repair the damage by regeneration of tissue, further damage as a result of infection must be prevented, and the debris must be cleared away. This is

achieved by the granulocytes and the macrophages, which have arrived at the site in the meantime.

Granulocytes: There are 20 to 50 billion of these cells present in the circulation, and an even greater number held in reserve in the bone marrow. They offer protection against bacterial invasion by means of phagocytosis (Fig. 2).

Phagocytosis is, however, only possible when opsonification of these bacteria has occurred, opsonification being the process by which a cell is rendered suitable for phagocytosis by opsonin, an antibody in the plasma. Protective antibodies are found in the wound exudate as a result of previous contact with these micro-organisms. These combine with the surface antigen to form the antigen-antibody complex, which activates the complement system. The cascade of enzymatic reactions which now follows, leads finally to membranolysis of the bacteria which are thus prepared for phagocytosis.

After the bacteria have been ingested and the walls of the granulocyte have formed a phagosome, this phagosome fuses with the lysosome already present, which
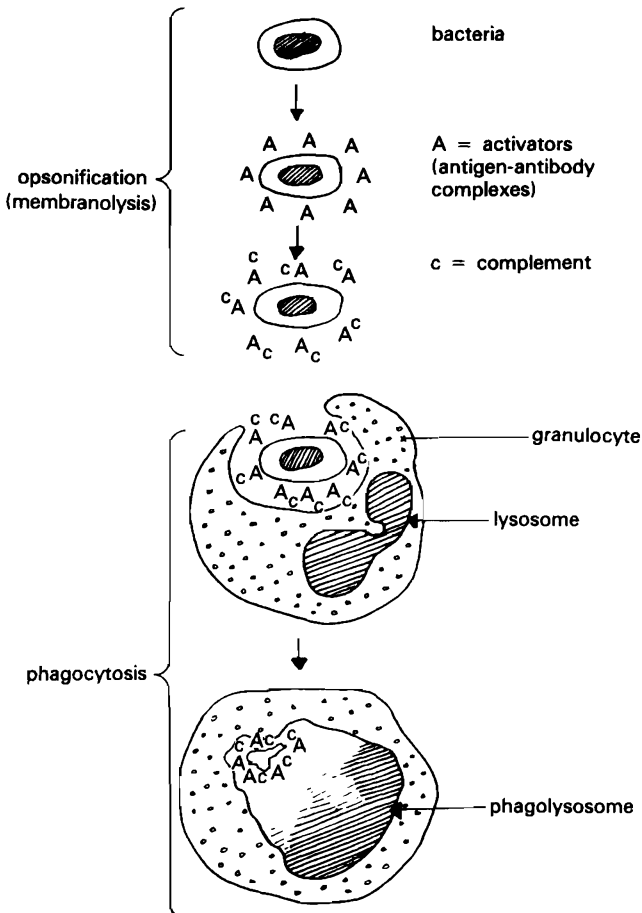

Fig. 2.   *Phagocytosis.*

contains a large number of hydrolytic enzymes such as lysozymes and lipases, proteases and nucleases. These enzymes thus end up in the phagolysosomes formed by the fusion (degranulation), and destroy the bacteria. In addition to these lysosomal enzymes, granulocytes also produce the enzyme collagenase, which is capable of breaking down collagen.

Macrophages: The granulocyte is the predominant cell in the wound for the first few days following trauma, but from the fifth day the macrophage dominates. These cells are large and mobile and, like granulocytes, are capable of surviving in a poorly nourished area. They ingest macromolecules, converting them to amino acids and sugars. They function as the digestive tract of the wound and hold a key position in the healing process in that they remove the damaged tissue by means of phagocytosis and the production of lysosomal enzymes. In addition, they produce substances which attract other macrophages to the wound by chemotaxis, and activate the formation of endothelial cells and fibroblasts. Their number is increased by the administration of vitamin A, but corticosteroids have an inhibiting action.

Both granulocytes and macrophages can survive in the wound by virtue of their anaerobic metabolism. Both produce collagenase, and both are activated by the ingestion of bacteria and other substances to produce lactic acid. Repair of the tissues is only possible when the wound has become clean, and then only to a limited extent, without complete restoration to the original state.

In man — unlike the salamander, which can regrow an amputated limb — the capacity for regeneration is limited to a few cells, which comprise the endothelial cells, the fibroblast and the epithelial cells.

Regeneration of endothelial cells

Oxygen deprivation in a wounded area can only be relieved by neovascularization. Capillary buds form in the walls of functioning vessels surrounding a wound, stimulated by hypoxia and activated by macrophages. These buds grow and combine with other similar offshoots to form a capillary loop. In principle, this neovascularization can occur in any of three ways: by the bridging of a large tissue defect; by forming a connection to a previously untapped circulation, as occurs in transplantation; and by forming a connection with other functioning vessels by apposition in a primarily closed wound.

