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POST-SURGICAL OEDEMA

ITS AETIOLOGY AND MANAGEMENT IN ORAL SURGERY

(with special reference to inflammation and early wound repair)

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PREFACE.

Much has been written on the subject of post-surgical oedema, but we still lack a clear understanding of the processes involved in its formation. The topic has been complicated by the different concepts held by various writers. By some, it is considered a physiological part of healing, whilst others claim it to be pathological in nature. Despite this, it is common practice for medications aiming to reduce oedema to be prescribed without consideration of the physiological basis of its formation.

Furthermore, the clinical presentation of post-surgical oedema shows considerable individual variability which occasionally seems out of proportion to the surgical insult. The chief components of this insult which are responsible for the formation of oedema however, are not clear. Nor is its aetiology. Hence the management of oedema is largely empirical.

In order to gain deeper understanding of the formation of oedema following surgery, it is necessary to explore basic aspects of microvascular physiology and the pathophysiology of inflammation; it is only by understanding the physiology of wound healing that one can explain the clinically observed responses to injury.

A difficulty in this area is that the aspects of inflammation particularly relating to post-surgical oedema have not been clarified. It has therefore been necessary to review all aspects of inflammation which may have some role. The volume of information covered is hence large. From this broad overview, the author has attempted to bring together those aspects which are most significant to inflammation in the post-surgical setting.

The bulk of the literature on wound biology is devoted to the study of this subject in organs other than the mouth, notably the skin, and for this reason, much of the material quoted has come from such sources. It has been necessary to extrapolate from this material and draw comparisons with the much smaller literature on oral wound healing.

This author's interest in this field stems from the age-old question - "Why is it so?" Just what does a surgical injury cause which promotes the almost universal response of fluid accumulation within the tissues? How do similar injuries cause such varied responses in different individuals? From a clinical viewpoint, the oral surgeon is faced with the problem of preparing patients for this response. The surgeon must also provide both a wound and subsequent therapy which is most able to facilitate speedy and uneventful recovery. This thesis attempts to come to terms with these and other questions surrounding post-surgical oedema.

The discussion opens with a review of the normal processes and restraints of healing, thereby establishing a framework in which the aetiology of post-surgical oedema may be considered.

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Chapter 1 NORMAL PROCESSES AND RESTRAINTS IN WOUND HEALING.

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A fundamental feature of living tissue is its ability to repair itself. Repair and regeneration occur constantly both at the subcellular level (with turnover of membranes and other organelles) and at the cellular level (where aged or lost cells are replaced). Upon this reparative capacity, surgery is intimately dependent, for "the body, not the surgeon, repairs wounds" (BURGESS 1974).

Surgery must always aim to provide circumstances which are the most conducive to prompt, uncomplicated healing with minimal functional disturbance. The surgeon's concern for these features can be translated into clinical practice only by understanding of the cellular and biochemical processes of wound healing (SHOSHAN 1981; BERLINGER 1982).

These processes, though basic to all tissues of the body, show variation in their expression. This variation is essentially related to the tissues involved, since repair is a "compromise between the competing needs of different cell types" (SILVER 1980).

In order to outline the context in which post-surgical oedema forms, this chapter describes the processes of repair as seen in the healing of the surgical wound. Skin wounds form the basis of this description since these wounds have provided the chief experimental model for wound healing studies. Numerous authors have reviewed this subject including NEWCOMBE (1972), SCHILLING (1976), CARPENTER et al (1977), HOWES and HOOPES (1977), POLLACK (1979a;b;c), SILVER (1979), (1982), BERLINGER (1982). Full discussion of this extensive field is not possible here. The essence of current thought is outlined; those aspects relating to acute inflammation and oedema formation are elaborated in later chapters and are hence only briefly described here.

It is important at this point to clarify the distinction between regeneration and repair. 'Regeneration' is the replacement of cells or tissues with new elements which are structurally and functionally indistinguishable from the original. In the higher animals, regeneration occurs only in a limited range of tissues (chiefly epithelial) such as epidermis, liver and the mucosal linings of the alimentary, respiratory and genito-urinary tracts. It is also seen in bone (SILVER 1979; 1982).

'Repair' occurs where there is filling of the wound space; however, the tissue is of a different type or structural arrangement from that which was lost. Integrity is re-established, but the form and function are different. Repair is usually seen as the "second-best" method of healing, almost always producing tissue which is less biologically effective than the tissue which it replaced. Known as "scar tissue", it may in fact produce deleterious effects on the surrounding tissue, through excessive wound contraction, for example (SILVER 1979; 1982).

In clinical practice, tissue and organ systems rarely originate solely from one embryonic tissue type. Thus, where the wound involves significant connective tissue (in addition to epithelium) regeneration to the original structure may be ineffectual. This is exemplified in skin: superficial skin wounds regenerate completely without scarring. Deep wounds, involving disruption of the connective tissue architecture, demonstrate scarring and frequently also show thinning of the overlying epidermis (SILVER 1979).

1.1 THE WOUND MODULE.

Following incision, some degree of tissue damage occurs - a "wound" is formed. This wound affects the organism in many ways. There is local loss of blood and fluid. Pain develops following direct stimulation of afferent nerve endings: a response is seen from the central nervous and endocrine systems. Cellular products are released into the circulation; catabolic metabolism is initiated (MOORE 1977).

Though initially merely a gaping cut, the wound rapidly becomes a functioning unit of repair. In simple terms, the wound has been termed a "fibroblast-capillary system", chiefly formed to produce connective tissue (FORRESTER 1976). HUNT and VAN WINKLE (1979) designate this fibroblast-capillary system as the "wound module". By this they refer to the intimate, ecological co-operative of new blood vessels, macrophages, granulocytes and fibroblasts which develops at the site of injury. The spatial relationships observed between these various cell types and the wound edge are expressions of their functional inter-relationships (NIINIKOSKI 1980b).

The important aspect of these inter-relationships is that, in effect, they constitute the formation of an independent organ system which is established solely to achieve repair. Former functions are subjugated to the requirements of repair; new processes commence. This independence is illustrated in the further development of the wound module: as its metabolic requirements for inflammation, neovascularisation and fibrogenesis overwhelm its nutrient supply capacity, the body responds by giving priority of nutrient supply to the wound (FORRESTER 1976; NIINIKOSKI 1980b; BERLINGER 1982). See 1.5.

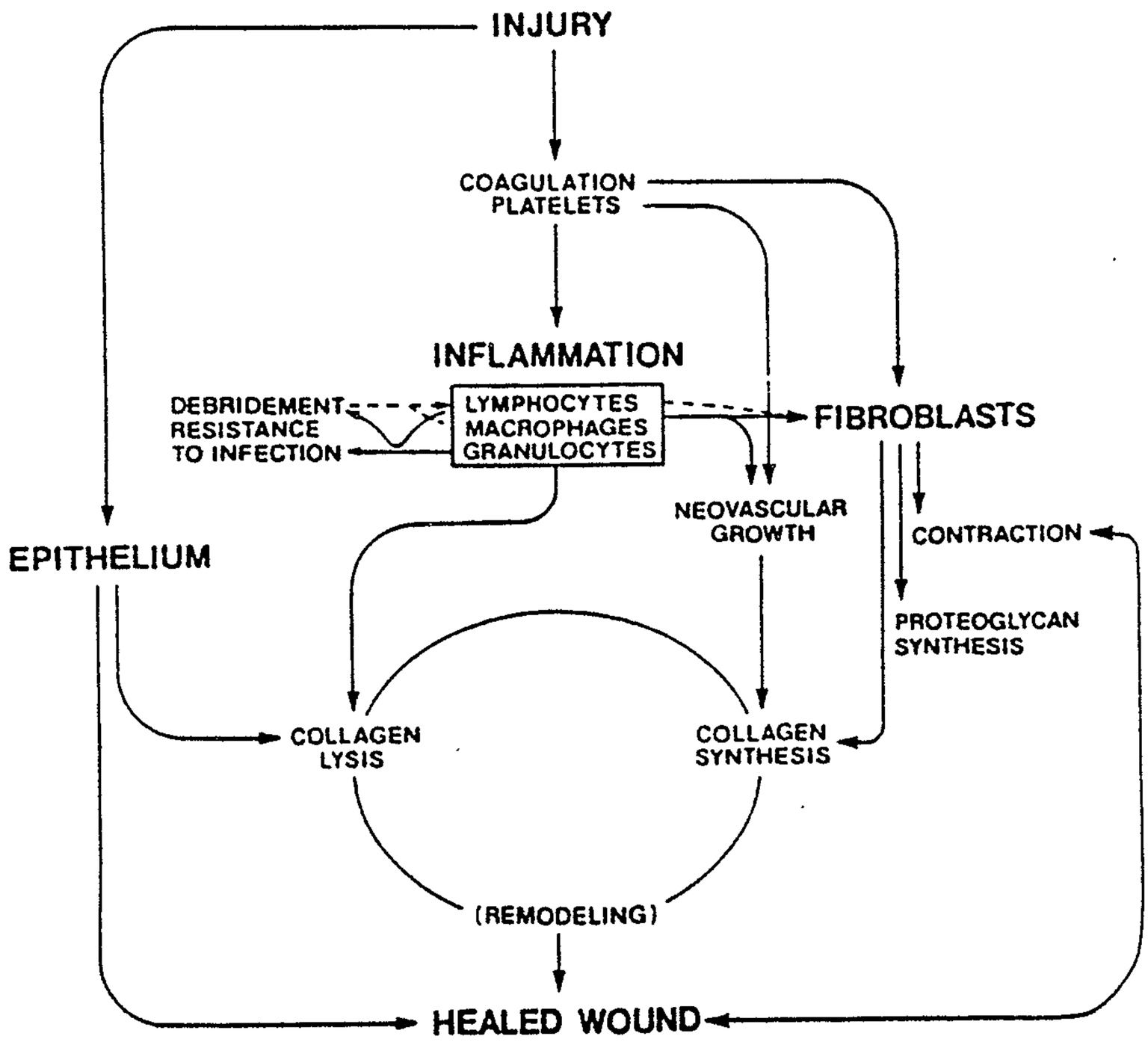
1.2 THE SEQUENCE OF HEALING.

The processes of wound healing can be observed to form an uninterrupted continuum from the moment an incision is made until healing is complete. PEACOCK (1974) describes this continuum as a "concert of simultaneously occurring processes" rather than merely a series of events. Some of these processes, such as sealing of the wound and regaining tensile strength, occur relatively quickly. Others, such as collagen remodelling, are long-term; up to two years, and perhaps even, indefinite (CARPENTER et al 1977).

METCHNIKOFF (1891) was first to recognise this sequence, noting that various characteristic cellular infiltrates appeared within the wound. Commencing immediately after injury, platelet thrombi form in the damaged vessels. Shortly after, leucocytes aggregate in the vicinity, later actively migrating into the area of injury. Fibroblasts and endothelial cells replace both after several days of intense metabolic activity. Neovascularisation also commences about this time, improving the supply of metabolic nutrients. As this so-called **"granulation tissue"** (1.5) grows to fill the wound, the epithelium migrates from the wound perimeter.

These processes are, in normal circumstances, physiologic; their quantitative extremes, however, may be pathologic. (SCHILLING 1976). Further development of this concept will be made later.

An outline of these events is given in TEXT-FIGURE 1.1.



TEXT-FIGURE 1.1 Schematic outline of repair (from SCHROCK 1982, p2).

Many authors have proposed classifications of the phases of wound healing with a variety of terminology. These classifications are primarily functional though somewhat arbitary due to the sequential nature of the subject.

Three phases are commonly identified: the substrate phase, the proliferative phase and the remodelling phase (COHEN et al 1975; FORRESTER 1976; WILKINS & KULWIN 1979; BERLINGER 1982). Although such classification provides a generally sound basis for discussion of the healing wound, its scope is limited in relation to the initial "substrate" phase.

This substrate phase is chiefly associated with the inflammatory process, and as such its nomenclature is appropriate. However, the substrate phase must also include consideration of haemostasis. This

tends to be somewhat minimised by the above classification. Hence, four phases have been identified here as a basis for discussion - haemostasis, inflammation, proliferation and remodelling. It is reiterated that such classification is primarily for convenience rather than any real segregation of events.

It is also noted that the events seen in wound healing are an obligatory sequence which may be initiated by a variety of agents including antigen-antibody reactions and other immune responses, and microbial infection, in addition to injury. There is therefore considerable overlap in the manifestation of these reactions. In the case of injury, the healing sequence appears to be initiated by activation of tissue complement and elements of the coagulation process (HUNT 1980).

It would appear from animal experiments that the cellular, biochemical and histological changes described here are confined to the newly formed granulation tissue and a narrow strip of tissue (approximately 5 millimetres wide) immediately adjacent to the wound (NEWCOMBE 1972; SILVER 1982).

1.3 HAEMOSTASIS.

The almost universal event seen immediately following tissue injury is rupture of the vasculature with resultant haemorrhage. Cessation of this haemorrhage is essential both to prevent serious blood loss (with its concomitant systemic effects) and to prevent accumulation of blood within the wound, causing separation of the wound edges. (Note: discussion of the effect of coagulation diatheses on haemostasis is not within the scope of this thesis).

Primary haemorrhage control is efficiently provided in small vessels by two mechanisms:

i) constriction and retraction of the damaged vessels, and

ii) plugging of these vessels with aggregates of platelets.

These mechanisms are also seen in larger vessels, but, due to vessel size and intralumenal pressure, they are inadequate to achieve cessation of vessel leakage. These mechanisms must be further supplemented (as in small vessels) to provide permanent haemostasis. This is seen with the formation of the "fibrin clot".

1.3.1 Vaseconstriction and Retraction.

Immediately following division, vessels constrict and retract (unless enclosed within bony canals) (BOHM 1977; CARPENTER et al 1977). These responses are postulated to result from a vasomotor reflex (BOHM 1977). Their effectiveness is limited where injury to the vessel is only partial (CARPENTER et al 1977); retraction at such sites in fact opens the vessel.

1.3.2 Thrombocytes.

Thrombocytes, or platelets, are cytoplasmic fragments derived from megakaryocytes (RATNOFF 1969). Platelets have long been known to be central to coagulation, both by their mechanical adherence and aggregation, and their provision of components and initiators of the intrinsic process of coagulation. More recent discoveries have identified other platelet functions in wound healing; these are discussed elsewhere (1.5.1.1; 3.2.2.5).

Under normal circumstances, circulating platelets are nonadherent to other blood elements and to intact endothelium. This **thromboresistance** of the vessel wall has been attributed in part to the

heparin-like activity of endothelial membrane glycocalx (see 2.3.2) and possibly to prostacyclin (see 3.3.2.2) (THORGEIRSSON & ROBERTSON 1978) Several other mechanisms for endothelial thromboresistence are postulated (see HARLAN & HARKER 1981). Vascular disruption, exposing blood to the subendothelial connective tissues, however, initiates interaction between the platelet and the vessel wall. The following account, although separated into subsections, describes a rapidly occurring continuum.

1.3.2.1 Platelet adherence.

When circulating blood platelets contact the subendothelial connective tissue of an injured vessel, they quickly adhere to its surface (CARPENTER et al 1977). This adherence involves the interaction of platelet surface glycoproteins with the connective tissue elements (particularly collagen). Von Willebrand's factor (Factor VIII) is an essential co-factor in this reaction (WEISS et al 1974, cited by HARLAN & HARKER 1981). This factor is reportedly synthesised by endothelium and megakaryocytes with the platelet glycoproteins possibly serving as receptor sites (HARLAN & HARKER 1981).

Adherence occurs irrespective of the cause of injury (CARPENTER et al 1977; SHOSHAN 1981). The nature of the subendothelial elements does however appear to be significant, since platelet adhesion and subsequent aggregation are not seen unless the collagen is in the mature, multimer form (1.5.2.1). Type IV collagen (basement membrane) appears to elicit only adhesion without aggregation (see SHOSHAN 1981). The question of platelet adherence is the subject of continuing research.

Following this interaction with the perivascular tissue components, platelets undergo "disc-to-sphere transformation" and surface alteration with the formation of multiple long, thin pseudopodia. These changes are the result of intracellular microtubule contraction (CARPENTER et al 1977; HARLAN & HARKER 1981).

The initial platelet aggregates and their morphological changes in response to collagen and early thrombin formation (1.3.4) are reversible and therefore described by some authors, as the "primary wave of aggregation" (ZUCKER 1973; HARLAN & HARKER 1981).

1.3.2.2 Platelet release reaction and adhesion.

With continued stimulation, collagen-induced platelets degranulate releasing adenosine di-phosphate (ADP), 5-hydroxy-tryptamine (serotonin, 5-HT), prostaglandins, thromboxane A2 and other factors. Such granule release leads to increased adherence of platelets to each other and to collagen, along with a secondary or recruitment wave of aggregation (RATNOFF 1969; CARPENTER et al 1977; HARLAN & HARKER 1981). See FIGURE 1.1.

The platelet release reaction is a secretory process leading to the active extrusion of the substances stored within the platelet granules. ADP, adrenaline, subendothelial connective tissues and thrombin are identified as the physiologically important agents inducing this release via specific glycoprotein receptor sites (HARLAN & HARKER 1981).