Initially, the basal membrane is still incomplete in these new vessels, and leakage occurs. Only later, when the new vascular network has been strengthened with collagen gel by the fibroblasts, is it able to withstand increasing pressure.

Regeneration of fibroblasts

Fibroblasts are formed in perivascular cells. They are mobile at the site of the wound, and migrate along the fibrin threads and other fibres. The migrating fibroblast is directly followed by newly formed capillaries, which possess a plasminogen activator. The enzyme plasmin thus formed then breaks down the fibrin network via the fibrinolytic system. The fibroblast begins production of collagen and ground substance within a few days of the trauma under certain circumstances.

Collagen synthesis is stimulated by lactic acid and vitamin C. Lactic acid is

formed by granulocytes and macrophages. Vitamin C in its reduced form is only found in a hypoxic environment. The two prerequisites, hypoxia and acidosis, are both satisfied in the wound area. However, oxygen is necessary for the production of collagen, since only part of the collagen molecule can be made in its absence. The solution to this problem appears to be relatively simple. Like the macrophage and the epithelial cell, the fibroblast is a facultative anaerobe. This means that any excess of oxygen available is used by the cell. The P_{O_2} in the region of the fibroblast therefore remains low and can thus stimulate collagen synthesis unaltered.

Interactions between macrophages, fibroblasts and endothelial cells can be illustrated using a 'wound module', which illustrates the succession of events at the wound edge (Fig. 3). The macrophages, which clear the way, lie to the fore of the module. They probably give the signal to the fibroblast to follow, and feed this cell with the amino acids released by the breakdown of the macromolecules. The young fibroblasts cannot begin the formation of collagen until the macrophages have produced sufficient lactic acid, and until oxygen, amino acids, glucose, vitamins and metals such as zinc, copper and iron, can be delivered by a new vascular system. The fibroblasts are therefore followed in turn by capillaries, the most distally functioning of which lie at a distance of 50-75 μm from the wound edge.

When the walls of the capillaries in this granulation tissue can withstand the arterial pressure and thus become more functional, and when two capillary loops with different pressure systems join up, more oxygen can be delivered from the system with the higher pressure. The front-line fibroblast, which is deprived of oxygen, then reacts by producing more collagen, which forms a matrix allowing the macrophage to advance further. The young fibroblasts then follow in their turn as far as the supply line will allow. The others remain behind to manufacture firm connective tissue.
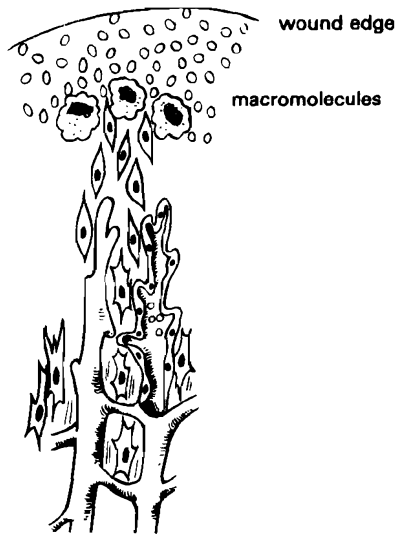

Fig. 3.   Wound module. P_{O_2} is lowest at the wound edge and gradually increases in the remoter areas under the influence of neovascularization. The lactic acid content, on the contrary, is highest at the wound edge and decreases in the better vascularized areas.

Production of collagen

The synthesis of collagen begins with the lining up of a number of amino acids according to the code stored in the deoxyribonucleic acid (DNA). Messenger RNA provides the correct sequence, and transfer ribonucleic acid (tRNA) transports the various amino acids to their place in the α-chain which occurs on the ribosome (Fig. 4). When assembly is complete, the ratio of amino acids is as follows: one-third glycine, one-third proline, and one-third X-Y-Z.

Hydroxyproline and hydroxylysine are still absent. These substances are unique because they occur nowhere else in the body; they are made on the ribosome by hydroxylation of proline and lysine by the enzymes proline hydroxylase and lysyl hydroxylase (Fig. 5). These enzymes are active in the supply of oxygen and of co-factors, such as vitamin C, α-ketoglutarate and iron.

The next step in the synthesis of collagen (see also Fig. 4) is the assembly of three α-chains (two similar α_1-chains and one different α_2-chain). Each α-chain represents a right-hand helix. Together, the three α-chains form a left-hand superhelix. The rather shorter tropocollagen is formed next from this procollagen molecule after glycosylation in the Golgi apparatus, and after separation of the terminal registration peptides.
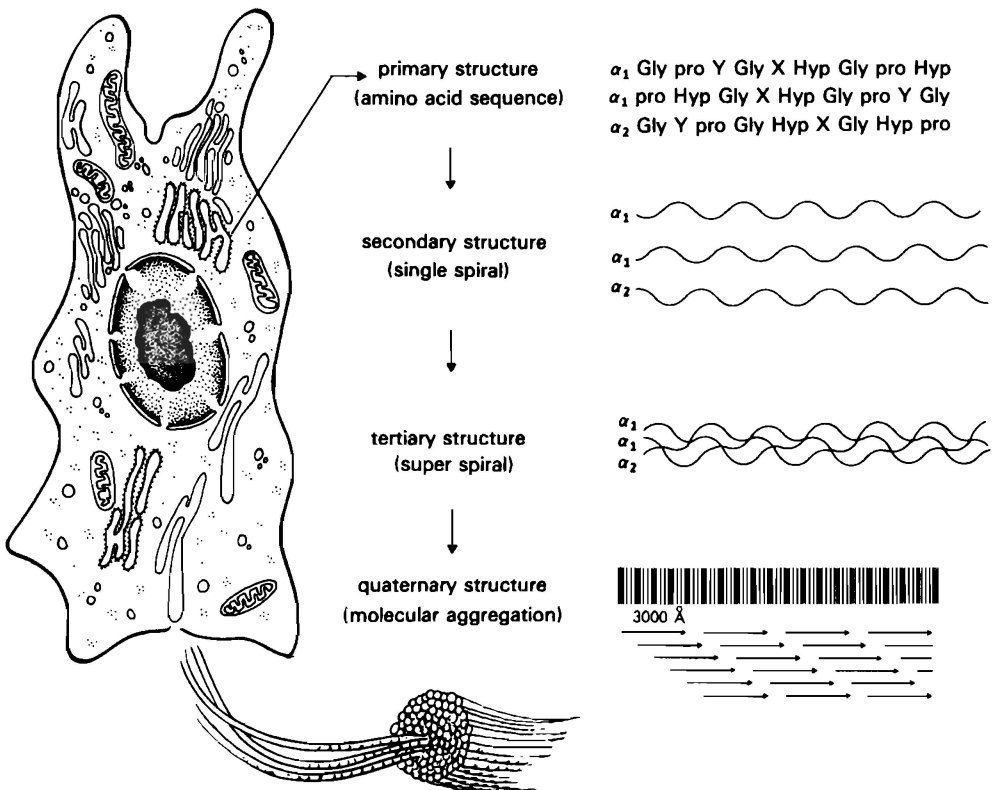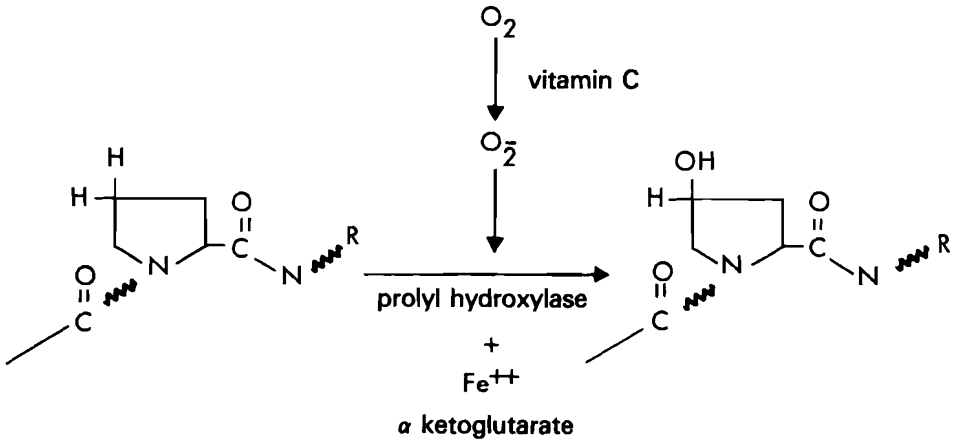

Fig. 4. Collagen synthesis.

Fig. 5.   Schematic representation of proline hydroxylation (after Hunt and Van Winkle).