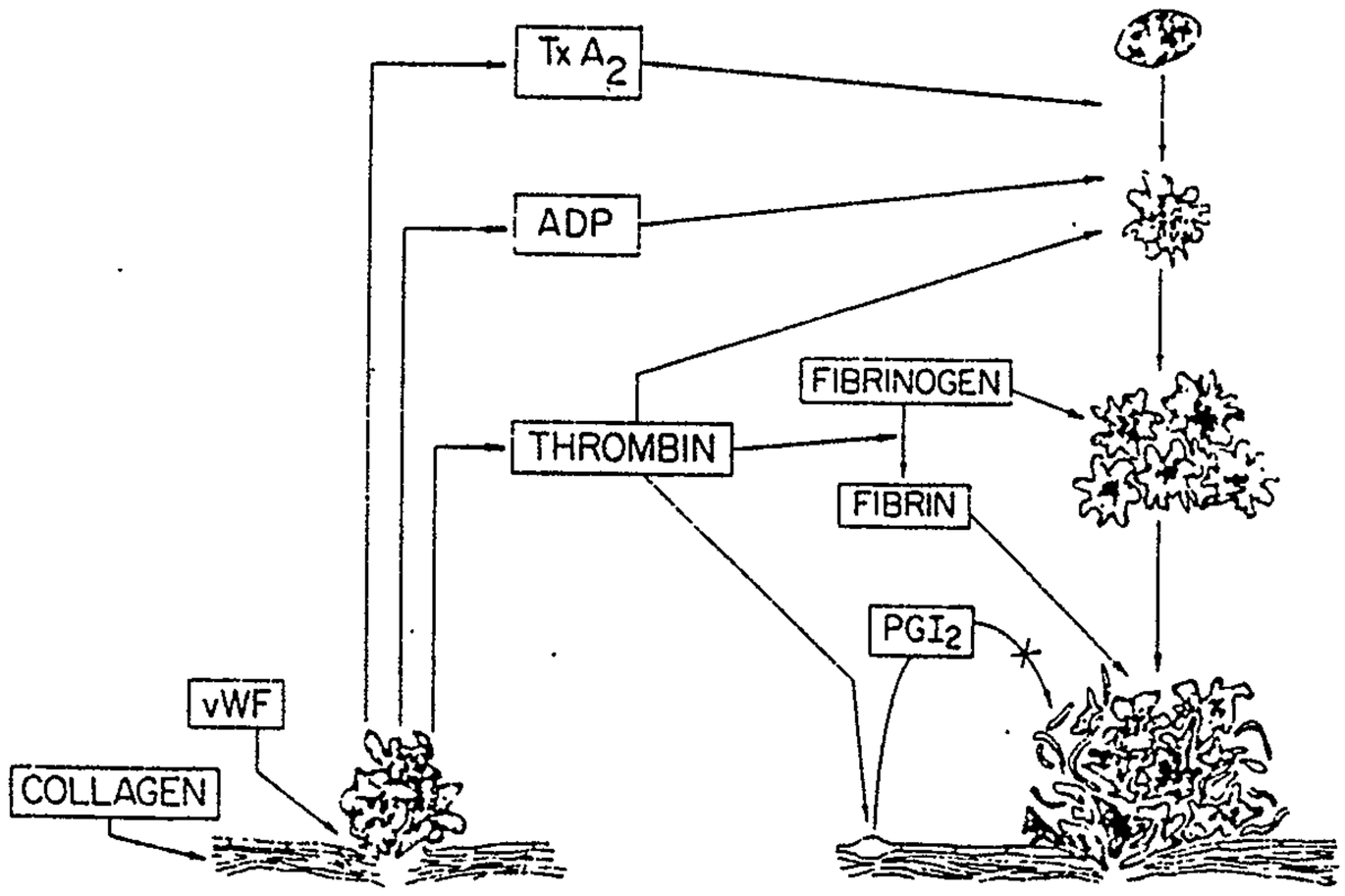
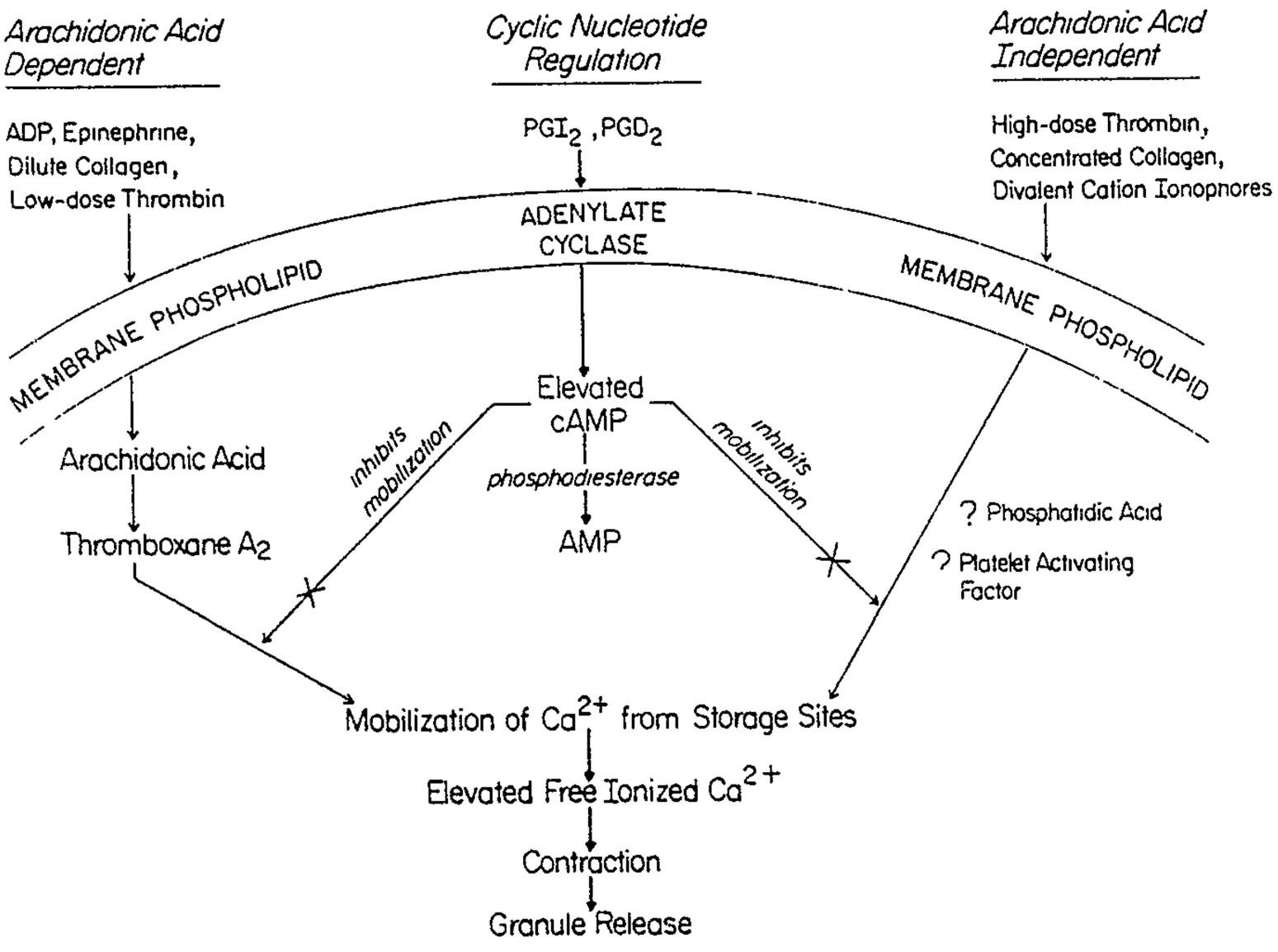


FIGURE 1.1 Haemostatic plug formation. Platelets are normally non-reactive to intact vascular endothelium. Vessel injury initiates platelet adherence with von Willebrand's factor (vWF) as an essential co-factor. Adherent platelets release granule contents including ADP Thrombin is generated locally through tissue factor, factor XIIalpha, and platelet procoagulant activity. Thromboxane A2 (TxA2) is synthesised from arachidonic acid liberated from membrane phospholipases. Released ADP, TxA2 and thrombin recruit additional circulating platelets to the enlarging platelet mass Thrombin-generated fibrin stabilises the platelet mass. PGI2 released by the vessel wall in response to thrombin limits thrombus formation by inhibiting further platelet aggregation (from HARLAN & HARKER 1981, p860).

These processes are modified by the functioning of prostacyclin (prostaglandin I2), which is produced by the vessel wall in response to thrombin. Such modification is suggested to occur through its interaction with the cyclic-AMP pathways (see also 3.3.2.6). Cyclic-AMP (cAMP) action is thought to result in regulation of the intracellular supply of free calcium; increased levels of cAMP decrease the levels of available free calcium thus inhibiting aggregation and degranulation. Intracellular calcium is thus the final mediator of platelet aggregation and degranulation (see TEXT-FIGURE 1.2). In the physiological circumstance, the action of prostacyclin is thought to limit thrombus formation by inhibiting further aggregation (HARLAN & HARKER 1981).



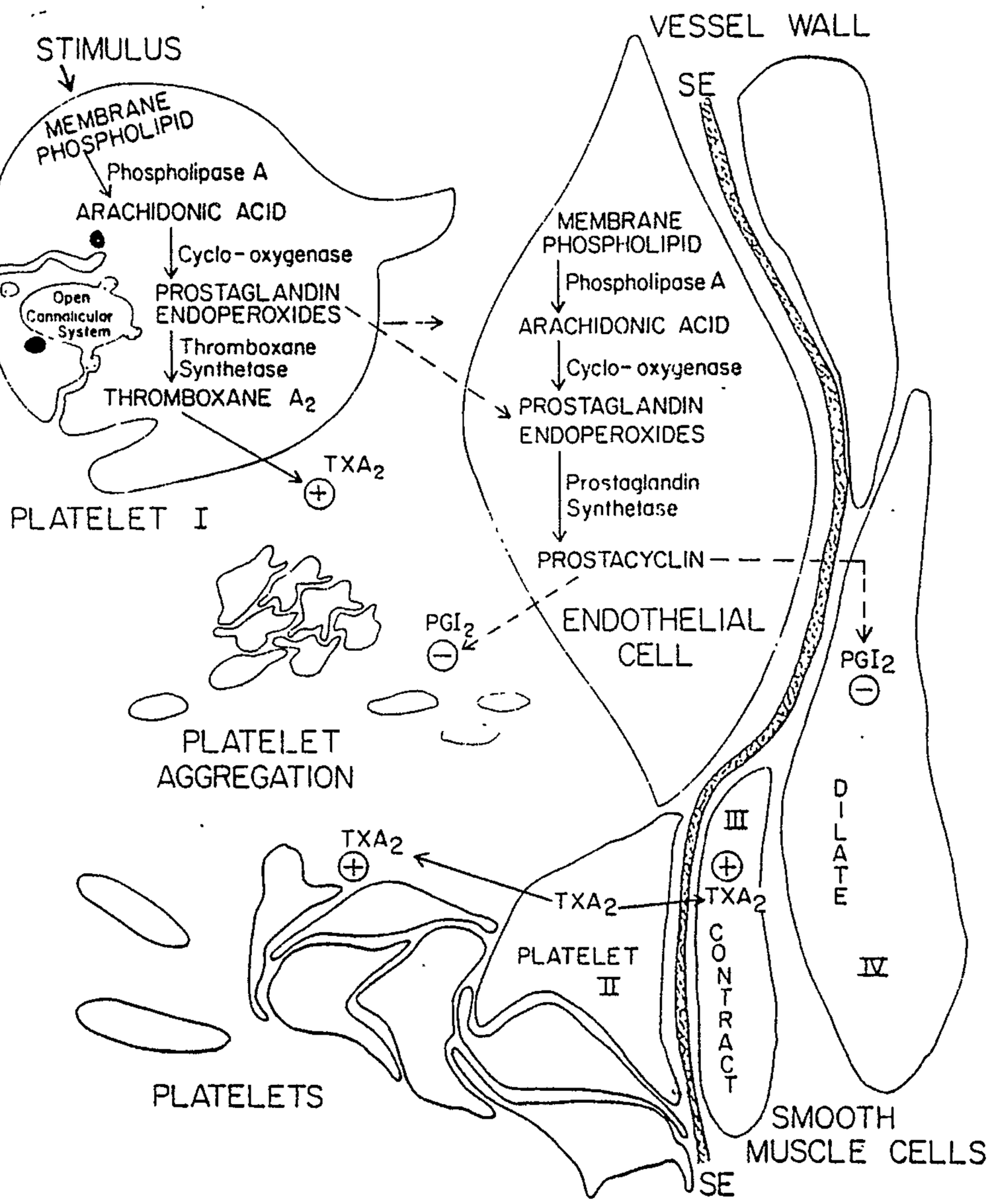
TEXT-FIGURE 1.2 Mechanisms of platelet release. In the arachidonic acid-dependent pathway, ADP, adrenaline, dilute collagen, or low-dose thrombin initiate membrane phospholipidase activity leading to the liberation of arachidonic acid and the formation of thromboxane A2 (TxA2). TxA2 mobilises divalent calcium ions from intracellular storage sites producing an elevation of free, ionised, cytoplasmic calcium. Elevated calcium ions trigger contraction and initiates secretion from both alpha and dense granules.
High dose thrombin, divalent cation ionophore, and concentrated collagen initiate granule release by an arachidonic acid-independent pathway perhaps involving other phospholipase products such as phosphatidic acid or platelet activating factor. Mobilisation of calcium ions again appears to be the final critical event triggering contraction and release.
Binding of PGI2 and PGD2 to membrane receptor initiates adenyl cyclase activity elevating cyclic AMP (cAMP). Elevated cAMP inhibits calcium mobilisation from storage sites and this prevents granule release by either arachidonic dependent- or independent- pathways (from HARLAN & HARKER 1981, p865).

Following this "recruitment wave", the aggregate is largely irreversible. The action of thrombin on the platelets themselves and through the conversion of fibrinogen to fibrin is important here (CARPENTER et al 1977; SHOSHAN 1981).

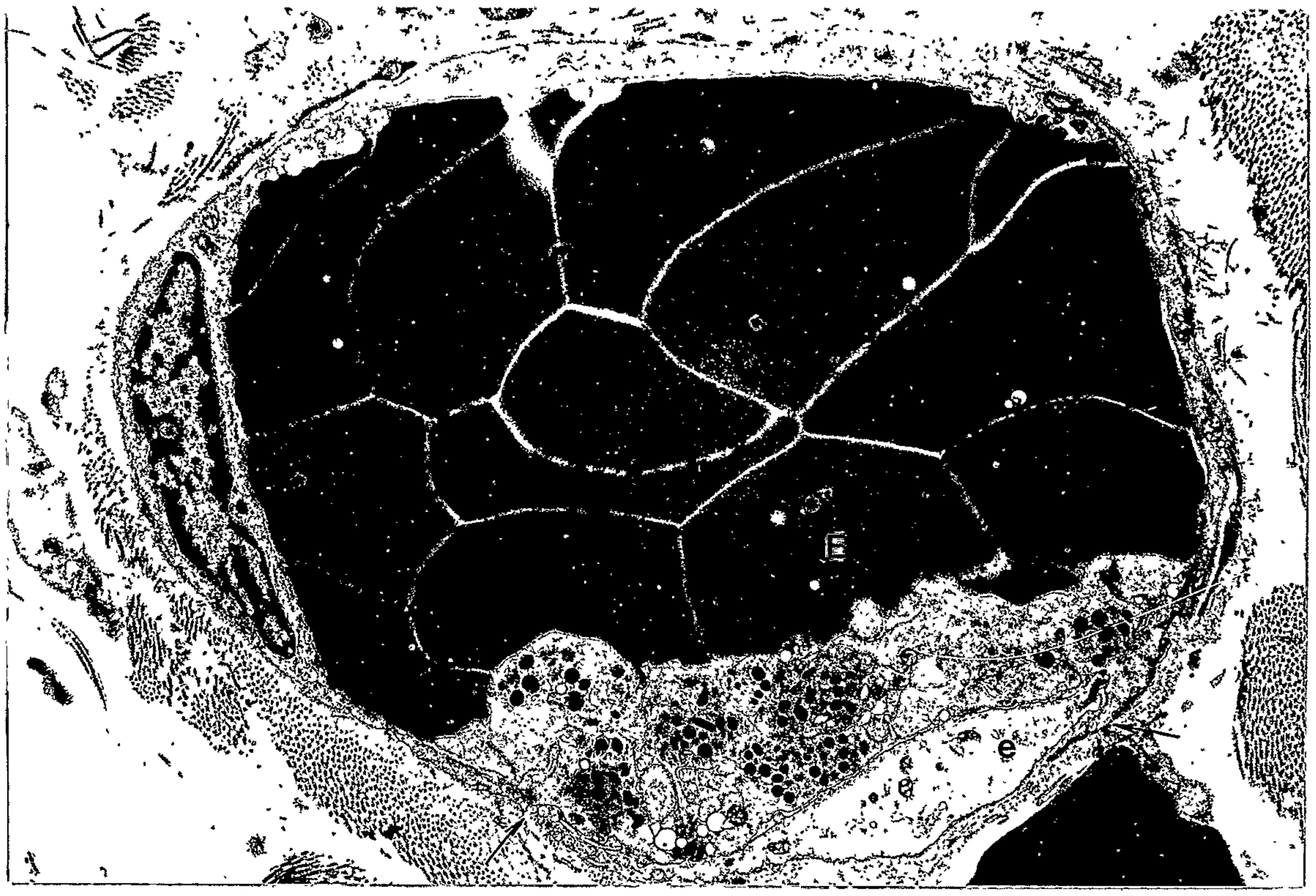
Concurrently, platelets are seen to fuse in a process known as **viscous metamorphosis** (CARPENTER et al 1977). As a result, their plasma membranes are disrupted and further discharge of cytoplasmic granules

occurs. With repeated stimulation of platelet aggregation and adhesion, these events lead to the formation of a compact, fused, amorphous structure firmly bound to the subendothelial collagen. This is known as the "platelet plug" (CARPENTER et al 1977).(See PLATE 1.1).

The interactions between platelet and endothelial cell, particularly relating to interactions between the prostaglandins, are illustrated in TEXT-FIGURE 1.3.



TEXT-FIGURE 1.3 A diagrammatic illustration of the interaction between platelets and endothelium, with special attention to the counter-balance mediated by thromboxane A2 (TxA2, platelet origin) and prostacyclin (PGI2, endothelial cell origin). TxA2 provokes platelet aggregation and has a vasoconstrictor role on smooth muscle (III) in the vessel wall). PGI2, a potent inhibitor of platelet aggregation, also produces vasodilation in vascular smooth muscle (IV). Symbols: (+) = positive influence; (-) = negative influence; SE = subendothelium (from NALBANDIAN & HENRY 1978, p115).



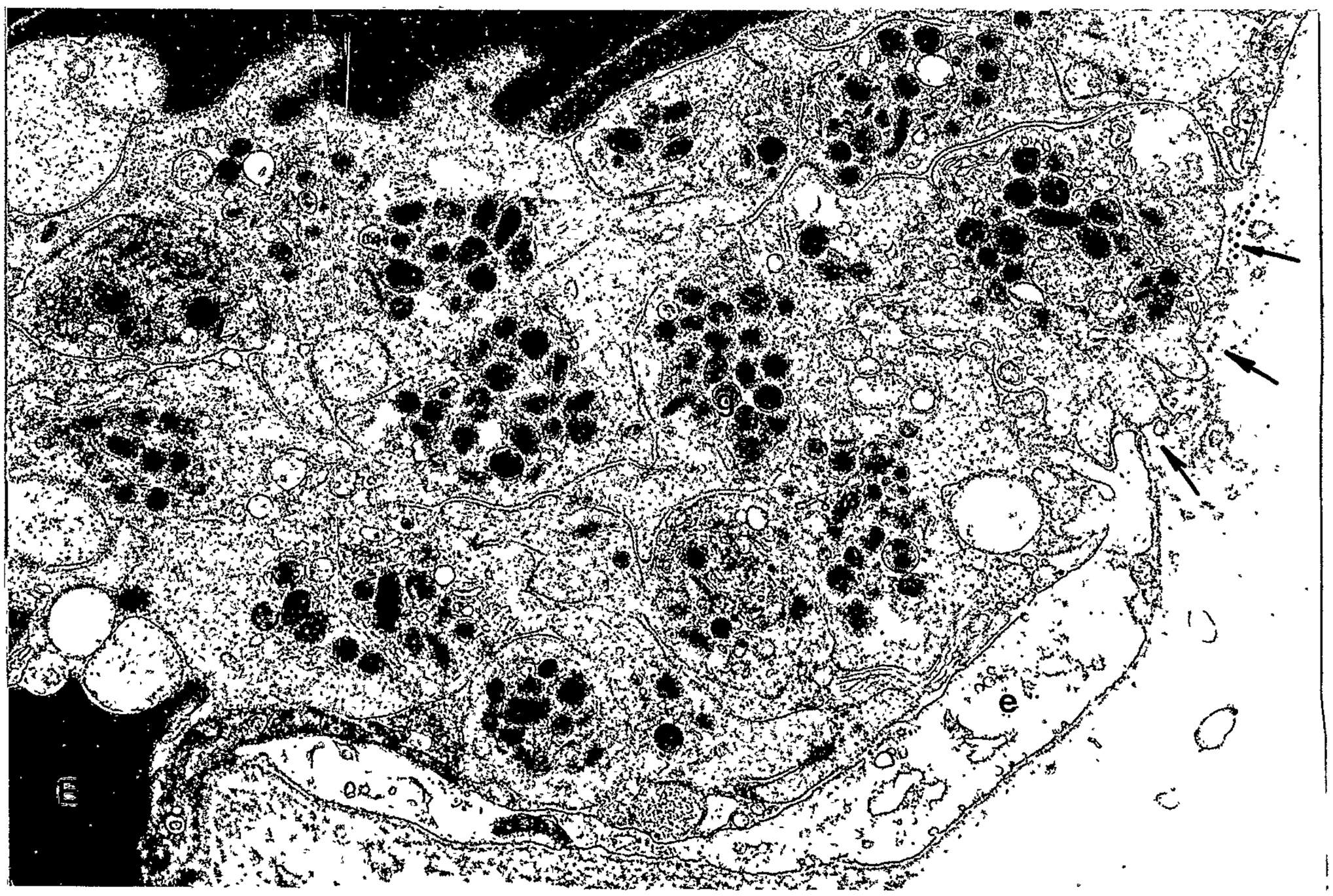


PLATE 1.1A & B Electronmicrographs from human attached gingiva distal to the mandibular second molar. This tissue was removed just prior to closure (that is, about 15mins after incision) of a buccal mucoperiosteal flap associated with the removal of a mandibular third molar tooth. In these vessels (possibly small venules) "platelet plugs" are evident adhering to areas of exposed subendothelium (arrows). The basal lamina would appear to be mostly intact (....). Intracellular granules (g) are clearly visible. Other symbols: e = endothelial cell; E = erythrocyte. (Magnification: PLATE 1.1A x6825; PLATE 1.1B x14,470).