This molecule, which is 3,000 Å long and 15 Å wide, is excreted into the extracellular space where aggregation occurs. The molecules range themselves in a sequence whose characteristic is that one molecule overlaps the next by a quarter of its length (quarter staggering). As yet, these microfibrils are not strong. The chains in the molecule and the molecules themselves are held together by weak hydrogen bonds, and disaggregation occurs in a saline solution. This situation changes within a few days. The solubility of the collagen decreases, while the stability of the intra- and intermolecular bonds, and thus the strength of the fibres, increases.

As far as is known, the process is as follows: Normal connective tissue contains aldehydes, which are formed by oxidative deamination of lysine (deamination being the splitting off of the amino group NH_2 from the organic bond). The deamination process is catalyzed by the enzyme lysyl amino-oxidase (LAO), an enzyme which only acts in the presence of copper ions. Aldehydes which react with each other form an intramolecular bond. Intermolecular bonds are formed by the reaction of aldehydes with other amino groups. The larger the number of cross-bonds, the stronger the collagen fibres. The formation of cross-bonds can be inhibited by the administration of beta-aminopropionitrile (BAPN). This substance, which specifically inhibits the action of lysyl oxidase, is found in sweet beans of the *Lathyrus odoratus* species. Lathyrism, a disease found in animals, is characterized by bone malformations, and by arterial aneurysms caused by BAPN.

Collagen lysis

Collagen is lysed by the enzyme collagenase, which is present in the wound from the start, being brought there by the granulocytes and macrophages. It is also produced by regenerating epithelial cells, and is found in the wall of the digestive tract and in the post-partum uterus. It is capable of breaking down the tropocollagen molecule in the triple helix, unlike the proteolytic enzymes, which only break down collagen when it has been denatured and broken down into α-chains. Local collagenolytic activity can be found up to approximately 7 mm from the wound edge. Under

certain circumstances, therefore, sutures lying within this zone may loosen easily. If dehiscence is expected, the surgeon can forestall this by using stay sutures. These pierce the skin at a safe distance from the wound, and are tied over a bolus.

Because lysis is a destructive process, less energy is used than in the synthesis of collagen. In extreme oxygen deprivation or extreme deficiency of protein or vitamins, lysis continues whereas synthesis stops. One can imagine what happens when the balance between the two is disturbed, if for example synthesis stops and lysis is intensified by the influence of corticosteroids or infections.

Anyone who has ever seen the fresh bright red granulation tissue of a burn wound converted within a few hours by sepsis to a dull grey jelly will never forget this dramatic picture. However, it is not only the local activity of collagen with which the surgeon has to reckon. The collagen is continuously broken down in the body, but this process is intensified in trauma. The greater the trauma, the greater the generalized collagenolytic activity.

Production of ground substance

In addition to collagen, the fibroblast also produces glycosaminoglycans (mucopolysaccharides) and proteoglycans. These are large molecules which take up a great deal of space and are about 4,000 Å long. The following glycosaminoglycans play an important part in the building and repair of tissues: chondroitin, chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate, heparin sulfate, keratan sulfate and hyaluronic acid.

The orientation and diameter of collagen fibres are presumably determined by this ground substance. In connective tissues of different fibre structure and architecture, glycans are found, independently or in combination, which are characteristic of that type of tissue; for example, dermatan sulfate is found in the skin, keratan sulfate in the cornea, and hyaluronic acid in the cartilage. Their function in the healing process of wounds is not fully understood, however. For instance, keratan sulfate is not found in corneal scars, in contrast to other wounds, while chondroitin sulfate and dermatan sulfate are, as in other wounds. Also, cartilage wounds are not repaired by the formation of new hyaline cartilage.

About 50% of cartilage consists of proteoglycans. These are largely responsible for this tissue's elastic qualities because they retain water and only release it slowly under pressure. The influence of time and a continuous load on our intervertebral discs is thus satisfactorily explained. Shortly after a burn, the scar still contains proteoglycans, but these later disappear, and with them the water which they contain. The scar then takes on its definitive aspect, which is determined by the presence of collagen fibres. The transformation of hypertrophic scars into atrophic scars is thus partially explained.

Regeneration of epithelial cells

Regeneration of epithelium is brought about by a proliferation of cells in the wound edge, migration, and maturation *in situ*. When contact between the epithelial cells migrating from the two wound edges is restored directly, this is described as healing by first intention. In those cases, however, where contact is only restored later by migration of epithelial cells over granulation tissue, which has to be formed first,

this is referred to as healing by second intention.

The skin consists of multi-layered squamous epithelium (epidermis) and a connective tissue layer (dermis) (Fig. 6). The epidermis consists of the following five layers: the *stratum basale*, i.e., the basal layer; the *stratum spinosum* or 'prickle-cell' layer, which is a broad layer of cells bound to each other by desmosomes; the *stratum granulosum* or granular layer, which is a narrow layer of flat cells; the *stratum lucidum* or clear layer, which is a transparent layer; and the *stratum corneum* or horny layer, a keratinized layer consisting mostly of keratin. The dermis consists of the following two layers: the *pars papillaris* or papillary part, named thus because of the papillae; and the *pars reticularis* or reticular part, which derives its name from its fibrous appearance.

Defects in squamous epithelium are closed by regeneration and the migration of cells in the basal layer. The migration begins within a few hours following the injury in those areas in which the epithelial cells can extend over vital granulation tissue. To ensure satisfactory nutrition, the epithelial cells cut a path for themselves over the already-formed granulation tissue and under the crust of dead cells, by means of the enzyme collagenase.

The migration of these epithelial cells and the regeneration of vessels in the substrate is intensified by an oxygen-rich environment. More or less in contrast to this, the healing of a closed wound proceeds more smoothly than that of an open wound. The reason is not clear. It is suspected that the conservation of heat and water, which are lost in an open wound, probably plays an important part.

If it should be thought, therefore, that it would be better to keep a wound closed, it must be remembered that the danger of infection is much greater in a closed wound than in an open wound, precisely because the retention of heat and water provides an ideal breeding ground for bacteria.

3. Remodelling phase

This phase, which partially overlaps the regeneration phase, is characterized by a reduction in size of the wound surface, an increase in the strength of the scar, and an alteration of the fibre structures.
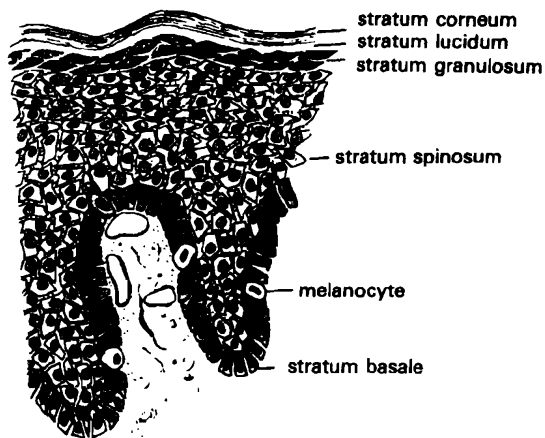

Fig. 6.   Microscopy of the skin.

fibres allows. There has for years been uncertainty as to the cause of this phenomenon, but recent investigations have shown that fibroblasts are responsible. These cells appear capable of transformation to myofibroblasts, which can form intercellular bonds by means of desmosomes, and furthermore are able to contract, due to the presence of contractile proteins (actomyosin). In fact, these cells, which are involved in many processes concerned with contraction of connective tissue, behave like smooth-muscle fibres.

Usually, contraction has no disadvantages, but there may be some when the body is not capable of compensation — when, for example, its reserves are used up during the healing of a defect. If circular scar tissue is formed, e.g. round a hollow structure, such a contracture may lead to stenosis.

During the first three weeks following the injury, the quantity of connective tissue in the wound and the strength of the fibres increase considerably. At the end of this period, the quantity of collagen stabilizes at a given level. The strength of the fibres, on the other hand, continues to increase for several months. There is thus no relation between the quantity and strength of the collagen. The increase in strength can be explained by a further increase in the number of cross-bonds, and the replacement of old molecules by new ones in another pattern.

This is made possible by a continuous turnover of collagen, although the quantity remains constant: new fibres are formed and old ones broken down. The rate of this turnover in the first three months definitely appears to be related to the increase in strength of the scar.

Four months after an injury, the turnover of collagen is still considerably greater than that in normal skin. However, it is not only the rate of turnover which determines the nature of the scar, but also the level at which the balance is struck between synthesis and lysis. When synthesis of collagen predominates, a hypertrophic scar is formed, while an atrophic scar results when the reverse is true.

The advantages of wound healing are generally known, but the disadvantages are unfortunately less well appreciated. One is that already mentioned, namely the possibility of contractions which can lead to serious disturbances of function. Another is the absence of selectivity of the healing process, so that tissue integrity is lost, and adhesions may form between several tissue structures which move relative to each other. Correction of a contracture is possible in many cases, for instance by supplementing the shortage. Repair of the tissue integrity which allows structures to move or glide relative to each other can only be accomplished by surgical means.

For this we are mainly dependent on a number of factors which influence the turnover process in such a way as to produce differentiation in the scar, varying from loose and areolar to stiff and polarized. These are: the age of the patient; the type of tissue; the quantity of scar tissue; the age of the scar tissue; the direction of the scar; the site of the scar; the pressure on the scar; and the contraction of the scar.