1.3.2.3 Platelets and coagulation.

Having considered the mechanics of the platelet response to endothelial injury, discussion is now briefly directed to the relationship of platelets to the clotting mechanisms. In blood deprived of platelets, "the coagulation time in glass tubes is prolonged, the activation of prothrombin is incomplete and the clots do not retract" (MACFARLANE 1973). Platelets are said to contribute to coagulation "by behaving as a phospholipid, thus providing a surface necessary for several of the reactions in the coagulation cascade" (ZUCKER 1973).

This action is given the name "Platelet Factor 3" (PF3). Such activity is not seen in circulating platelets but develops upon their aggregation. It does appear however, that only small amounts of free PF3 are released into the plasma. Rather PF3 activity primarily seems to represent a change in the platelet membrane (ZUCKER 1973).

1.3.3 The Fibrin Clot.

The platelet aggregate may be adequate to prevent blood flow from the smallest vessels (as seen in congenital afibrinogenaemia, RATNOFF 1969). However, a second mechanism is required to complement primary haemostasis by the transformation of the blood clot into a solid gel.

This gel is predominantly composed of the fibrous protein, fibrin, with entrapped blood cells and serum. Fibrin is formed by the action of thrombin on the soluble precursor, fibrinogen (RATNOFF 1969; MACFARLANE 1973). Thrombin acts to hydrolyse the fibrinogen molecule releasing two pairs of small polypeptide fragments (fibrinopeptides A and B, see 3.3.1.2). The major fragment which remains - termed the "fibrin monomer"- then polymerises in the presence of thrombin, fibrin-

stabilising factor (Factor XIII) and calcium ions to form the insoluble fibrin clot (RATNOFF 1969; MACFARLANE 1973; STAINDL 1979).

1.3.4 The Formation of Thrombin.

Both the formation of fibrin and the activation of fibrin-stabilising factor, (together with platelet aggregation and clot retraction), are initiated by thrombin. This proteolytic enzyme is not found in fresh normal blood. Under appropriate conditions, it rapidly evolves from the inert, soluble plasma protein precursor, prothrombin, the existence of which was first postulated by MORAWITZ (1905, cited by MACFARLANE 1973).

Considerable information is recorded concerning the processes leading to this conversion; two major pathways have been identified and are briefly outlined here. In one, the **extrinsic pathway**, clotting is initiated by the interaction between the injured tissue and the blood with which it comes into contact. This results in the production of an ill-defined complex of protein and phospholipid known as tissue thromboplastin. The second sequence which results in thrombin formation - the **intrinsic pathway** - occurs independently of the action of tissue thromboplastin. Rather, the activation of Hageman Factor leads to its initiation. The last steps of each of these pathways coincide.

Knowledge of much of these processes remains only superficial despite intensive research. In both pathways, evidence suggests that pairs of the involved factors relate in a sequential chain of reactions, the product of each becoming a participant in the next stage. In this long chain of reactions, there is considerable overlap in time between the stages, with several stages being activated concurrently.

Another significant feature of both clotting sequences is that the presence of the active factor amplifies its initiation reaction. Thus, the sudden appearance of fibrin follows an almost explosive generation of thrombin in a sequence which was initially activated by the minute physical stimulus of surface contact. This rapid appearance of fibrin following a short latent period, appears to be critical in the effectiveness of the haemostatic processes. The more gradual appearance of fibrin seen in haemophilia is a less effective haemostatic measure (MACFARLANE 1973).

Hageman Factor (or Factor XII in the intrinsic pathway) was first discovered by RATNOFF in 1954 (see RATNOFF 1966). It is a plasma glycoprotein present in normal blood in an inactive form. Hageman factor (HF) can be activated in the fluid phase, or by contact (solid phase) (COCHRANE et al 1973, 1974). From the work of MARGOLIS (1963) it is well established that solid phase activation of HF occurs following its contact with negatively charged surfaces. This includes exogenous substances such as glass and kaolin along with a variety of biological substances including collagen and basement membranes. Bacterial lipopolysaccharides (endotoxins) are also implicated (RYAN & MAJNO 1977). Trypsin, plasmin and plasma kallikrein have been shown to activate Factor XII in the fluid phase (see MOVAT 1979).

It is noted that this separation into fluid and solid phases is somewhat arbitary considering that activation in the fluid-phase is greatly augmented when Hageman factor is bound to a surface. This suggests that binding alters its three-dimensional configuration thereby exposing an active site, or facilitating cleavage of the HF molecule (KAPLAN et al 1982). See 3.3.1.4.

Hageman factor is critical in initiating three of the early healing process: i) triggering the clotting cascade as discussed here, with activation of Factor XI, ii) fibrinolysis (through the activation of plasmin) and iii) prekallikrein activator activity (leading to kinin activation). Discussion of HF in relation to these aspects is reviewed in 3.3.1.4.

1.3.5 Clinical Significance of Haemorrhage Control.

Cessation of haemorrhage is highly significant in the schema of wound healing. As stated by HUNT (1974), "without normal haemostasis, repair has, at the very least, a difficult beginning". It is now recognised that an extravascular fibrin clot is, in fact, regarded by the body as a collection of necrotic tissue.

This is primarily a matter of degree since the presence of extravascular fibrin is important in providing an immediate, if poor, seal of the wound against the ingress of foreign materials such as debris and bacteria (SCHILLING 1976; POLLACK 1979a; SILVER 1980). This clot also weakly unites the wound edges (POLLACK 1979a) and it may provide a lattice framework for later cell migration (COHEN et al 1975; SCHILLING 1976) (see 1.5.1).

However, the extravascular clot increases the extent of dead and devitalised cells and debris which must be removed by the body's defences. Large collections also place additional load on liver and kidney for metabolism and excretion of breakdown products (MOORE 1977). In addition, the extravascular clot enlarges the size of the wound to be repaired, and it provides an excellent culture medium for microorganisms which have contaminated the wound (SCHILLING 1976; CARPENTER et al 1977). Further, a large clot significantly compromises

the nutrient supply to a wound which is already ischaemic as a result of initial vessel division (SCHROCK 1982).

It is the intravascular clot which is considered to be the physiologically essential requirement (SCHILLING 1976).

This subject will be discussed further in relation to haematoma (see 6.3.5).

1.4 ACUTE INFLAMMATION.

(Discussed in detail in Chapter 3)

1.4.1 Historical Aspects.

From ancient times, description of the body's response to physical trauma has included the idea of comparing "red, hot, swollen skin" to something "ablaze". (Examples can be found in the cuneiform script of Mesopotamia and in the hieroglyphic notation of Egypt). Several such terms can be translated as "inflamed" or "inflammation" - a word which itself is derived from the Latin, "inflammare" meaning "to flame within" (RYAN & MAJNO 1977; THOMAS 1981).

The classical description of inflammation is that of the Latin author, Celsus (c.AD30). Celsus provided a description of the characteristic signs of inflammation, namely redness and swelling with heat and pain (rubor et tumor cum calor et dolor) (LYONS & PETRUCELLI 1979). These so-called cardinal signs stand to this day, however understanding of the processes which lead to their expression remains far from complete. HURLEY (1972; 1981) and WEISSMANN et al (1980) provide a lucid discussion of current knowledge of the biological basis of these signs. Some clarification is presented throughout.

1.4.2 The Inflammatory Phase.

Inflammation represents a collection of processes which serve to protect tissue in the event of injury. It commences immediately upon occurrence of tissue damage and becomes established following stabilisation of the vascular supply to the surgical site, as seen at the completion of haemostasis (MILLIKAN 1981). This phase is considered to encompass the first three to four days (BERLINGER 1982). The nature of the response varies with the type and intensity of the injurious agent, the site which is damaged and the physiological response of the host. It results in the accumulation of cells and fluid exudate at the site of injury (RATNOFF 1969).

Classically, the inflammatory phase of wound healing was termed the "latent" or "lag" phase since little seemed to be occurring to the observing eye. The inaccuracy of this terminology was soon demonstrated with the advent of the microscope when it was revealed to be a period of intense metabolic activity (WILKINS & KULWIN 1979; BERLINGER 1982).

Inflammation is primarily a vascular reaction in which local vessels become dilated and show increased permeability to macromolecules and cells. This results in two features which are characteristic of acute inflammation.

Firstly, a fluid exudate is formed. This contains a variety of biologically active substances including vasoactive proteins and immunoglobulins. It also contains nutrients required for healing, thus providing an environment in which emigrating leucocytes can survive (POLLACK 1979a).

Secondly, leucocytes are attracted to the area in large numbers as a result of the early disruption of vessels and the release of

chemotactic factors (MILLIKAN 1981). Polymorphonuclear leucocytes appear first (within minutes of wounding in some experimental models, WILLIAMS 1983) and engulf any bacteria which may be present. Polymorphs are followed by monocytes which enter the tissues and rapidly transform into tissue macrophages (see 1.5.1) (POLLACK 1979a; ROSS 1980).

The end result of the inflammatory phase is the "preparation of the optimal environment for the expeditious repair of the defect" (MILLIKAN 1981). It is only when bacteria, foreign bodies, surgical trauma, medications, or the accumulation of proteolytic enzymes cause extensive tissue necrosis that inflammation may become a deterrent to healing (PEACOCK 1974). From a clinical viewpoint, each of these features (other than the accumulation of proteolytic enzymes) may be minimised or maximised by the surgeon (see Chapters 5 and 6). However, the role of inflammation in healing is still debated: the extent to which it is normal and that which is excessive. This subject is discussed in Chapter 9.

It is emphasised that the phases of acute inflammation and repair are mutually exclusive and that acute inflammation must begin to subside before repair begins (CARPENTER et al 1977; SCHROCK 1982). Persistence of the acute inflammatory reaction leads to persistence of neutrophils in the wound, so that other changes are delayed (CARPENTER et al 1977).

1.4.3 Differential Diagnosis.

When considering the formation of oedema which occurs as a result of post-surgical inflammation, it is essential for the clinician to outline the differential diagnosis of post-surgical swelling.

1.4.3.1 Angioneurotic oedema.

Hereditary angioneurotic oedema (HAE) is a fairly uncommon condition resulting from an inherited deficiency (qualitative or functional) of the serum inhibitor of the first activated component of complement (C1). This deficiency follows an autosomal-dominant inheritance pattern and results in the sometimes rapid development of localised oedema. Eyelids, face, lips, tongue, larynx, extremities and genitals are the most frequently involved sites (CHUE 1976). It is frequently initiated by pressure and trauma such as with dental extractions and surgical procedures.

Diagnosis of HAE is simplified by its distinctive presentation. It is differentiated from acute infection and acute inflammation by the absence of pain, heat and erythema. Erythema also differentiates HAE from contact dermatitis (CHUE 1976; PENCE et al 1974). It is noted that acute angioneurotic oedema is reported as occurring independent of familial association, apparently induced by many foods and drugs. The appearance of this form is clinically indistinguishable from the inherited form.

1.4.3.2 Infection.

Facial swelling may also be indicative of acute post-operative infection. Infection is generally characterised by fever, erythema, pain and malaise. Such swelling is frequently described as "brawny", suggesting tense, musky-red swelling. In this aspect, swelling resulting from acute infection may be differentiated from acute inflammatory oedema. The latter is more normally soft, and though (as noted in the cardinal signs), inflammatory vasodilatation is accompanied by localised erythema and heat, the patient is essentially afebrile.

In view of the fact that oral wounds are invariably contaminated by potentially pathogenic microrganisms, the subject of wound infection is closely related to the biology of wound healing in the mouth and thus to post-surgical oedema (6.2.3). Infection itself, however, is a peripheral concern to this thesis.

1.4.3.3 Surgical emphysema.

BARCLAY (1970) draws attention to surgical emphysema - swelling due to air in the tissue spaces - as an additional aetiological factor in post-surgical swelling. Surgical emphysema is a fairly uncommon complication of minor oral surgery. It may result from inappropriate use of high-speed air turbine handpieces and from ineffective wound closure in the presence of vaccuum drains. Such swelling is differentiated from oedema by its characteristic "crackling" sensation to palpation.

1.4.3.4 Haematoma and oedema.

There remain the two chief aetiological factors of postoperative swelling - haematoma and oedema. Of these, oedema will dominate this discussion, it being the central concern of this thesis.

The aetiology of haematoma is theoretically clear, resulting from post-operative haemorrhage. Though its aetiology is unrelated to oedema, the two are frequently found together, and any discussion of oedema must also consider haematoma. (See 6.3.5).

1.5 PROLIFERATIVE PHASE.

The dominant features of the proliferative phase of healing are rapidly increasing wound tensile strength and wound contraction. These features result from the prolific functioning of the "fibroblast-capillary" system which leads to increasing synthesis and deposition of collagen and ground substance (FORRESTER 1976).

A variety of factors may affect the functioning of this "wound module". The supply of oxygen in the wound environment is the governing factor. Other important factors include nutritional factors (such as the availability of zinc, and vitamins A and C), disease factors (such as diabetes), and physiological changes (such as hormonal fluctuations and ageing). These factors are reviewed in Chapters 5 and 6.

Any injury which destroys more than the superficial epithelium evokes formation of a new tissue - called "granulation tissue" - which becomes progressively transformed into a mature, fibrous scar. The amount of tissue formed, the time sequence of its maturation and the rate of epithelial coverage vary depending upon the site and type of injury and the size of the defect. However, certain features are common, namely that wherever granulation tissue is formed, it consists of three basic elements - cells, fibres and ground substance (NEWCOMBE 1972).

1.5.1 Cells.

During the phase of acute inflammation, the wound is held together by a small amount of fibrin aided by sutures (CARPENTER et al 1977). After 24 to 36 hours, the first signs of connective tissue proliferation are seen with rapid mitotic division of fibroblasts and endothelial cells (SILVER 1980). The early dominance of neutrophils

also declines at around this time, being replaced by mononuclear cells which rapidly transform into macrophages (ROSS 1980; SILVER 1980).

These three cell types - the macrophage, the endothelial cell and the fibroblast - are the predominant cellular elements of granulation tissue in all organs, except those of epithelial origin (SILVER 1980). They form the functional unit of the "wound module" (1.1). The interactions between them are exemplified by their spatial relationships which are principally governed by nutritional and environmental factors (NIINIKOSKI 1980b), see below.

Concurrent with this connective tissue repair is the re-epithelialisation of the wound (consideration of which follows in 1.5.1.2).

1.5.1.1 Connective tissue cells.

Macrophages.

"As the granulation tissue grows into the wound space, the leading edge is occupied by a vanguard of macrophages and other phagocytic cells" (SILVER 1982), the latter being principally neutrophils (POLLACK 1979a). At this site, the tissues are acidic with a very low (almost zero) oxygen tension. Although macrophages are capable of extended periods of survival in complete anoxia, their destruction of bacteria is somewhat less efficient here since it is chiefly achieved through the production of hydrogen peroxide and super-oxide (see 3.2.2.5). Their phagocytic capabilities however are not impaired (SILVER 1982).

In the past, most researchers considered that the role of macrophages in healing was limited to phagocytosis. It has however recently become apparent that they have multiple functions and are in

fact crucial to the wound system. This was clearly demonstrated by the widely reported studies of LEIBOVICH and ROSS (1975). In these studies, skin wounds were made in animals who were monocyte-depleted and who were injected with anti-macrophage serum subcutaneously around the wound. In comparison with a variety of controls, the healing of the wounds in these animals was considerably retarded, particularly in relation to fibroblast migration and proliferation. These studies were later followed by in vitro experiments in which medium from stimulated macrophages was found to stimulate resting fibroblasts to logarithmic growth (LEIBOVICH & ROSS 1976).

In contrast, previous studies on neutrophil-inhibited wounds showed no alteration of healing except where large numbers of bacteria were present. In this case, infection developed, progressing to death due to septicaemia (SIMPSON & ROSS 1972, cited by ROSS 1980). Similar results are seen if lymphocytes are experimentally excluded from the wound (SCHROCK 1982). (See also 3.2.2.5).

Several functions relating to the promotion of connective tissue proliferation have been ascribed to macrophages. Firstly, (as previously stated), the macrophage acts by microscopically debriding the injured tissues. Secondly, and resulting from the first, the macrophage processes the ingested macromolecules to release the primary biochemical units (amino acids, simple carbohydrates, free fatty acids). Thirdly, the macrophage secretes chemotactic factors attracting further macrophages to the area. Fourthly, the macrophage is thought to secrete factors which stimulate fibroblast growth and activation, and neovascularisation (POLLACK 1979; SHOSHAN 1981; BERLINGER 1982).

Fibroblasts.

As the macrophages move into the wound space, they are closely followed by undifferentiated connective tissue cells. These cells have been shown to produce soluble collagen and its precursors and are therefore classified as a type of fibroblast (SILVER 1980; 1982).

The origin of fibroblasts was for some years the subject of debate between two opposing views (NEWCOMBE 1972):

i) that fibroblasts are derived from the perivascular, pluripotential mesenchymal cells and from local fibroblasts in the immediate vicinity of the wound, and

ii) that they arise from transformation of leucocytes (particularly monocytes) which have migrated to the area of injury.

The balance of scientific opinion now is that the first theory is correct. This is chiefly the result of studies by ROSS and others using monocyte-depleted animals and labelled monocytes from donor animals. Labelled monoctyes, neutrophils and lymphocytes were observed to migrate freely into the wound, yet no labelled fibroblasts were seen in the wound amidst the large numbers of unlabelled fibroblasts (cited by ROSS 1980).

Various factors which stimulate fibroblast growth and synthesis have been proposed. These chiefly originate from the macrophage (as mentioned above) (LEIBOVICH & ROSS 1976). Recent studies however, suggest that potent growth factor(s) are also derived from platelets (RUTHERFORD & ROSS 1976; SCHROCK 1982). ROSS (1980) also suggests that perhaps this platelet-derived growth factor is a principal, initiating factor in fibroblast proliferation and wound repair. Collagen (Types I, II and III) also seems to be chemotactic for fibroblasts (SHOSHAN 1981).

Restoration of tissue continuity following injury and strengthening of the ensuing repair tissue is chiefly dependent upon fibroblasts (SHOSHAN 1981). These cells synthesize not only collagen, but also elastin and proteoglycans. They also provide enzymes necessary to wound metabolic processes including those for cholesterol synthesis, the Kreb's cycle and glycolysis (POLLACK 1979a).