II. WOUND-HEALING DISTURBANCES

The wound-healing process is a complicated one, and can therefore be disturbed in numerous ways. Various factors are important to the surgeon.

1. Systemic factors

Coagulation disorders

The coagulation mechanism can be disturbed by a number of different factors, including treatment with anticoagulants, haemophilia, thrombocytopenia, and fibrinogen deficiency (e.g. due to liver cirrhosis or disseminated intravascular clotting).

Oxygen

It has now been confirmed that an increase in arterial P_{O_2} is accompanied by an increase in collagen synthesis in the wound. Clinically, this indicates that a lowering of the oxygen tension by ischaemia or hypovolaemia can disturb wound healing, and that wounds in a vascularized area, such as the face, heal more rapidly than those in a poorly vascularized area, such as the leg.

Wound healing is thus not disturbed by anaemia. A decrease in the haemoglobin content is accompanied by a decrease in the viscosity of the blood, a decrease in peripheral resistance, and an increase in cardiac output. The oxygen tension therefore remains constant.

Proteins

The synthesis of collagen is also delayed by protein deficiency. One of the most important building blocks for the fibroblast is the amino acid cystine, which is formed by conversion from methionine and which plays a part in the formation of the triple-helix configuration. A cystine residue is found in the non-helical registration peptides which lie at the end of the procollagen molecule. Allowances must always be made for wound-healing disturbances in the event of a negative nitrogen balance.

Figure 7 gives an illustration of cheek ulcer in a patient with ulcerative colitis.

Corticosteroids

Cortisone and adrenocorticotropic hormone (ACTH) have a stabilizing effect on the lysosome membrane of granulocytes and macrophages, and impede the migration of macrophages into the wound. They inhibit the regeneration of endothelial cells, fibroblasts and epithelial cells, and also have a delaying influence on the synthesis of collagen and proteoglycans. Although the administration of corticosteroids in the surgical clinic is not condemned, extreme care is indicated in the treatment of an injury in a patient receiving steroids (see also Fig. 8). Vitamin A may be used in such a case.

Vitamins

Vitamin C: Vitamin C is indispensable for the rapid synthesis of collagen. As is well known, it plays a part in the hydroxylation of lysine and proline, and accumulates in scar tissue, in which there is a prolonged turnover of vitamin C, in addition to the turnover of collagen. This explains why scars can rupture after a prolonged interval in the presence of vitamin C deficiency (scurvy!).

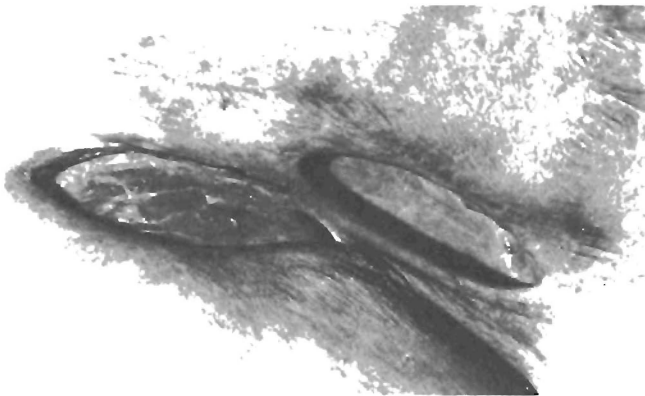*Fig. 7. Ulceration of cheek in a young boy with ulcerative colitis.*

Fig. 8. Disturbed wound healing after lymph node excision in the right groin of a patient treated with corticosteroids (courtesy Prof. M. Frenkel).

Vitamin A: This vitamin promotes the presence of granulocytes and macrophages in the wound. Furthermore, it reinforces the inflammatory reaction by exerting a labilizing effect on the membrane of the lysosome. The anti-inflammatory action of corticosteroids is corrected by vitamin A.

Vitamin E: Wound healing is delayed by the administration of vitamin E, which — like corticosteroids — has a stabilizing effect on the lysosome membrane.

Chemotherapy

Antiphlogistics: Drugs such as acetylsalicylic acid and indometacin owe their anti-inflammatory effect to the inhibition of the synthesis of prostaglandins E_1 and E_2, the mediators which induce vascular dilatation and chemotaxis. The influence of phenylbutazolidine and the salicylates on the inflammatory process is probably explained in the same way.

Cytostatics: It is obvious that wound healing can be disturbed by cytostatic agents, because cell proliferation forms an important part of this process. However, reliable data are not available on this point.