Some fibroblasts also develop numerous intracellular myofibrils, as well as undergoing structural, compositional and functional changes (SHOSHAN 1981). These contractile cells are then called myofibroblasts; they appear to function in wound contraction (see 1.5.1.4) rather than in connective tissue synthesis (POLLACK 1979a; SILVER 1982).

To some degree, fibroblasts obtain their high metabolic requirements through phagocytosis and degradation of debris in their environment (SILVER 1982). However, this is inadequate to meet all their mitotic and metabolic needs. Thus, fibroblast proliferation is critically determined by the growth of endothelial sprouts and their supply of oxygen: mitosis is only seen where the oxygen tension is 25 mmHg or above, that is, only immediately adjacent to the leading edge of the capillary (NIINIKOSKI 1980b; SILVER 1982). (See 6.2.1.4).

Endothelium.

When a new capillary sprout first develops, it is solid. Its growth is directed towards a neighbouring sprout with which it fuses, forming an arch. The stimuli which determine the direction of this growth remain far from clear.

The traditional opinion held that necrotic tissue and blood clot provided the stimulus for neovascularisation (THAKRAL et al 1979) and that new fibroblasts and capillaries grew along the fibrin strands.

However, this has recently been questioned. Although it is certainly correct that these elements provoke an inflammatory response (THAKRAL et al 1979), it would appear that large collections of clotted blood form a barrier to healing and that the removal of tissue debris and the remains of the clot are essential prerequisites before repair occurs (ORDMAN & GILLMAN 1966a; SILVER 1980; 1982). (See also 1.3.5).

Some suggest that the growth of new vessels may be directed towards an area of low oxygen tension, or following a pH gradient, or perhaps some chemotactic factor secreted by macrophages. Presumably, the capillary buds are able to join each other and pre-existing vessels by means of chemotactic attraction between such cells (SILVER 1982). It is possible that all these factors may play some role. Most authors would agree, however, that the macrophage is the most likely cell to "orchestrate" the repair process. This is supported by various studies (cited by THAKRAL et al 1979) reporting that macrophages directly stimulated growth in some areas of this process (including neovascularisation), and they appeared highly significant in others.

Neovascularisation into the wound follows the removal or liquefaction of debris and clot by macrophages (SILVER 1980; 1982). Endothelial cells then contribute to this dissolution, apparently by their secretion of plasminogen activator (COHEN et al 1975; VAN DER MEULEN 1982).

The new vessels exhibit unusual properties, including marked permeability to macromolecules (and even to particles), and easy penetration by a variety of cells (SILVER 1982). This is, in part, related to incomplete formation of the basal lamina (VAN DER MEULEN 1982). These vessels do not contribute to wound nutrition until some consistency of flow is established (HUNT et al 1969).

Their spatial orientation is initially profuse and irregular. However, as the wound matures and its metabolic requirements stabilise, many of these newly developed vessels regress (POLLACK 1979a). (See 1.6). Vascular neogenesis is illustrated in FIGURE 1.2.

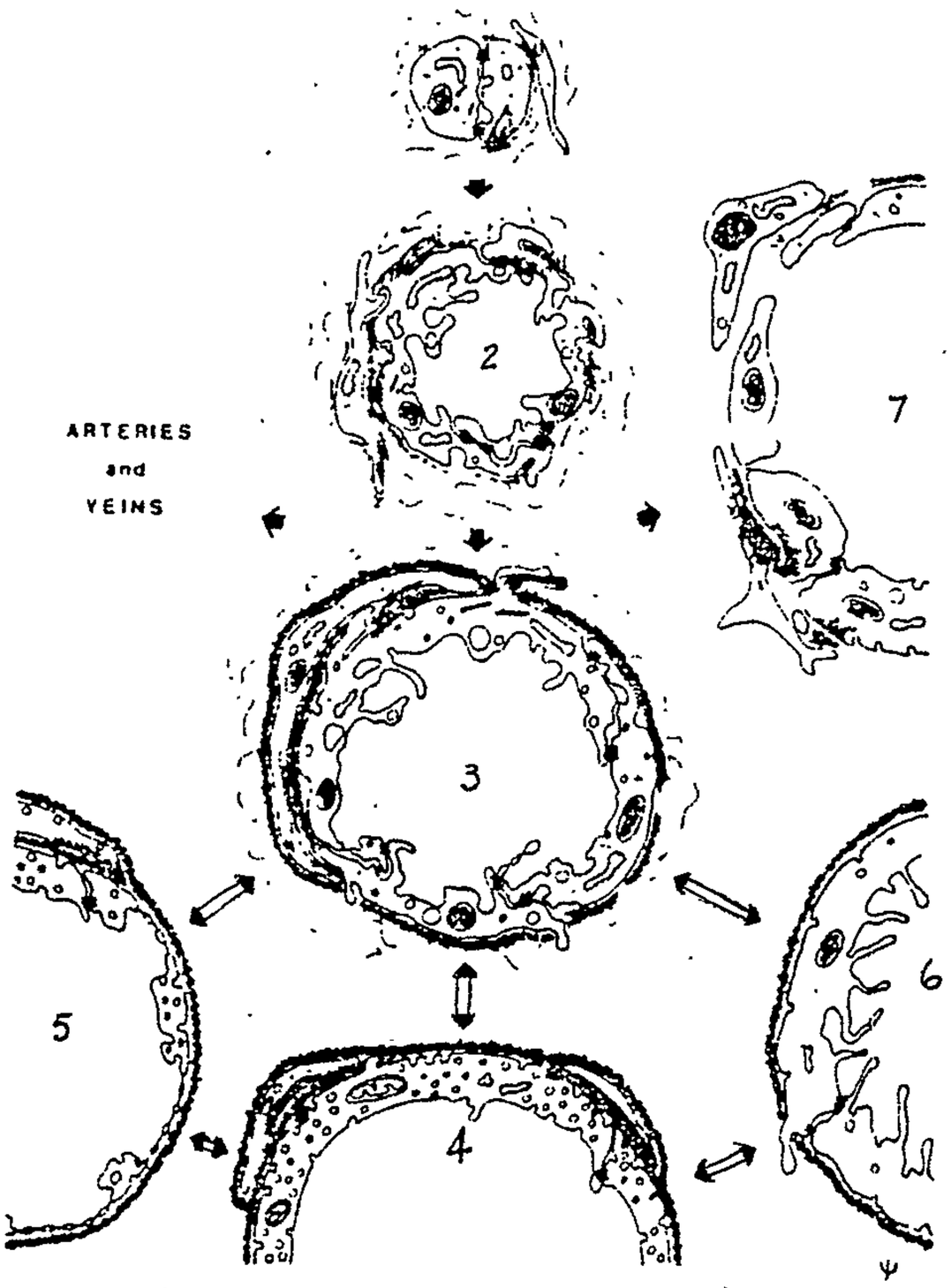


FIGURE 1.2 Diagram summarises various developmental stages from sprouts (1) to immature capillaries that may occur in inflammation and granulation tissue and tumours (3). From this precursor, all types of vessels and capillaries are formed, those with continuous (4), fenestrated (5), microvillous (6), and discontinuous endothelia, so-called sinus or sinusoids (7). The possibility of transformations from one type to another is indicated by arrows (from WOLFF 1977, p123).

Mast cells.

Another cellular feature of early connective tissue wound healing is the presence of mast cells which appear to surround the newly formed capillaries. Later they are found to be scattered throughout the granulation tissue filling the wound. Mast cells are characterised by their metachromatically-staining (with toluidine blue) cytoplasmic granules which have been shown to contain heparin, histamine and serotonin. Their role in wound healing however, remains speculative (NEWCOMBE 1972).

"Granulation tissue".

Before epithelialization is complete, considerable gross changes can be observed in the centre of the wound. The surface, which once was greyish-white (on oral mucosa) / yellow-brown (on skin) becomes bright red and granular. This gross morphological appearance results from the "extravagant proliferation of richly perfused capillary loops" and it was from this appearance that the term "granulation tissue" was derived (PEACOCK 1974).

This tissue grows to fill all defects created by the injury. It does not usually, however, grow beyond the epithelial line, except in cases where continued irritation is present. The reason for this is unknown but appears to be in some way associated with the nature of epithelial migration (SILVER 1980). It also appears to be related to so-called "contact inhibition": when cells of a similar type come into contact, further multiplication is suppressed (POLLACK 1979a).

1.5.1.2 Epithelial cells.

One of the major characteristics of epithelium is that it forms a barrier to the evaporation of water and to the entry of organisms. Re-establishment of epithelial continuity is thus the next major requirement following haemostasis (SILVER 1982). This occurs concurrently with the above connective tissue events.

Morphological changes in wounded epithelium.

In linearly incised wounds on mouse or human skin, epithelium is seen to undergo various morphological changes. Basal and suprabasal cells become flattened and lose many of their junctional complexes, developing instead, "ruffled", interlocking borders (POLLACK 1979a).

Various intracellular changes also occur including the development of banded, contractile protein filaments and of inclusion bodies, presumed to be phagolysosomes. In addition, these cells become greatly hypertrophic as a result of increased material storage and synthesis (FISCHER 1971; ODLAND 1977; POLLACK 1979a).

Having undergone these morphological changes, these cells no longer proceed towards keratinisation/cornification. Rather, their functioning is directed towards migration and multiplication by division (POLLACK 1979a). This alteration of function is also indicated by other intracellular changes suggestive of a "loss of differentiation" (ROSS & ODLAND 1969).

Epithelial migration.

Within 20 to 24 hours of wounding, the epidermis mobilises and commences to migrate inwards (GIACOMETTI & MONTAGNA 1969; ODLAND 1977). The route of this migration depends on the state of the wound. In a simple, well-sutured incisional wound there is minimal dead space and tissue dehydration and the cells migrate just beneath the surface of the fibrin clot (FISCHER 1971; SILVER 1982).

In skin wounds healing by secondary intention, where superficial dehydration is more extensive, the advancing epidermal cells migrate deep to the blood clot, within the intact dermis (GIACOMETTI & MONTAGNA 1969; SILVER 1982). The depth at which this migration occurs is dependent upon the "water table" within the wound - dessicated tissues being unable to sustain cell vitality (POLLACK 1979a). The deep path is obviously more difficult and takes considerably longer (SILVER 1982). In the primarily closed oral mucosal wound, it is unlikely that surface dessication occurs.

Migration involves the "tumbling of (loosely attached) epidermal cells over one another" (FISCHER 1971) (see also WINTER 1964; JOLLY 1964). Once an advancing basal cell has formed an attachment to any residual basal lamina or to fibrin strands via hemidesmosomes, its trailing edge advances, carrying with it the overlying cells. Extensions of these cells then overtake the first and repeat the process (FISCHER 1971). It is suggested that the intracellular contractile filaments are significant in this movement, since they are only evident in migrating cells (POLLACK 1979a; SHOSHAN 1981).

A critical feature of epithelial migration is that although these cells are actively motile and the epithelial sheet often only one to two cells thick, the cells do not wander freely within the tissue; contact is always maintained with the last cell in line (ROSS & ODLAND 1969; SILVER 1982).

The factors directing epithelial movement remain unclear. GIACOMETTI and MONTAGNA (1969) suggest that guidance is provided by fibrin strands which are aligned across the wound, perhaps by the retraction of the margins. This concept - called **"contact guidance"** - is supported by other authors including HARRIS (1979a) and POLLACK (1979a), and is considered to provide partial explanation of the observed limitations under which epithelial migration occurs (FISCHER 1971).

Early reseach gave little consideration to the way in which epithelial cells achieved migration. More recent studies have demonstrated these cells to have various capabilities which facilitate their movement. This includes active phagocytosis of substances such as whole erythrocytes, fibrin, serum proteins and cytoplasmic debris (FISCHER 1971; ROSS 1971; ROSS & ODLAND 1969; ODLAND 1977). This

phagocytosis apparently operates in a similar manner to that of the macrophage, with lysosomal enzymes playing an important role (ROSS 1971). Collagenase and other proteolytic enzymes also appear to be produced and released by these cells, enabling cleavage of a path of migration within dermis (PEACOCK 1974; HARRIS 1979a). The production of collagenase may also be significant in the interactions seen between the epidermis and mesenchyme (see 1.5.2.3).

Mechanisms of epithelialization: migration vs mitosis.

The mechanism by which epithelium attempts to close wounds has been the subject of considerable speculation for many years. Some previous descriptions of the process, which assumed that mitosis was not prominent, have been shown to be incorrect: the studies of GILLMAN and PENN (1956) and by BULLOUGH (1965), for example, conclusively demonstrated significant mitosis occurring during epithelialization.

Certainly, mitotic figures are not common in the advancing edge of the epithelium. Migration is however followed by an increase in mitosis (up to 17 fold, PEACOCK 1974), in the cells just behind it. It is emphasised that cells undergoing mitosis do not also migrate; mitosis only occurs when the cells have completed their individual migration (ROSS & ODLAND 1969).

Cell migration however predominates over proliferation in epithelialization, as evidenced by the diminishing thickness of the cell margin to monolayer proportions. The significance of this feature of the normally healing wound becomes more evident when it is compared with the gross piling up of cells seen in epidermoid carcinoma (PEACOCK 1974). Thus, in normal epithelial regeneration, it would appear that, although mitosis is contributory, cell "dedifferentiation" and migration are more important (PEACOCK 1974).

Contact inhibition.

Epidermal migration is seen to continue until these cells come into contact with epidermal cells moving across the wound from other directions (FISCHER 1971; POLLACK 1979a). Like that described for connective tissue cells, this cessation of growth following contact between like cells is termed "contact inhibition" (HARRIS 1979a; POLLACK 1979a). (See also PEACOCK 1974).

The loss of "contact inhibition" is also cited as a possible stimulus for epithelial migration (POLLACK 1979a). This concept and various other factors concerning epithelialization - including wound hormones ("trephones") are the subject of continuing research which is beyond the scope of this thesis. Cursory discussion is given to "chalones" (which are said to inhibit mitosis) in Chapter 5.

Basal lamina (EM terminology)/**Basement membrane** (LM terminology).

As the wound progresses in age to between 3 and 5 days, the basal lamina is re-established (apparently secreted by basal epithelial cells - see FISCHER 1971), the regenerated epithelium becomes hyperplastic and intercellular junctional complexes are re-formed (FISCHER 1971; ODLAND 1977). The cells are later seen to gradually resume their previous level of differentiation and function so that the various layers of normal, resting dermis/mucosa are formed (FISCHER 1971; POLLACK 1979a). Concurrently with basement membrane formation, some stimulus appears to exist which orients fibroblasts parallel to this membrane, also facilitating functional collagen deposition (FISCHER 1971).

Complete epithelialization of the wound surface is greatly assisted, after 4 to 5 days, by the process of wound contraction (PEACOCK 1974). This contraction considerably reduces the wound surface area (particularly in open wounds). See 1.5.1.4 below.

1.5.1.3 Epithelio-mesenchymal interactions.

Interactions between epithelium and mesenchyme, as seen in normal embryological development, are also significant here. Repair and regeneration are, in fact, aspects of developmental processes combining cell proliferation, cell migration (individual or en masse) and cell differentiation particularly the interaction between various tissue components (GROBSTEIN 1969; FISCHER 1971; PEACOCK 1974).

Various interactions are described, including a stimulatory effect exerted on connective tissue regeneration by epithelial migration, division and subsequent re-differentiation (GILLMAN & PENN 1956). Some have also noted the close association between regenerating, migrating epithelium and macrophages. Collagen also appears to be significant (FISCHER 1971; SHOSHAN 1981) (see above).

1.5.1.4 Wound contraction.

Contraction is seen clinically in wounds healing by secondary intention through the movement of full-thickness skin towards the centre of the wound, thus decreasing the wound area. This much debated subject is critically significant in skin wounds since, in excess, contraction may lead to disfiguring and functionally incapacitating contractures (PEACOCK 1974).

The success of wound contraction is dependent upon the flexibility of the skin over the underlying tissues. If it is relatively closely bound, with multiple "fixed" structures, as for example, on the face, the defect will be less sucessfully closed (BERLINGER 1982). This is because the healing of wounds in which integumental tissue has been lost is the result of movement of adjacent tissues to close the space and not of new tissue formation (COHEN et al 1975). Wound contraction is of similar importance to oral mucosal wounds; wounds healing by secondary intention may be the site of severe contacture which may, for example, obliterate the buccal sulcus.

The significance of wound contraction is minimised in primarily closed wounds where minimal active granulation tissue is produced and little loss of integument has occurred. Some suggest that myofibroblasts (the cells presumed to be responsible for contraction) are not actually present in linearly incised and primarily closed wounds, perhaps because of differing tensile forces across the wound (COTTIER et al 1976).

1.5.2 The Fibrous Component of Granulation Tissue.

Following preparation of the wound environment by macrophages, the development of renewed vascular supply and the migration and proliferation of fibroblasts, the "fibroblast-capillary system" begins to synthesize the "scar tissue" which will ultimately re-establish the continuity of the integument, permitting the cell-to-cell interactions which are required for normal function.

Fibroblasts are responsible for synthesizing both fibres and ground substance. For some years, it was believed that the fibrous component of granulation tissue consisted entirely of collagen

(NEWCOMBE 1972). As will be seen, collagen is certainly the single, most important entity in ultimate repair, however, recent studies have also demonstrated elastin fibres to be present in wounds of 60 days or older (BHANGOO et al 1976). Its presence is also attributed to fibroblasts (POLLACK 1979a). Ground substance also plays a significant role in the repair of functional integrity in the injured tissue. However, in function, the completed scar does not permit the normal elasticity and resistance to abrasion seen in similar unwounded tissue (see 1.6).

As a result of fibroblast synthetic activity, there is a rapid increase in wound strength.

1.5.2.1 Collagen.

Collagen is the principal structural protein of the body, forming up to 30% of the total body protein mass (SCHILLING 1976). It is the principal component of mature scar tissue (BERLINGER 1982); without it there is no strength or permanence in the healing wound (SCHILLING 1976). As such, its synthesis and characteristics must be understood in order to appreciate the details of repair.

Various types of collagen are identified. Type I is the most common, being found in bone, fascia, tendon and adult skin. Type II is only found in cartilage. Type III is characteristic of embryonic connective tissue but is also found in small amounts in adult tissue, such as wounds, dermis and aorta. This type is laid down in fine fibres (also termed reticulin, REPESH et al 1982), which are mechanically inferior to the thicker fibres of Type I. Type IV is found in association with basement membranes and may be secreted by certain types of epithelial and endothelial cells (SILVER 1982). Some variation

is seen in the amino acid sequencing and chain arrangement between these types. (See also BAILEY 1978 and SHOSHAN 1981 re collagen types). When fibroblasts are active in wounds, it is Type III collagen which is produced. This is subsequently removed or modified and replaced by Type I over a period of weeks to months (SILVER 1982).

All types consist of three separate linear peptide chains twisted into left-handed helices to form the basic collagen molecule, "tropocollagen". These structures lie parallel to each other and are then twisted into a right-handed super-helix. This compound helical formation is unique to collagen; it accounts for its rigidity even along the considerable length of the molecule (SILVER 1982).

Most of the collagen extrusion from fibroblasts occurs during the proliferative phase (FRYKBERG & ERBEN 1977) following completion of the triple helix tropocollagen molecule. The tropocollagen then aggregates to form thin, randomly oriented collagen fibrils which have the consistency of a gel. At this stage, the fibrils are held together only by electrostatic forces (WALTER 1976; POLLACK 1979a).

As such they contribute little mechanical strength to the wound. The subsequent formation of multiple stable cross-linkages (WALTER 1976) and the "remodelling" of the collagen fibres (POLLACK 1979a) provides this strength, as discussed in 1.6.

1.5.2.2 Collagen synthesis.

Collagen is a unique protein both in structure and composition (FRYKBERG & ERBEN 1977). As mentioned, its basic molecular unit - tropocollagen - is a triple helical structure. The three chains are united primarily by intramolecular hydrogen bonds and covalent links (FRYKBERG & ERBEN 1977). This is facilitated by the very high content

of the amino acid, glycine, which is present, allowing close bonding of the polypeptide chains (NEWCOMBE 1972).

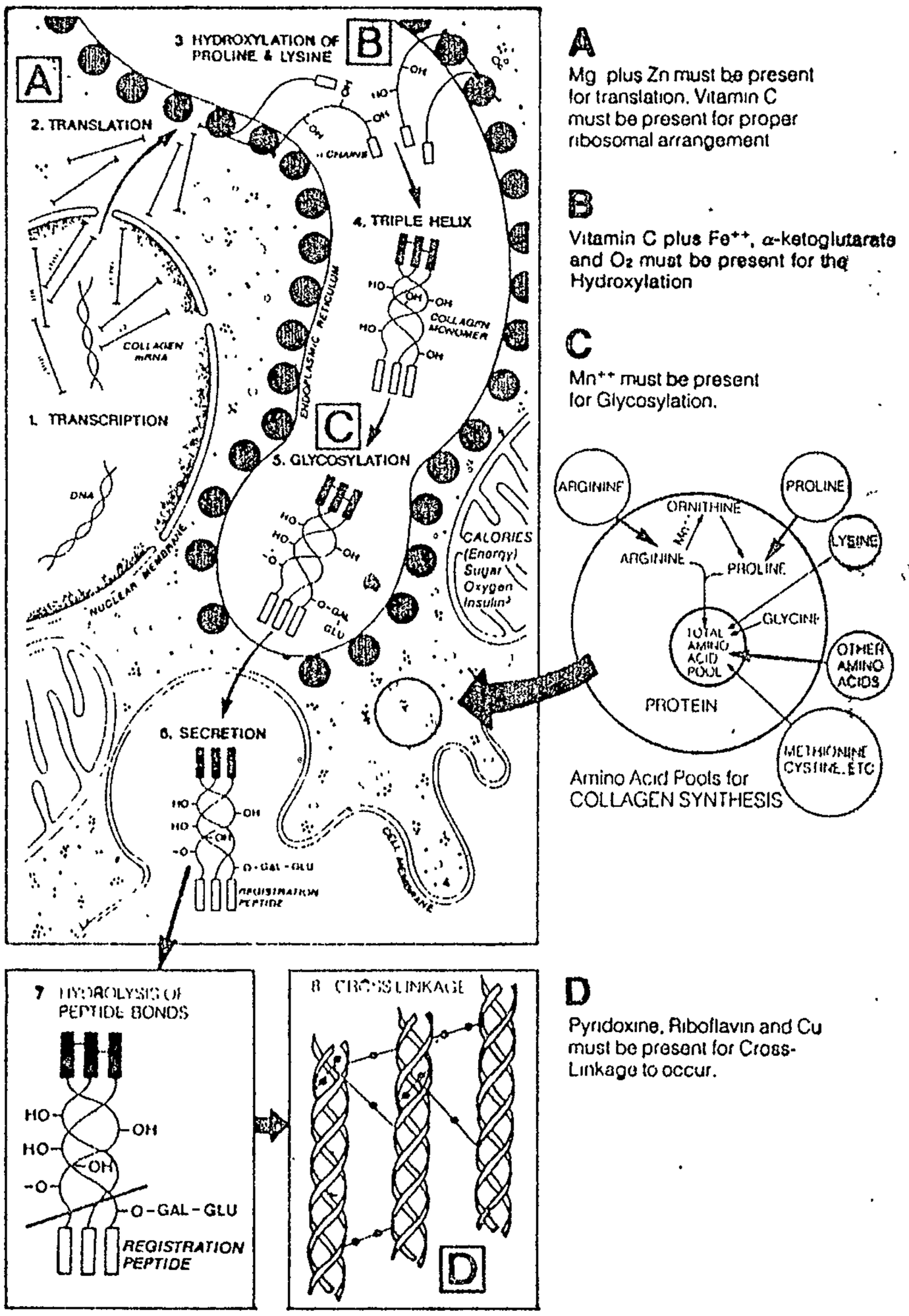
In addition to this high glycine content, collagen is unique in that it contains hydroxyproline and hydroxylysine, neither of which are found in appreciable amounts in any other animal tissue (NEWCOMBE 1972; FRYKBERG & ERBEN 1977). Research suggests that these amino acids are not incorporated into the peptide chain in the usual manner which utilises a specific transfer RNA for each amino acid. Rather, it is proline and lysine which are incorporated into the protocollagen peptide chain. The protocollagen molecule (probably while still attached to a ribosome) then undergoes hydroxylation via specific enzymes, thus hydroxylating many proline and lysine residues (FRYKBERG & ERBEN 1977; SILVER 1982).

This hydroxylation requires the presence of dissolved molecular oxygen with ascorbic acid (acting as a reducing agent), ferrous iron and alpha-keto-glutarate as co-factors (BERLINGER 1982; VAN DER MEULEN 1982). As the supply of these co-factors is tenuous in the wound (6.2), it is this hydroxylation reaction of proline molecules which is the rate-limiting step in collagen synthesis (NEWCOMBE 1972).

The hydroxylated forms of these amino acids are thought to have important roles in the functioning of collagen: hydroxyproline in stabilization of the triple helix and hydroxylysine in providing sites for attachment of variable amounts of carbohydrate in the form of glucose and galactose (ROSENBLOOM & PROCKOP 1969).

Once hydroxylation is completed, the procollagen chains are extruded (FRYKBERG & ERBEN 1977). Alignment of the fibres prior to polymerisation is achieved via the sulphur-containing amino acid

terminal end, which is then removed leaving the "tropocollagen" molecule (WALTER 1976; SILVER 1982). Tropocollagen then aggregates so that each molecule overlaps the next by one quarter of its length producing the characteristic banding pattern of collagen fibrils seen under electron microscopy - the "quarter stagger" (ROSS & BENDIT 1966; VAN DER MEULEN 1982; SILVER 1982). These fibrils become woven into fibres with increasing intra- and intermolecular bonds, thereby increasing fibre strength (FRYKBERG & ERBEN 1977; VAN DER MEULEN 1982). These steps are outlined in TEXT-FIGURE 1.4.



TEXT-FIGURE 1.4 Diagramatic representation of steps involved in collagen synthesis showing various nutritional requirements (from POLLACK 1979c, p29).

Collagen production lasts from around the third or fourth day (when it is first detectable in the wound), until wound healing is complete. Its deposition is reported to increase up to about the sixtieth day (HARRIS 1979a).

1.5.3 "Ground Substance".

Even into the 1950's, some treatises on wound healing made no mention of "ground substance". This material has long been a mystery; even modern definitions have done little to clarify the nature of this amorphous material which lies in and around the collagen fibres (PEACOCK 1974). In current usage, the term "ground substance" usually refers to a "continuous non-fibrillar matrix, including water and electrolytes through which metabolites diffuse between blood vessels and cells" (PEACOCK 1974). It is primarily, a complex of conjugated and unconjugated proteins - namely glycoproteins and mucoproteins - along with a variety of high molecular weight mucopolysaccharides. These are now given the collective name of "glycosaminoglycan" (HARRIS 1979a) or "proteoglycan" (BENTLEY 1980).

The major structural feature of the polysaccharide component is the repetition of the three disaccharide units of chondroitin sulphate and non-sulphated hyaluronic acid (NEWCOMBE 1972). The non-sulphated group appears to be the principal component of the amorphous gel fraction of ground substance with the sulphated group being most closely associated with the fibrillar elements.

In the phenomenon of healing, ground substance gains its significance from its association with collagen. Various chemical linkages have been postulated to occur between these two (PEACOCK 1974). Some suggest that the proportional concentration of the various

components in the surrounding tissue in fact determines the size and orientation of the collagen which is formed (COTTIER et al 1976; FRYKBERG & ERBEN 1977; SILVER 1982). It is certainly true that the assembly of the collagen subunits into fibrils, and thence fibres, is dependent upon many environmental conditions, and ground substance is likely to predominate in these (BENTLEY 1969; PEACOCK 1974).

It is significant that collagen does not occur "naked" in biological tissue. In cross section, each fibril is seen to be enclosed within a proteoglycan sheath which isolates it from its neighbours and environment (GIBSON & KENEDI 1969; COTTIER et al 1976). FORRESTER (1976) describes collagen fibres as interlacing "both physically and chemically" with the ground substance in which they are embedded. It would appear that this glycosaminoglycan sheath, in combination with the interstitial fluid, provides the skin with its general qualities of extensibility and mobility (GIBSON & KENEDI 1969).

Whatever the association, it is clear that as the quantity of collagen increases, so the relative volume of ground substance diminishes (NEWCOMBE 1972; CARPENTER et al 1977). The reason for this reciprocal relationship has not been elucidated.

1.6 CONSOLIDATION.

In the process of maturation the highly cellular nature of granulation tissue is gradually transformed into a fibrous, relatively acellular scar (CARPENTER et al 1977; ROSS 1980). After 5 or 6 days, wound strength is seen to increase - rapidly for the first two to three weeks and then more slowly (CARPENTER et al 1977). These changes are primarily related to maturation and remodelling of the collagen fibres. Maturation involves two processes: the transformation of the fragile,

soluble fibrils into strong, insoluble fibres, and their remodelling from a disorganised, random, inefficient arrangement to an oriented, organised structure which provides tissue with mechanical strength (PEACOCK 1974).

The direction of the stresses operating across the wound is thought to be chiefly responsible for determining which fibrils are lysed and how the newly deposited fibrils are orientated (POLLACK 1979a). Such stresses are suggested to produce electrical charges which aid in this orientation, perhaps via the strongly charged glycosaminoglycans (BERLINGER 1982). Generally speaking, it is the fibres which are orientated parallel to lines of tension in the vicinity which are retained (POLLACK 1979).

Collagenolysis of early fibres also permits their replacement with adult (Type I) collagen (SILVER 1982). It is also significant at the wound margins in the earlier stages of remodelling, perhaps allowing unification of the new and old collagen (GRILLS et al 1969). All these factors tend towards providing scar tissue which is at least partially able to restore function to the injured tissues. Although there may be no increase in the quantity of collagen present, remodelling leads to the continued increase in wound tensile strength (CARPENTER et al 1977). The remodelling phase is thus a dynamic equilibrium between collagen synthesis and collagen lysis (HUNT 1974; BERLINGER 1982).

As the wound matures, several other changes are seen to occur: the wound becomes less cellular, fibroblast synthesis diminishes and the wound's metabolic requirements decrease (WILKINS & KULWIN 1979). In response to these changes, much of the newly-formed prolific vasculature, begins to regress. The vessels which remain, mature to

produce the full range of vascular structure (POLLACK 1979a; SILVER 1982) (see FIGURE 1.2).

Normal healing is characterised by this regulation of the prolific reparative processes to body requirements. "Cell production is balanced by cell death; collagen production by hydrolysis, degradation and absorption; capillary formation by capillary obliteration" (SCHILLING 1976). Though the processes which lead to this "shut-down" are poorly understood, they are most important. Without them, wound repair would lead to uncontrolled, neoplastic-like overproduction of cells and cell products (SCHILLING 1976). Discussion of these concepts is beyond the scope of this thesis. They are, however, significant areas of current scientific investigation.

As a result of these cellular and vascular reductions, along with the gradual loss of glycosaminoglycans and water from the wound (the latter by wound contraction), collagen fibrils come into closer approximation. This increases the sites available for cross linkage, and promotes the formation of covalent bonds between lysine residues, significantly increasing both the tensile strength and insolubility of collagen (BORNSTEIN 1969; FRYKBERG & ERBEN 1977; POLLACK 1979a).

The mature scar is composed of dense mats of collagen fibres and is relatively avascular when compared with unwounded tissue. Normal microscopic patterns are not restored and the "final" scar is often more densely packed and containing more collagen than comparative unwounded connective tissue (FORRESTER 1976; POLLACK 1979a).

From a functional viewpoint, scar tissue is inert except for providing mechanical continuity and strength (SILVER 1979). However, because of the differences in chemical composition (SILVER 1979), and

in the arrangement of the fibres (FORRESTER 1973), even the strength of the scar is less than that of unwounded tissue (FORRESTER 1976; 1980b; CARPENTER et al 1977; SILVER 1979; SILVER 1982). Most authors report, in fact, that wounded tissue never regains more than 70 - 80% of its original strength (DUNPHY & JACKSON 1962; FORRESTER et al 1969; FORRESTER 1976; WALTER 1976; HARRIS 1979a).

That the mature scar which forms following healing can in no way be considered to restore full normal function is exemplified in the following. Epithelium over scars is considerably less thick when compared with unwounded epithelium. Its attachment to the underlying dermis is also less effective since the rete pegs are short and sparse compared with normal. These features imply that the epithelium over the scar remains more easily eroded by friction. Also, as its blood supply via the more densely fibrous scar tissue is poor, it will easily sustain pressure necrosis (CARPENTER et al 1977; SILVER 1979).

1.7 CONCLUSION.

A brief overview of the processes involved in the healing of wounds (particularly surgical) has been presented. It is stressed that though some divisions have been made, these are relatively arbitrary for ease of discussion and that, in the living organism, the processes of haemostasis, acute inflammation, proliferative repair and remodelling form a continuum in the normal healing situation.

Also, the functioning of these processes as the "fibroblast-capillary" system of FORRESTER 1976 or the "wound module" of HUNT (1980) is introduced.

This chapter thus sets the context within healing in which oedema develops. It also introduces the physiological framework and basis for this oedema. In following chapters, particular attention is given to the acute inflammatory process and its relation to the formation of clinical oedema.

Preparatory to these subjects, Chapter 2 considers the microcirculation - its normal structure and function.

CHAPTER 2 MICROCIRCULATION IN NORMAL FUNCTION.

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2.1 INTRODUCTION.

It is said that anatomy is to physiology as geography is to history: it describes the theatre of events. This is particularly so in the study of inflammation, as has been revealed by the advances made through the examination of normal and "inflamed" tissue under the electron microscope and with vital microscopy.

Since acute inflammation in response to surgical (and other) injury is primarily characterised by a vascular response (SILBERBERG 1979), appreciation of the changes seen in this response, and of the physiological and clinical effects which follow, thus requires clear understanding of the normal structure and function of the microvasculature.

This was pointed out by some of the earliest modern investigators of inflammation. COHNHEIM (1882), for example, gave vivid descriptions of the events occurring in the microcirculation of frog tongue and mesentery during inflammation, and on the basis of these observations, he published several major papers on inflammation. The work of EBBECKE (1917), KROGH (1924) and LEWIS (1927) later revived interest in COHNHEIM's attention to these vascular changes (cited by EBERT & GRANT 1973). These authors (but Krogh and Lewis in particular) went on to publish works which have dominated the study of inflammation since.

The literature written on the subject of normal microcirculatory structure and function is vast and an exhaustive review is not possible here. This chapter provides a general review of this literature, with specialised consideration of the microvasculature of the oral cavity. Discussion of the specialised variations seen in lung, kidney, liver,

for example) are outside the field of this thesis. Many areas discussed remain the subject of intense controversy and debate; some distillation of these ideas has been attempted.

2.2 THE MICROVASCULATURE.

The term "microvasculature" applies to three systems, all of which are concerned with the transport of fluids, nutrients and metabolic products to and from tissue cells. The microcirculation is identified separately, not because of the extent of the network of microscopically small vessels, but because the behaviour of these vessels is significantly different from that of large vessels (ZWEIFACH 1977b).

The first of these systems is the blood vascular system consisting of arterioles, capillaries, and post-capillary venules. The second system is the lymphatic (here including the collecting lymphatics and the initial lymphatics). Between the two is the system of tissue channels (the "ultracirculatory system", CASLEY-SMITH 1979a) and though often omitted from discussions on the microvasculature, it is, in fact, the ultimate path of communication between the blood, the cells and the lymphatics. Discussion of the microvasculature will here consider each system separately with later reference to their functional interrelationships.

2.3 THE BLOOD MICROVASCULATURE: MORPHOLOGY AND FUNCTION.

"Over three centuries have passed since Malphigi (1661) demonstrated that the 'porosities in the flesh', whereby blood flows from artery to vein, were rather long, thin-walled tubes, which he termed capillaries" (BAEZ 1977b). Despite intensive study since, the minute size of the microcirculation, and its complexity in both structure and function, have made advances in this field painstakingly slow.

Some progress has however been made in recent years, primarily a result of the development of two forms of study. One of these uses in vivo light microscopy, where thin, transparent, living tissue in accessible locations is examined under light microscope - termed "vital microscopy". The other involves intensive direct comparisons between light and electron micrographs (LUDBROOK 1981). These observations have then been correlated with studies of microvascular function in areas such as dynamic pressure and pressure gradients, flow rates, and exchange functions within the various components of the microvasculature (LUDBROOK 1981).

Significant discrepancies are present in the literature with respect to microvascular terminology. Definition of some of this terminology is hence presented where appropriate, based on a consensus of the literature. The reader is referred to the review article by BAEZ (1977a) for further discussion of terminology.

Discussion here is confined to the microcirculation, consisting of terminal arterioles, capillaries and venules. The term "terminal arteriole" is the least well defined of these. Here it is used to designate the final arterial ramifications (from 50 to 7 microns

diameter) endowed with a continuous single layer of smooth muscle cells (BAEZ 1977b). This is based on the widely reported proposal of RHODIN (1967, 1968). (See also YEN & BRAVERMAN 1976). FIGURES 2.1 and 2.2 outline the microstructure, arrangement and histological features of arterioles, capillaries, venous capillaries and venules.