Minerals

Zinc: It is known that the content of zinc in the blood can fall after serious injury, such as burns, and that the regeneration of fibroblasts and epithelium is disturbed by this. Proliferation of these cells is not possible without the enzymes DNA-polymerase and reverse-transcriptase, which both contain zinc. Although migration is not disturbed, epithelialization and collagen synthesis are unsatisfactory. Healing is restored to normal by replacement of the zinc content. The influence of zinc on wound healing can, however, be disadvantageous, because zinc is said to inhibit the migration of macrophages and phagocytosis, and also reduces the activity of the copper-containing enzyme lysyl oxidase, due to a copper-zinc antagonism.

2. Local factors

Temperature: Wounds heal more quickly at a temperature of 30°C. If the room temperature is reduced to between 20°C and 12°C, the strength of the wound decreases, possibly as a result of reflex vasoconstriction. This effect can be nullified by denervation of the skin.

Trauma: The type of injury naturally has an influence on the course of wound healing. A sharp wound almost always heals without problems, whereas the process is markedly inhibited by the presence of dead tissue, foreign bodies, a haematoma or a seroma. The debris must first be removed before revascularization and healing can occur.

Radiotherapy: Wound healing is often difficult in an irradiated area. This is undoubtly due to poor vascularization, an abnormal inflammatory reaction, and the absence of regeneration of endothelial cells, fibroblasts and epithelial cells.

Denervation: In animal experiments, denervation can lead to spontaneous ulceration. The same phenomenon can occur in patients with transverse lesions of the spinal cord. A minimum of pressure and ischaemia are sufficient to cause an

enormous decubitus ulcer. It appears that the collagenase activity in these wounds is particularly high, and it is suspected that an insufficient supply of collagenase inhibitors during the brief ischaemia is the cause.

Wound infection: An open wound is by definition contaminated. Everybody's skin carries bacteria on the surface as well as in the depths of the pores. Complete sterilization of the skin is not possible, and it is even less possible for a wound to be sterile at the end of an operation. In a study of 50 surgical wounds, bacteria were found in all cultures taken before the wound was closed [1]. Forty-six wounds appeared to contain coagulase-positive staphylococci. In a further study, in which the staphylococci found in the operation wound were typed, the following sources were found: the hands of the surgical team, 6%; the nose and skin of the surgical team, 14%; the nose and skin of the patient, 50%; and the air above the wound, 68% [1].

Although contamination does not always lead to infection, it does increase its possibility. If a patient has to wait longer than three weeks for an operation, rather than two days, this possibility increases by more than 100%. A long operation also appears to increase the possibility of subsequent infection. Much has been done over the years to reduce bacterial infection, but despite all the precautions taken by operating theatre personnel (such as wearing masks and special clothing, careful washing of the hands, etc.), the presence of air conditioning in many modern operating theatres, and the careful sterilization of all instruments used, and despite all the reports that have been written on this subject (see, for instance, the report of the Dutch Health Council on hospital infections), infections still occur regularly. For that reason, attention is now being paid to the various and diverse factors which are important in the genesis of infection.

3. Causes of infection

Bacteria

Naturally the type and virulence of bacteria play an important part in the establishment of an infection. Table I lists the micro-organisms found in a study of 100 consecutive wound infections [1].

The number of bacteria is also important. Formation of pus after contamination with *Staphylococcus pyogenes* only occurs when the number of organisms exceeds one million, from which it appears that under normal circumstances the body is capable of destroying large quantities of bacteria.

The patient

The resistance of the patient plays an important part in the struggle between the body and bacteria. It is generally known that advanced age and such diseases as diabetes, cirrhosis, uraemia and leukaemia contribute to a reduction of this resistance. The reason for this is less well known, but the cause must lie in factors listed in Table II.

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Table I.   Micro-organisms found in a study of 100 consecutive wound infections [1].

Micro-organism	No. of cases
Staphylococcus aureus	48
Enterococcus	33
Escherichia coli	20
Proteus	8
Pseudomonas	8
Aerobacter aerogenes	8
Bacteroides fusiformis	4
Streptococcus	3
Clostridium welchii	2
Corynebacterium	2
Bacillus subtilis	1

Table II.   Factors contributing to impairment of the patient's resistance to bacterial infection.

a) Disturbances in the deposition of phagocytosing cells

Arising in the mediators:	— complement deficiency
	— age factors (e.g. old age and prematurity)
Arising in the vascular reaction:	— nutritional deficiency
	— radiotherapy
	— intoxication
Arising in vascularization:	— hypovolaemia
	— vasopressors
	— thrombosis

b) Disturbances in the opsonification of bacteria

Arising in antibody deficiencies:	— absence of exposure
	— hereditary disturbances (agammaglobulinaemia, dysgammaglobulinaemia)
	— acquired disturbances (reduced synthesis, increased breakdown)

c) Disturbance in phagocytosis

Caused by:	— unsatisfactory production of cells
	— absent ingestion
	— absent digestion

The surgeon

Treatment by the surgeon can also form an important source of infection: (A) if the factors already mentioned as promoting wound infection are not recognized; (B) if he devitalizes tissue health, for example by insufficient knowledge of anatomy, by traumatic use of instruments, by the use of an antiquated instrument, by the introduction of thick sutures, by the making of ligatures which include too much tissue, or by the coarse use of diathermy for coagulation or dissection; and (C) if he uses suture material which is conducive to infection. In the presence of one silk suture, only 100 pyogenic staphylococci, rather than one million, will suffice to produce a wound infection. Due to the presence of this foreign body, the hundred bacteria multiply to one million within seven hours.

What procedures are now available to prevent infection?

4. Prevention of infection

The bacteria

These can be combated directly by immunotherapy and a large number of antibiotics.

Immunotherapy: For many years, *tetanus toxoid* has been used to protect against tetanus. If a patient has not been immunized, a satisfactory prophylaxis can be achieved by antitoxin. *Pseudomonas vaccine* has demonstrated its value in the treatment of seriously burned patients, and *gammaglobulin* is used for the prevention of infection in patients with agammaglobulinaemia or dysgammaglobulinaemia.

Antibiotics: The prophylactic use of antibiotics in clean elective operations is in general not indicated. The chance of a wound infection is then less than one per cent. Furthermore, a patient's normal flora and eventually the flora of a hospital are altered in favour of resistant micro-organisms.

During the Yom Kippur War, a comparative study was made of a group of Israelis transported from the battlefield to a hospital within hours, and a group of Egyptians who were only admitted days or weeks later with seriously infected wounds [2]. In both groups complications due to hospital infection with Gram-negative resistant organisms occurred. However, the course of these complications was more serious in the Israelis than in the Egyptians, because the latter group showed a much greater resistance to infection in the hospital, apparently due to their already having a predominantly Gram-positive flora.

Only in those cases in which serious contamination cannot be prevented, or those in which even mild contamination can have serious results, is the use of antibiotics to be considered, for example: in injuries to the bowel, either non-elective (due to perforation) or elective (due to surgery on an unprepared colon); in wounds with extensive devitalization of tissue, such as burns, if the tissue cannot be or has not yet been removed; when operating on patients with pre-existing or active infection; when operating on patients with pre-existing valvular heart disease, to prevent endocarditis; for implantation of some prostheses, such as heart valves and total hips; and for transplantation of bone.

Whenever the decision is taken to administer antibiotics, it is important to ensure that administration is begun before the operation. The treatment should be directed towards destroying the bacteria before colonization has taken place, or within two to three hours if this is not possible, because the seriousness of the infection is determined during this period. There is, in general, no point in administering an antibiotic six to twelve hours after an operation.

The patient

An increase in the patient's resistance would form an important contribution to the prevention of infection. Unfortunately, this is not often possible in practice because the diagnosis of abnormal healing is usually made only when it is too late to be prevented.

The surgeon

The surgeon is in a better position than anybody else to influence wound healing favourably, because of the knowledge he has. Naturally, it is important that he changes his gloves when there is a hole in them. It has been shown that in a period of 20 minutes, 18,960 staphylococci can pass through a small puncture wound.

It is even more important, however, that he is aware of the unfavourable consequences which the formation of a haematoma or the leaving of dead tissue or a foreign body in the wound can have on wound healing, and how the development of an infection is encouraged by these factors. As mentioned before, simple silk sutures allow infection to be established in the presence of only 100 pyogenic staphylococci, rather than one million, which provides an obvious example of this process.

Preventive surgery should be directed at: (1) the removal of foreign bodies; (2) the removal of dead tissue; (3) the prevention of tissue necrosis by an atraumatic technique; (4) the prevention of haematomas by careful haemostasis; (5) the prevention of dead spaces by the apposition of tissue; (6) the prevention of infection by the use of inert suture material; and (7) the prevention of complications by drainage, in the following circumstances: (A) when closing a wound which is endogenously infected, such as an abscess cavity, (B) when closing a seriously contaminated wound, and (C) when leaving open a seriously contaminated wound.

References

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2. Siegal, B., Adam, Y. and Oland, Y. (1976): Blessures de guerre et infections. Etude comparative des blessures chez les combattants israéliens et égyptiens de la guerre d'octobre 1973 (guerre de Kippour). *Ann. Chir., 30,* 10, 781.