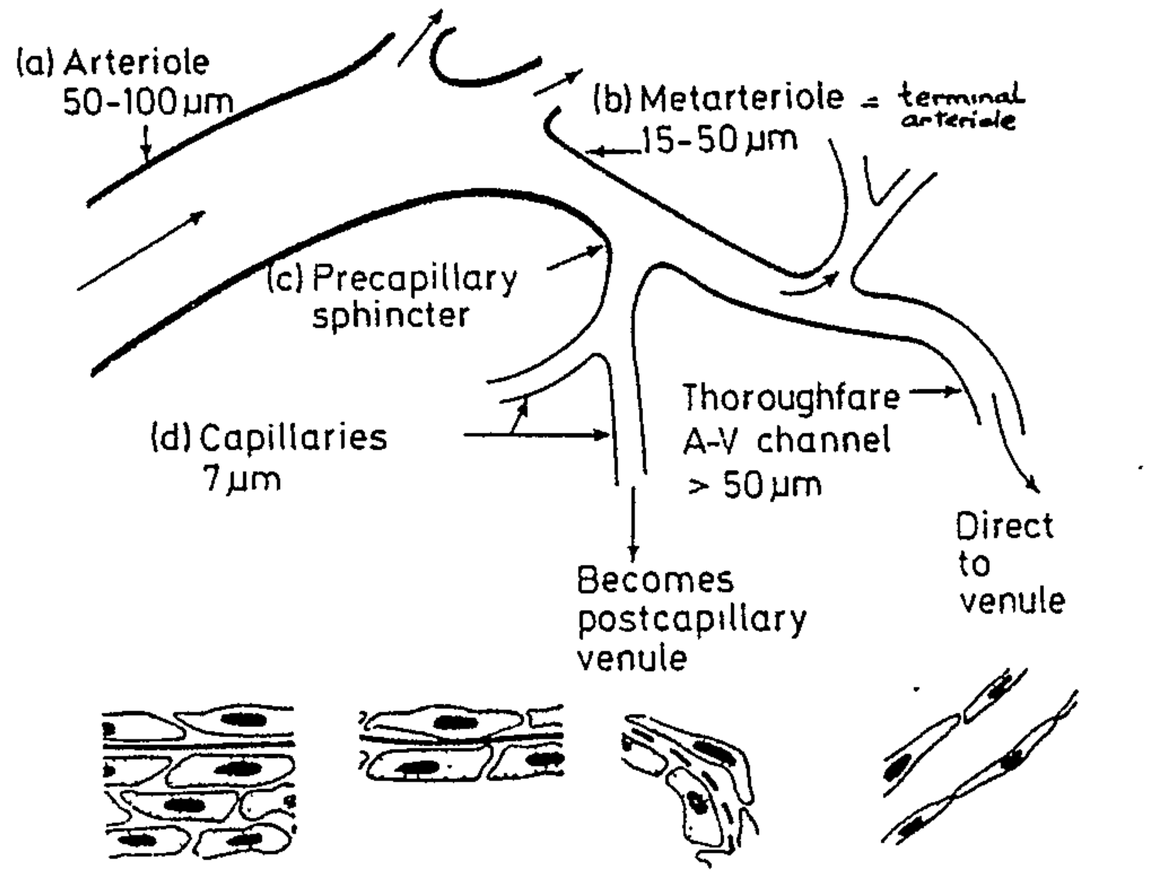

FIGURE 2.1 The microstructure and arrangement of arterioles and capillaries (from LUDBROOK 1981, p261).

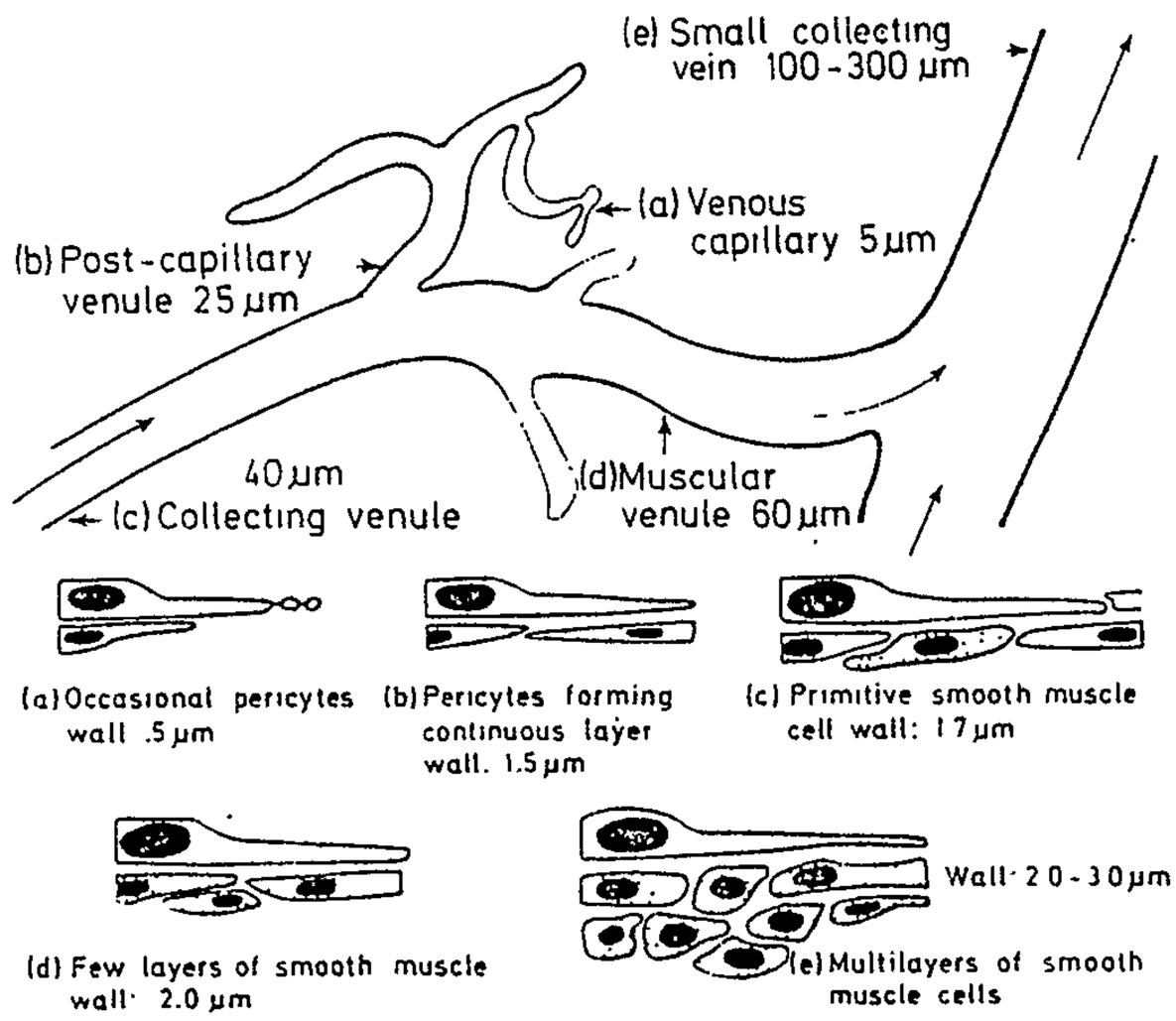

FIGURE 2.2 The microstructure and arrangement of venous capillaries and venules (from LUDBROOK 1981, p261).

For the purposes of this thesis, several structural features of microvessels are of particular importance, namely the intracytoplasmic vesicular system, the intercellular junction, the endothelial basement

membrane and the endothelial cells themselves. These are now reviewed. (See also CASLEY-SMITH 1983a).

2.3.1 The Endothelium.

The endothelium is an extensive, thin cellular layer which forms the ultimate barrier between the blood and the tissues. Microcirculatory endothelium forms the largest portion of this surface (GABBIANI & MAJNO 1977).

The classical view that endothelial cells merely formed a semipermeable monolayer which pertained to the maintenance of haemostasis, has now considerably evolved. It is becoming increasingly apparent that these cells are capable of many related, and apparently unrelated, functions including complex interactions with platelets and leucocytes (both stimulation and inhibition) (BARNHART & BAECHLER 1978), synthesis and secretion of endothelial basement membrane and mucinous surface coating (GABBIANI & MAJNO 1977; TRIPATHI & TRIPATHI 1977), the transport of macromolecules across the endothelium (TRIPATHI & TRIPATHI 1977; BARNHART & BAECHLER 1978), and the synthesis of Factor VIII moities (BARNHART & BAECHLER 1978) and of other substances which modify haemostasis and thrombosis (PEARSON 1983).

In addition, endothelial cells are responsive to the influence of local haemodynamic events, aligning themselves principally along the axis of blood flow "except at bifurcation orifices and perturbed areas" (BARNHART & BAECHLER 1978). Furthermore, endothelial cells are pharmacologically active, demonstrating marked morphological and biochemical changes in response to chemical and physical stimuli (NORTHOVER et al 1981) (see Chapter 3). This is because of the large number of receptor sites present on the endothelial surface.

Knowledge of endothelial metabolism has rapidly increased in the last decade. It is now realised that endothelial cell functions are essential in at least three aspects of vascular homeostasis: the regulation of leucocyte traffic (through alterations in white cell adhesion to the endothelium), the modulation of haemostasis and thrombosis (by regulation of thromboresistance and inactivation of thrombin), and the control of vascular tone and permeability (PEARSON 1983).

Nonetheless the main purpose of the endothelial lining probably may still be said to lie in "its control of the passage of substances into and out of the channels which it lines". The various morphological organisations of the endothelium determine whether selective, or relatively unselective transport occurs across it (TRIPATHI & TRIPATHI 1977). Endothelium is classified on this basis as continuous, fenestrated or discontinuous (WOLFF 1977). (FIGURE 2.3 illustrates the differences between these endothelia). Discussion here does not include high or discontinuous endothelium since this has not been described in oral tissues. PLATE 2.1 (a & b) illustrates some aspects of endothelial ultrastructure (see 2.7.3).

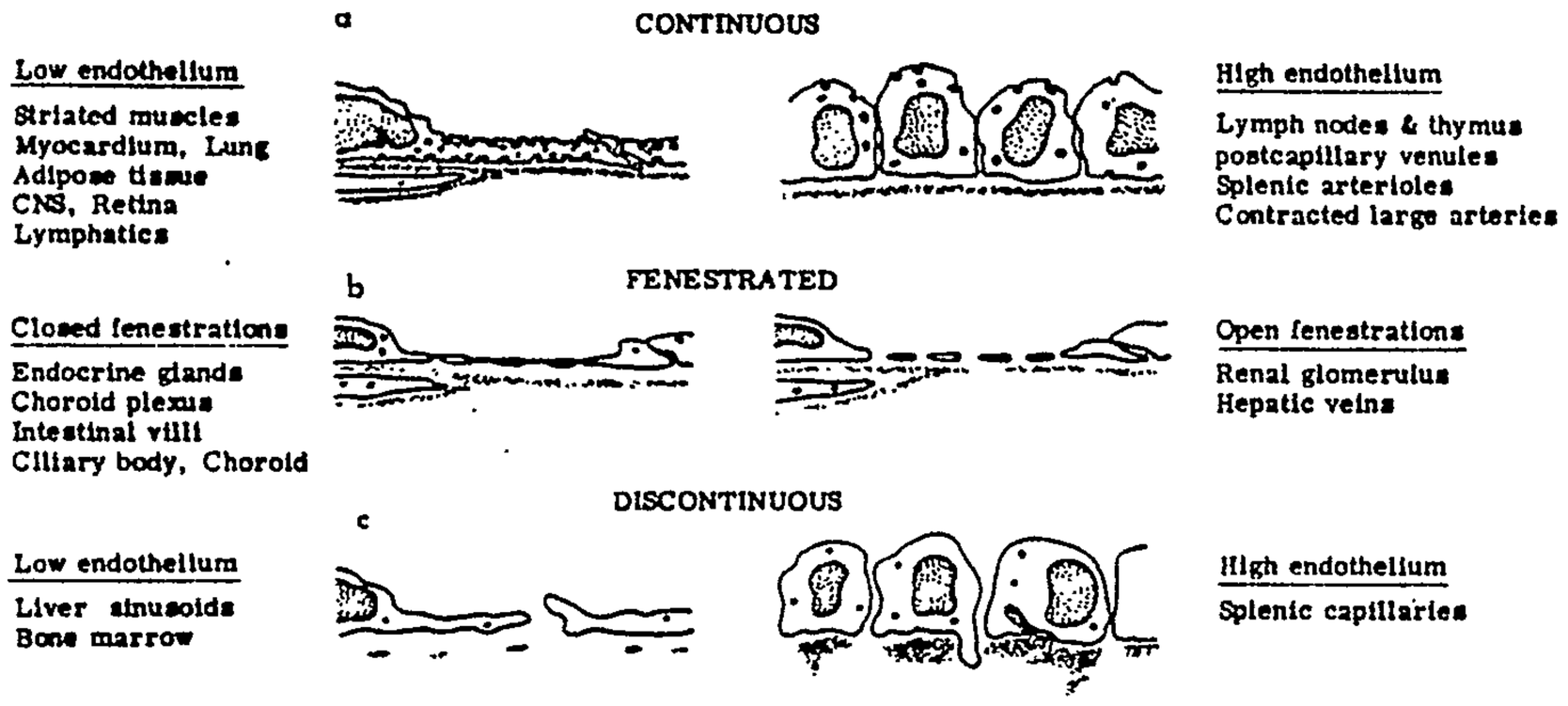

FIGURE 2.3 Diagrammatic illustrations of various types of vascular endothelium and a representative list of their locations (from TRIPATHI & TRIPATHI 1977, p309).

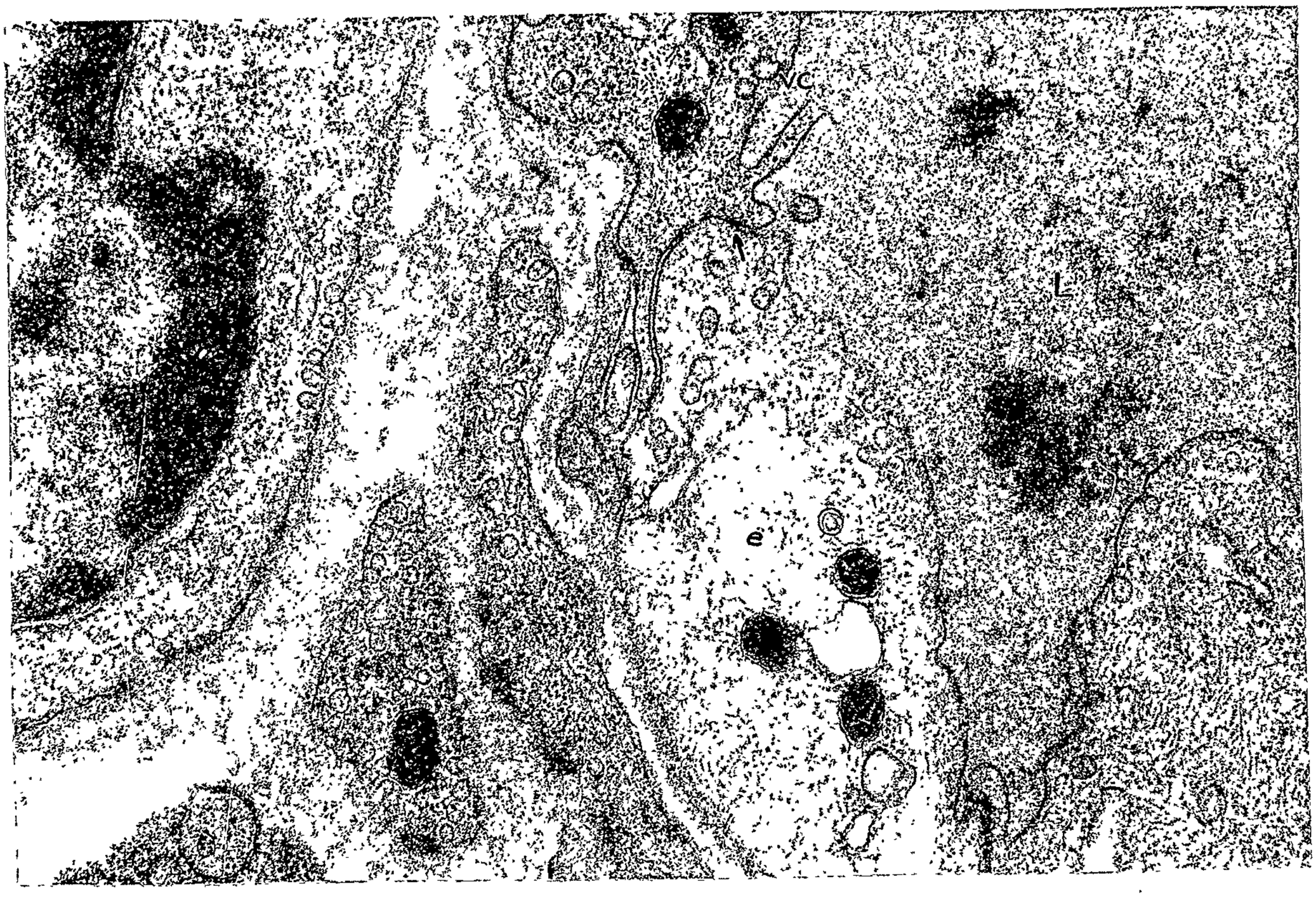

PLATE 2.1A & B Electronmicrographs from human attached gingiva (site as per PLATE 1.1) demonstrating aspects of endothelial ultrastructure (see 2.3.1). These sections would appear to include segments of relatively un-inflamed vessels lined by continuous, low endothelium. L = lumen; n = nucleus; v = endothelial vesicles; vc = vesicle cluster; mt = microtubules (both longitudinal jc = junctional complex; .... = basal lamina (demonstrating lamina rara (R) and lamina densa (D)); and transverse section). (Magnification in both plates x39,245). (Other symbols as with PLATE 1.1).

2.3.1.1 Endothelial vesicles.

Perhaps the most characteristic microscopic feature of continuous endothelia of microvessels is the presence of intracellular vesicles (WOLFF 1977). These vesicles were discovered by the work of PALADE (1953), who suggested that they were probably significant in transporting fluid and dissolved substances across the endothelial cells.

Such vesicles are very numerous, of uniform diameter (60-70nm) (GABBIANI & MAJNO 1977) and are surrounded by a membrane which is identical in appearence to the endothelial plasma membrane (FLOREY 1970b; CRONE 1973). Early studies reported that a high percentage of them (approximately 30%, BRUNS & PALADE 1968a) were fused to the luminal surface of the endothelium with about 40% fused to the basal surface; the remainder being suspended within the cytoplasm (WOLFF 1977).

While this concept of vesicle distribution is supported by other authors (CLOUGH & MICHEL 1981; CLOUGH 1983), investigations by Bundgaard and Frokjaer-Jensen in Copenhagen using ultrathin serial EM sections suggest that the vesicles are permanent structures which form a system of invaginations of the endothelial surface. In these observations, apparently "free" vesicles are seen to connect with other vesicles and/or the cell surface membrane in adjacent sections. The number of vesicles in these clusters varied but less than 1% appeared free within the cytoplasm (BUNDGAARD, FROKJAER-JENSEN & CRONE 1979; BUNDGAARD 1980; FROKJAER-JENSEN 1981; 1983). (See FIGURE 2.4).

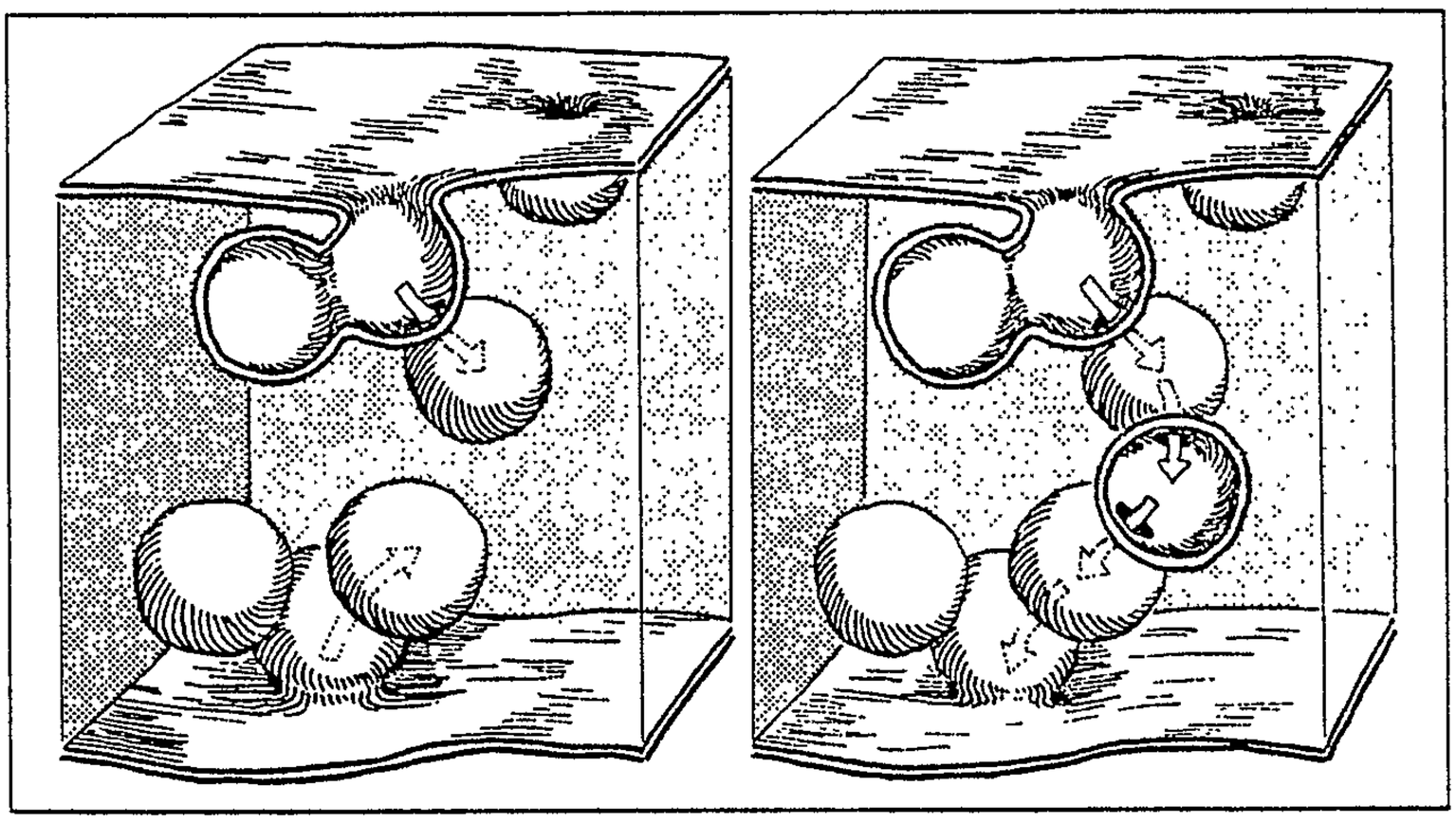

FIGURE 2.4 Schematic tridimensional representation of clusters of sessile vesicles communicating with each other (arrows), and with the blood or tissue front of the endothelial cell (left). Occasionally, the vesicular clusters communicate with each other (right), thus forming a transcellular pathway, possibly representing a "large pore" (from HAMMERSEN et al 1983, p5).

These two morphologic descriptions form the basis of current scientific thought. As will be seen these descriptions lead to quite different theories of vesicle function.

It is significant to note that various physiological and pathological factors may influence the numbers of these vesicles and the level of their fusions. (See WOLFF 1977). This is particularly relevant in relation to inflammatory changes in the microcirculation (3.2.1).

The functional significance of the vesicles has been further confused by the description of transendothelial channels as a regular feature of continuous capillary endothelium. Such channels, predominantly described by SIMIONESCU et al (1975b), are supposed to form when neighbouring, but opposing vesicle clusters contact and fuse.

This description and the functions which have been proposed to accompany it have recently received criticism, chiefly because the observation of channels has been extremely rare in the work of other

investigators (MICHEL 1979; HAMMERSEN et al 1983). It is suggested that they are likely to be artefactual as a result of chemical fixation procedures (CASLEY-SMITH 1979a;b) and possibly stimulated by the hemipeptide tracer molecules used. Such channels are more commonly observed in some pathological conditions, such as experimental oedema (HAMMERSEN et al 1983).

2.3.1.2 Endothelial Cell Junctions.

Much attention has also been given to the endothelial cell junctions. Postulations concerning the nature of these junctions have shown considerable evolution and the definitive understanding of their nature has still to be discovered.

Silver nitrate staining under light microscope suggested that cells were held together by cement in a similar way to bricks in a wall (CHAMBERS & ZWEIFACH 1940, cited by FLOREY 1970b). The advent of the electron microscope however, demonstrated that in fact the intercellular space was very narrow and therefore appeared not to provide sufficient room for the cement proposed by LM observations (FLOREY 1970b).

Subsequent investigations, and particularly the development of the "freeze-fracture" technique, have enabled increased understanding of the ultrastructure of intercellular junctions. In comparision with other monolayer epithelia, the junctional system of the vascular endothelium appears to be considerably simplified, generally consisting only of regionally modified zonulae and maculae occludentes (synonym: "tight junctions") and maculae communicantes (synonyms: communicating or gap junctions) (BARNHART & BAECHLER 1978; PALADE et al 1979).

The positioning and frequency of occurrence of these junctional complexes varies along the length of the microvasculature and between

different organs. For example, in vessels in the brain and retina, 'tight junctions' form an uninterrupted seal which is virtually impermeable to molecules (GABBIANI & MAJNO 1977) and probably constitues the 'blood-brain' and 'blood-retina' barriers (TRIPATHI & TRIPATHI 1977).

The segmental differences in numbers and differentiation of junctional devices have been most clearly delineated by SIMIONESCU et al (1975a). Arterioles (30-100µm diameter) were found to have the most elaborate system with ridges of z. occludens and intercalated maculae communicans. Capillaries however show only tight junctions. The ridges formed in capillaries are lower, and may end abruptly, allowing lanes of communication from one side of the cell to the other. Post-capillary venules demonstrate the simplest endothelial junctions with shallow, discontinuous, linear creases of the membrane. Adjacent cells interdigitate but frequent transverse free lanes are seen. Larger venous vessels show tight junctions and a system of ridges which is similar to but less elaborate than that in arterioles. Such vessels are therefore more permeable than arterioles (SIMIONESCU et al 1975a). See also BOHM 1977; PALADE et al 1979.

In addition to these junctional complexes and ridges, the cells frequently overlap and/or deeply interdigitate (FLOREY 1970b; TRIPATHI & TRIPATHI 1977). Apart from providing physical stability, such interdigitations may allow a certain degree of "sliding" between the cells (yet maintaining cell contact) in the maximally stretched state of the cell (vessel vasodilatation) (see FIGURE 2.5). Thus, while having high structural stability, the endothelium behaves as an elastic sheet, becoming thinner as the vessel dilates (WOLFF 1977).

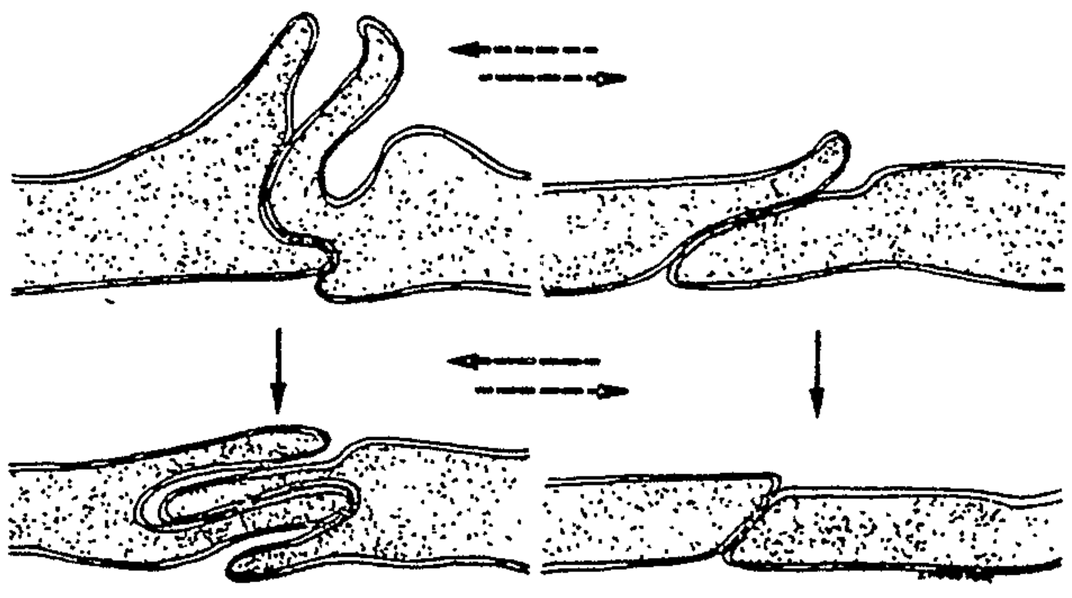

FIGURE 2.5 Diagrammatic representation of vascular endothelium and junctional modifications in dilated (lower) and undilated (upper) states of the vessel. Note the interchangeable configuration of cell contacts and increase in the surface area on vascular dilatation (from TRIPATHI & TRIPATHI 1977, p308).

2.3.1.3. Basal lamina

Surrounding the small vessels, and closely conforming to them, is a fine connective tissue sheath, the basal lamina (BL). It is probable that the basal lamina is elaborated by the endothelial cells themselves (as mentioned above) or that it results from stimulation of extracellular materials by substances released from these cells (FLOREY 1970b; GABBIANI & MAJNO 1977). Considerable variations of opinion regarding the precise nature of its composition are recorded.

Initial studies suggested that the vascular basal lamina was composed chiefly of polysaccharides (GERSCH & CATCHPOLE 1949). However, the finding that collagenase almost completely hydrolysed this structure (SPEIDEL & LAZAROW 1963) indicated that collagen was a major component) (both cited by GABBIANI & MAJNO 1977).

Recent opinion is that the basal lamina is a "condensation of the ground substance containing collagen and reticular fibrils" (ZWEIFACH 1973b). CASLEY-SMITH identifies this structure as a "fibrillar matrix with pores of approximately 5nm". Two zones are

identified: the outer of which is electron dense and apparently fibrillar in nature (the lamina densa), and the inner which is electron transparent (the lamina rara).

This is surrounded by collagen fibrils embedded in glycosaminoglycans. BOHM (1977) describes the ultrastructure of the lamina densa as a thick, fibrillar sheet of 50nm polygonal mesh which overlaps to result in a tight felt with 5-10nm pores. This 'felt' is anchored to the plasmalemma by perpendicular fibrils which form the lamina rara (see FIGURE 2.6). Considerable variation of this schema is identified in specialised organ systems for example, lung and kidney (BOHM 1977).

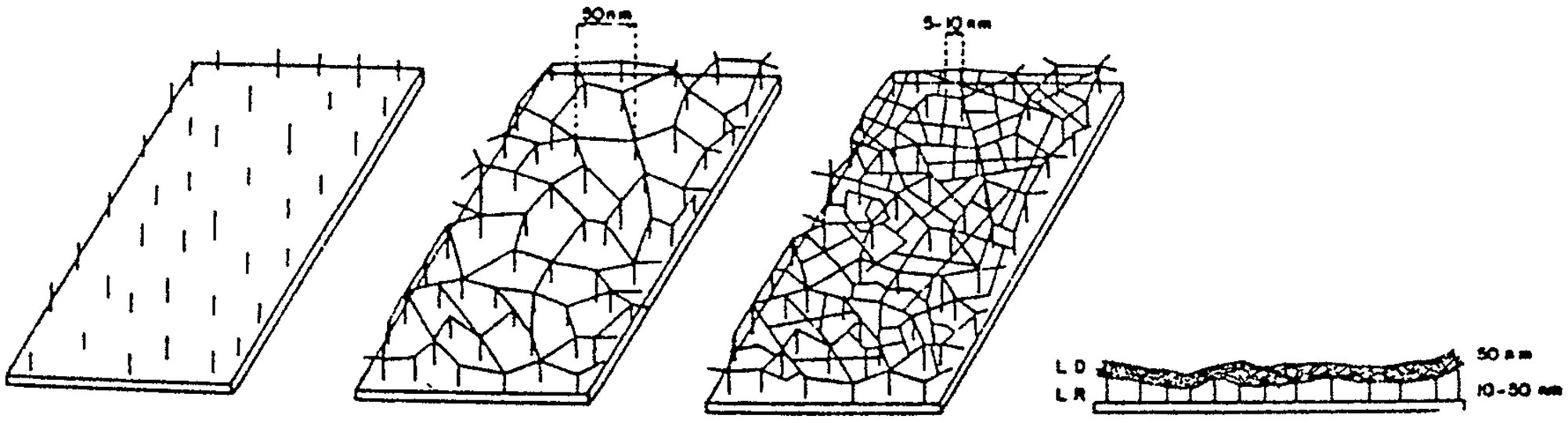

FIGURE 2.6 Schematic representation of the basal lamina. The lamina rara (LR) consists of vertical fibrils anchored to the plasmalemma and supports the lamina densa (LD), which is constituted of fibrillar nets with 50nm polygonal meshes. These overlap and result in a thick felt with 5-10nm pores. The height of both sections varies between different species and tissues (from BOHM 1977, p45).

In normal function it is suggested that the BL acts as a physical barrier for exchange and also as a supporting structure affording rigidity to the vessel (BAEZ 1977a), probably by virtue of its intimate association with collagen fibrils.

2.3.2 Barrier Function.

Despite the many postulated expansions of endothelial function, recent observations confirm the importance of the first conceived role of endothelium as a barrier controlling exchange across the vessel wall. A large portion of this transcapillary exchange is passive in nature (GABBIANI & MAJNO 1977) and as such, exchange is dependent upon diffusion, filtration, hydrostatic and osmotic pressures (PAPPENHEIMER et al 1951; LANDIS & PAPPENHEIMER 1965).

These exchange pathways chiefly relate to lipid-insoluble material since lipid-soluble molecules and respiratory gases are presumed to diffuse through the endothelial cytoplasm (LANDIS & PAPPENHEIMER 1965; HAUCK 1971; BOHM 1977). Such lipid-soluble diffusion is beyond the scope of the present review, however, the specialised routes of passage of lipid-insoluble molecules are highly significant in the later discussion of vascular permeability, both in normal function (2.3.3) and in inflammation (3.2.1.4).

It is now over thirty years since PAPPENHEIMER et al (1951) proposed the "pore" theory of transendothelial exchange, based on theoretical considerations and physiological data. This theory suggested that the capillary wall was perforated by water-filled channels or "pores", which small molecules traversed more easily than large (WAGNER & CASLEY-SMITH 1981).

GROTTE (1956) modified this theory, postulating a double mode of such molecular transport: a small-pore system permeable to water and small molecules (radius 35-45 Angstroms), and large pores which were also permeable to large molecules including proteins (radius 12-35nm) (BOHM 1977; GABBIANI & MAJNO 1977; ARFORS et al 1979; WAGNER & CASLEY-SMITH 1981).

From a morphological view, four endothelial constituents have been advanced as counterparts of these "pores" determining the particular permeability characteristics of a given capillary, namely: cytoplasmic vesicles, transendothelial channels, endothelial junctions, and the endothelial glycocalyx (HAMMERSEN et al 1983). As will be seen, significant debate continues on this subject.

On discovery of the intracytoplasmic vesicles, PALADE (1953) envisaged these vesicles to be in a constant state of motion "sequestering fluid and solutes from the extracellular medium and shuttling them across the endothelium" (WAGNER & CASLEY-SMITH 1981). This discontinuous mode of transport was not the continuous pore which Pappenheimer had postulated, but it did provide a conceptual means whereby large molecules could penetrate the wall (WAGNER & CASLEY-SMITH 1981).

However, no satisfactory data has yet been presented concerning the mechanisms whereby vesicle transport occurs (INTAGLIETTA 1977; ARFORS et al 1979) and in the light of other experimental evidence, it would seem that this original concept of freely moving vesicles is now at least partially obsolete (HAMMERSEN et al 1983). Based upon the two descriptions of the pattern of vesicular arrangement (see 2.3.2), two alternative conclusions have been proposed.

The first is based on the presence of a small but significant percentage of "free" intracytoplasmic vesicles; molecules are transferred from one vesicle to another by a continuing process of vesicle fusion and fission. The concentration of the transported material is gradually "equalised", so transporting material according to concentration gradient across the endothelium (MICHEL 1979; CLOUGH & MICHEL 1981; CLOUGH 1983; HAMMERSEN et al 1983). (See FIGURE 2.7).

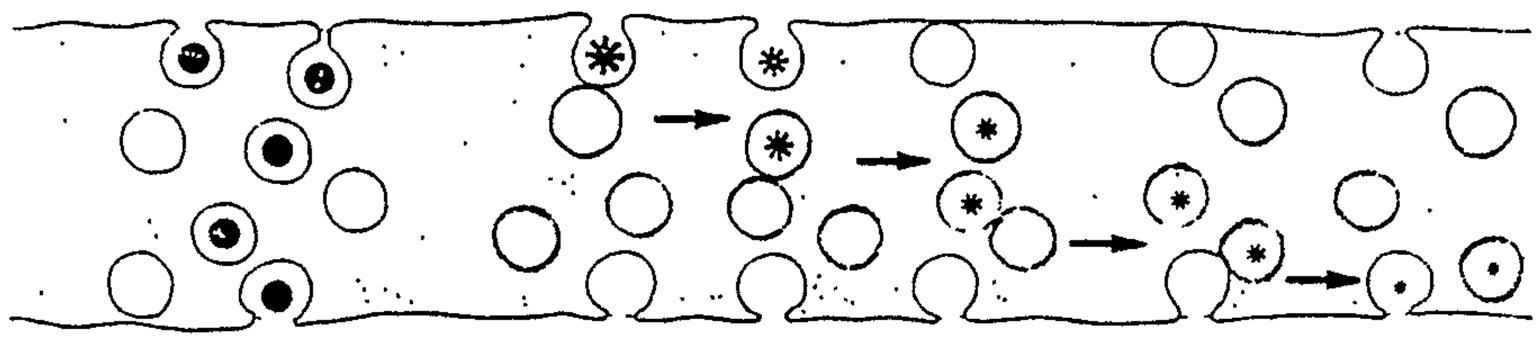

FIGURE 2.7 Schematic representation of two different modes of macromolecular transport via endothelial vesicles. On the left side 'vesicular transport' in a true sense is depicted, whereas the rest of the diagram illustrates the consecutive steps of the 'fusion-fission' concept. Vesicular transport implies that the vesicles are filled on either side of the endothelium and are then pinched off. They move freely through the cytoplasm, thereby ferrying their contents to opposite endothelial surfaces. The content of the vesicles (symbolised by the dots) did not show any concentration gradient during endothelial passage (indicated by the identical sizes of the dots). The 'fission-fusion' concept assumes that the material is transferred from one vesicle to another by a continuous processes of vesiclar fusion and fission. This results in a steady decrease of the transported material (symbolised by the decreasing sizes of the stars) (from HAMMERSEN et al 1983, p4).

The second concept implies that vesicles are permanent, sessile structures residing at the blood and tissue fronts of the endothelium where they commonly form inter-communicating racemose clusters. Rarely, transient cross-endothelial channels form by the fusion of opposing clusters. Such channels allow macromolecules to traverse the endothelial barrier (BUNDGAARD et al 1979; BUNDGAARD 1980; FROKJAER-JENSEN 1981; 1983; HAMMERSEN et al 1983).

Other proposals have also been made and it can only be said that there are several, markedly varied interpretations of electron microscope data which have led to profound differences of opinion with respect to the postulated functioning of the vesicular system in transendothelial transport (WAGNER & CASLEY-SMITH 1981). As stated by ZWEIFACH (1973b), the broad functioning of this system "as the mechanism for macromolecular transport between blood and tissue compartments remains largely unproven". It may be (as suggested by CLOUGH (1983), FROKJAER-JENSEN (1983) and CRONE (1983)) that the main physiological role of the vesicular system may relate to functions other than transport (for example, in endothelial secretion, or as a membrane reservoir) (see WOLFF 1977; PALADE et al 1979). WAGNER and CASLEY-SMITH (1981) and HAMMERSEN et al (1983) provide excellent review articles on the question of vesicular function.

The relatively rare occurrence of transendothelial channels appears now to offer little aid in clarifying the morphological structure contributing to transendothelial transport. Calculations relating to the most common form of channel (those with thin diaphragms across their opening) suggest that such channels could conceivably represent Pappenheimer's "small pores" with the even fewer truly open channels (without diaphragms) may similarly function as "large pores" (PALADE et al 1979). However, that few channels are found by most investigators under normal physiological conditions suggests that they are not of great physiological importance (HAMMERSEN et al 1983).

The endothelial junctions represent another structural component of the endothelial wall which has been closely linked to its permeability. This was postulated even when the endothelial cell borders could only be seen as a polygonal pattern of silver lines. Since this time, several investigators (KARNOVSKY 1967 and WISSIG 1979) have demonstrated these junctions to be permeable to horseradish peroxidase (low molecular weight tracer) thus implying that, in many endothelia, the occluding zonules do not completely girdle the cells. Rather they represent either spot-like or ridge-like sites of membrane fusion with interspersed narrow openings 4-6nm wide. Most authors agree that the junctional lengths where this distinct gap occurs correspond to the small pores of the Pappenheimer model (CASLEY-SMITH 1976; BOHM 1977; HAMMERSEN et al 1983).

The variations between different segments of the microvasculature form the basis of the functional integrity of the endothelial cells and the degree of intercellular seal (TRIPATHI & TRIPATHI 1977). Such variations become particularly significant in pathological states such as inflammation (3.2.1.4).

The function of endothelial cell junctions as small pores may relate to the proposed "intercellular cement" of CHAMBERS and ZWEIFACH (1940, cited by FLOREY 1970b) and LUFT (1965), interest in which has been revived by some more recent studies. BARNHART and BAECHLER (1978) describe an "extracellular glycocalyx" lining the luminal surface. This material is believed to consist of acid glycoproteins which form a network upon which serum proteins are adsorbed. It is now held that this glycocalyx represents the "intercellular cement" of light microscopists (BAEZ 1977a; BUNDGAARD 1980).

Recent studies suggested that the molecules in this glycocalyx form a fibrous network within the endothelial ultrastructure (including the vesicles and cell junctions) (CURRY 1980; U. RYAN 1983). These molecular networks are suggested to be the size-limiting structures which produce the observed sieving of solutes according to size (CURRY 1980).

Calculations concerning transendothelial diffusion show that the basal lamina has negligible effect on the movement of small molecules. It does however, exert an increasingly important effect as molecular size increases, particularly in fenestrated capillaries. It is thus extremely important in the regulation of protein passage into the interstitium (CASLEY-SMITH 1976b). Significant alterations of these functional properties occur in situations of altered permeability, where it demonstrates considerable flexibility and resilience.

A diagrammatic illustration of the various proposed transport pathways is given in FIGURE 2.8.

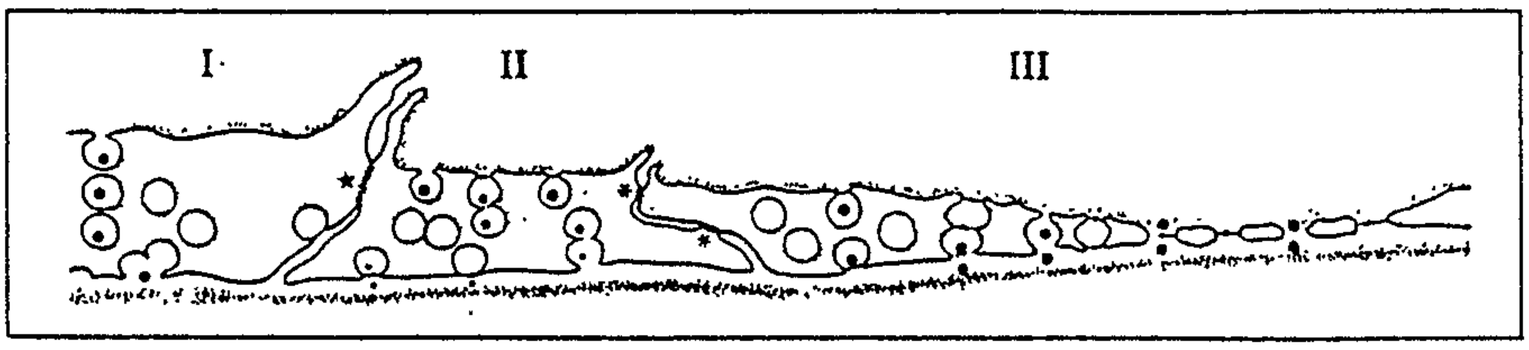

FIGURE 2.8 Schematic representation illustrating the various concepts regarding the functional significance of endothelial vesicles as vehicles for macromolecular transport (see also FIGURE 2.7). I Vesicular Transport Concept (G.E. Palade): the material is transferred by freely moving vesicles. II Fusion-Fission Concept (G. Clough & C.C. Michel): the probe molecule is transferred from one vesicle to another by a continuous process of vesicle fusion and fission. III Concept based upon the close interconnection of vesicles, channels, fenestrae and diaphragms (M. Simionescu & N. Simionescu). The endothelial cells are linked by either continuous occluding zonules or focal sites of membrane fusion (maculae occludentes). The luminal aspect of the endothelium is covered by a surface coat, the glycocalyx, which might also play a major role in endothelial permeability (from HAMMERSEN et al 1983, p2).

2.3.3 Vascular Permeability.

"The main purpose of the vascular endothelium lies in its control on the passage of substances into and out of the channels which it lines" (TRIPATHI & TRIPATHI 1977). Many concepts of such permeability attempt to account for transendothelial exchange on the basis of a simplified general mechanism. Such an approach, however, is not feasible in view of the wide range of substances of differing size, shape, solubility and charge being exchanged (ZWEIFACH 1973a).

In addition, as has been shown, the endothelial barrier "is not a homogenous, monostructured unit, but a mosaic of interlocking elements woven into tubular shape" (ZWEIFACH 1973a). Furthermore, considerable variety is seen in the segmental morphological organisation of the endothelium such that permeability is primarily limited, in normal function, to capillaries and post-capillary venules (SIMIONESCU et al 1975a).

"Used in the biological sense, the concept of permeability is a physiological phenomenon and is a measure of the rate of penetration of a substance through some sort of barrier" (LUFT 1973). "Permeability"

is thus normally a dynamic term which does not generally apply to morphonometric considerations. When speaking of the microvasculature, however, its meaning is commonly broadened to include these intimately related areas (LUFT 1973).

The Starling Hypothesis.

Broadly speaking, most authors agree that the original proposal for fluid exchange, made by STARLING (1896), is probably correct (HADDY et al 1976; ASCHHEIM 1977; CIVETTA 1979; TAYLOR 1981). Starling considered that the mass transfer of fluid across the capillary barrier ultimately depended upon the balance between the hydraulic force of the blood and the osmotic pressure of the plasma proteins which are unable to pass through the capillary barrier. (See also ZWEIFACH 1973a; INTAGLIETTA 1977).

These large molecular weight proteins are suggested to have two effects. Firstly, they diminish the net hydrostatic pressure, thus maintaining the intravascular volume of circulating fluid (INTAGLIETTA 1977). Secondly, they are also presumed to determine the reabsorption of fluid from the tissue back into the intravascular compartment in the venous portion of the microcirculation (as was suggested by Starling), (LANDIS & PAPPENHEIMER 1965).

Thus, in the generally accepted explanation of this process (LANDIS & PAPPENHEIMER 1965), blood enters the microcirculation at a comparatively high pressure and exits at a lower pressure as a result of small vessel viscosity. These input and output hydraulic pressures straddle the blood plasma colloid osmotic pressure such that outward filtration in the arterial (high pressure) portion of the network is largely balanced by absorption in the venous (low pressure) component (INTAGLIETTA 1977).

Note that the mathematical analysis of these physiological parameters is not considered to be within the realms of this thesis. The reader is referred to analyses of these formulations by PERL (1975); ROSELLI & HARRIS (1979); WIEDERHIELM (1979) and KATZ (1981).

It is generally agreed that this transendothelial exchange takes place "solely by processes which require no energy transformations on the part of the capillary endothelial cells" (PAPPENHEIMER et al 1951). Rather, random molecular diffusion (of individual molecules) and bulk fluid flow (driven by hydrostatic and osmotic pressure gradients (or convective forces)) are the principal mechanisms governing the movement of solutes across the capillary wall (PAPPENHEIMER et al 1951; LANDIS & PAPPENHEIMER 1965; ROZTOCIL et al 1980; WAGNER & CASLEY-SMITH 1981).

In this exchange, diffusion is considered the most important in transporting the substances which are central to capillary physiology: the small hydrophilic molecules and gases (DIANA & FLEMING 1979). "The paracapillary circulation (associated with convective bulk flow) plays virtually no role" in either tissue nutrition or the removal of metabolic waste products (CRONE 1973).

This transcapillary fluid flux is however important in:

i) maintenance of the osmotic equilibrium in the tissue milieu,

ii) facilitation of the movement of large molecules (for example, protein) through the interstitium, and

iii) maintenance and regulation of the intravascular fluid volume (INTAGLIETTA 1977). The interaction of these factors is well reviewed by FLEMING & DIANA 1977. (See also ZWEIFACH 1973c and Chapter 4).

Understanding of exchange processes must also include consideration of the so-called "gradient of permeability" in which the

greatest degree of permeability is seen in the venous portion of the microcirculation. This gradient was first proposed in 1930 by ROUS et al (HAUCK 1977) and more recently its existence was quantitatively confirmed by ZWEIFACH and INTAGLIETTA (1968), HAUCK (1971) and DIANA and FLEMING (1979). Such differences in permeability are most likely associated with the structural differences in the intercellular junctions (SIMIONESCU et al 1975a). (See 2.3.1.1).

"Thus the comprehensive analyses of fluid movement include intravascular forces, the characteristics of the capillary barrier, the properties of the interstitial gel in which the blood capillaries lie, and what is essentially an extension of the interstitium, the microscopic beginning of the lymphatic system" (INTAGLIETTA 1977).

It should be emphasised that the Starling model is only a general description. Thus, when tissue is examined with this model as the base, the specialised characteristics of each capillary area must also be considered. These forces are reviewed in more detail in 4.2. Further discussion of permeability is included in Chapters 3 and 4.

2.3.4 Terminal Vascular Bed - a Functional Unit.

"It is axiomatic that form and function of the circulatory system are interdependent, and that the anatomy clearly reflects the physiology of the part. The microcirculation is a paramount example of this" as will be seen from even a cursory examination of such vessels in different organ fields (SOBIN & TREMER 1977).

In 1885, BERNAUD (cited by SOBIN & TREMER 1977) proposed concepts regarding the external and internal environments. Based on these principles the circulation (the internal environment) can only

function through the maintenance of steady states in the body. The maintenance of these steady states results from several varied physiologic reactions, the sum of which is termed "homeostasis" after CANNON (1929, cited by SOBIN & TREMER 1977).

Flow in the exchange bed of an organ at any instant in time includes only a small fraction required for nutrition and maintenance of homeostasis of the cognate tissue; a larger fraction of flow relates to the particular function of the organ in reference to the needs of the organism (SOBIN & TREMER 1977). These concepts were originally proposed in 1940 by KNISELY (SOBIN & TREMER 1977), who suggested that the organ microcirculations could therefore be classified as "i) those which are primarily nutritional to the tissue, and ii) those whose functions are regulatory, or homeostatic, or operant" (SOBIN & TREMER 1977). This classification is the basis of understanding of the functioning of the three-dimensional organisation of the terminal vascular bed. Skin is considered to come within the "operant" group (SOBIN & TREMER 1977). See also 2.7 re the oral mucosa and facial skin.

2.3.4.1 Spatial arrangement of the microcirculation.

The discovery of both muscular and non-muscular vessels of capillary dimensions led to closer investigation of the small vessels of various tissues. From these investigations, ZWEIFACH and colleagues (1953) concluded that the microcirculation was somewhat more complex anatomically than the classical view of an undifferentiated capillary bed fed on one side by the arterial circulation and emptied on the other by the venous pathways. He proposed that the terminal vascular bed was built around a modular framework which is followed in all tissues but demonstrates variation as it is modified to conform with

the architecture of the parenchymal tissue (BAEZ 1977a; ZWEIFACH 1977a).

In this concept, the microvascular network is built up around a centrally placed terminal arteriole, from which multiple side branches arise. The central terminal arteriole itself ultimately divides into 2-3 capillaries which, after a short perfusion length, form confluent post-capillary venules (ZWEIFACH 1977a). The venous component of this framework lies on the outer rim of this three-dimensional network.

It is noted that the development of this structure is less regular in relatively two-dimensional tissues such as skin (BAEZ 406a). The terminal vascular bed is represented in FIGURE 2.9.

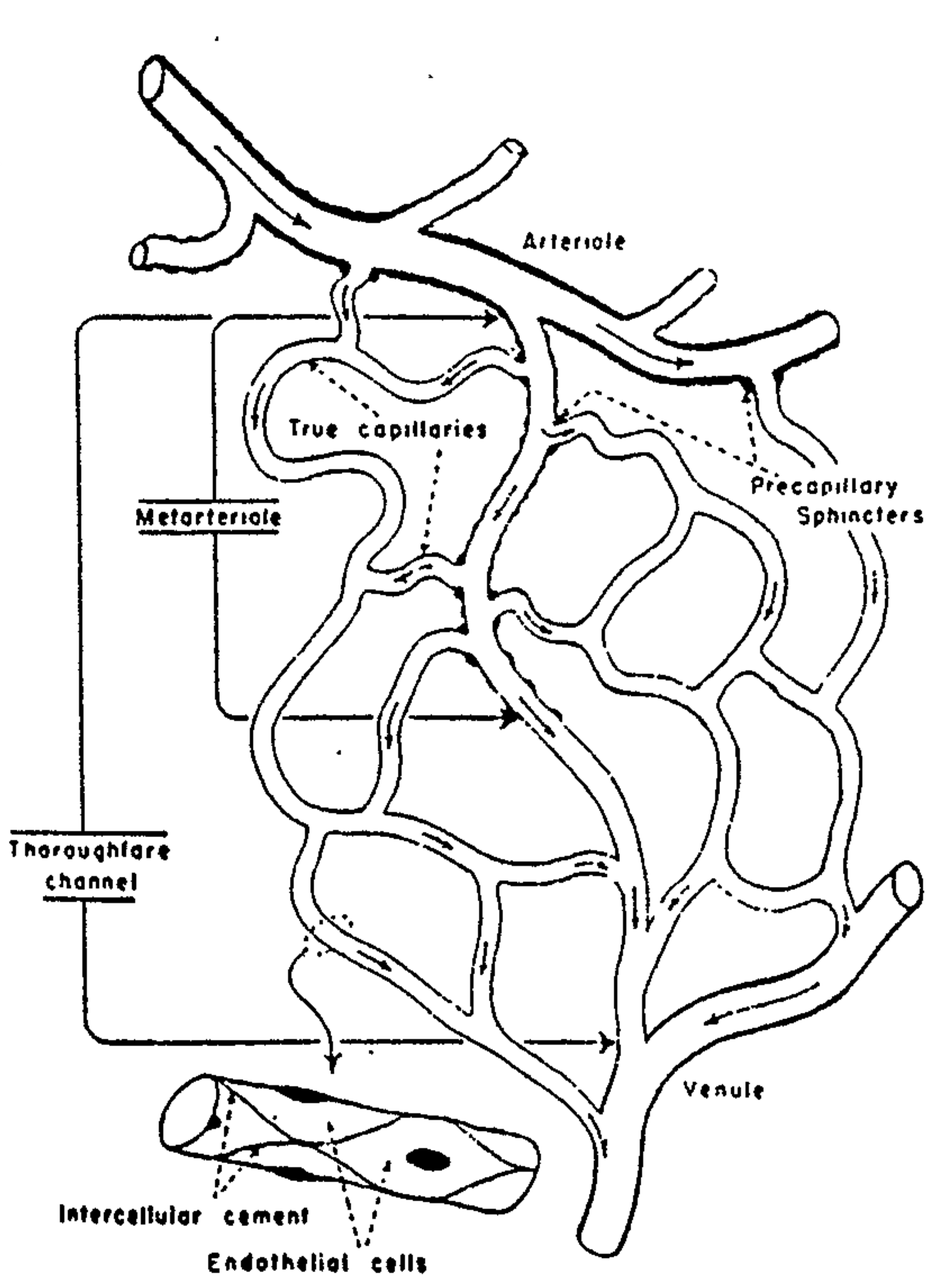

FIGURE 2.9 Diagrammatic representation of the basic structural pattern of the terminal vascular bed as visualised in mesentery. ("metarteriole" = terminal arteriole). The muscular coat is indicated by heavy scalloping of the vessel walls (from ZWEIFACH et al 1953, p627).

2.3.4.2 Precapillary sphincters.

In tracing the distribution of smooth muscle associated with the microvasculature, ZWEIFACH and others (1944, cited by INTAGLIETTA 1981) found that these cells were on the whole restricted to the terminal arterioles and their immediate pre-capillary extensions. A unique configuration is identified at the branching points so that the junctional segments act as valves - termed "precapillary sphincters" (CHAMBERS & ZWEIFACH 1944). In action, these sphincters serve to control distribution of blood flow within the terminal vascular bed, thus enhancing the "shunt" effect outlined below (ZWEIFACH 1977b).

There is some tissue variation in the pattern of diminution of these smooth muscle cells. The "pre-capillary sphincter" may in some situations be a zone of smooth muscle cells rather than single clusters of such cells solely at the origin of the arterial capillaries, as was originally described. In vessels of mesentery and fascial connective tissue, the smooth muscle cells are however thought to represent the classical morphological cluster arrangement (BAEZ 1977a). There is not, however, a sudden loss of muscle cells from muscular arteriole to non-muscular capillary. Rather, a gradual diminution is seen with scattered muscle cells providing for control of blood flow (ZWEIFACH 1977b).

2.3.4.3 Terminal vascular bed in function.

The measurement of pressure gradients has confirmed earlier postulations that arterioles provide the major site of peripheral resistance at rest (LUDBROOK 1981). Vital microscopic studies of the microcirculation reveal a characteristic continual fluctuation in the velocity and distribution of blood flow (BAEZ 1977b). These periodic

alterations are usually seen to occur synchronously in groups of several capillaries, further indicating the role of the arterioles, and in particular, the terminal arterioles and pre-capillary sphincters (INTAGLIETTA 1981).

In considering the movement of blood through this network, it will be seen that flow is primarily controlled through the action of the terminal arteriole since even moderate narrowing of its lumen will reduce flow to side branches, the majority of the flow continuing along the arteriole to its termination (ZWEIFACH 1977a).

In this context the few terminal capillary branches serve, in function, as a "flow-through shunt". Such vessels appear to have a higher flow rate and higher haematocrit and have thus been termed "preferential channels" (ZWEIFACH 1937, cited by BAEZ 1977b; ZWEIFACH 1977a).

With careful observation, the precapillary sphincters are seen to exhibit a slow cycle of contraction and relaxation, permitting and ceasing flow in the capillaries which they serve (BAEZ 1977b). This intermittent activity was termed vasomotion by CHAMBERS and ZWEIFACH (1944). Vasomotion may be limited to the "sphincter" or it may also include the supplying arterioles, thus varying the area of the microvasculature involved (BAEZ 1977b). The action of the sphincters is chiefly seen to enhance the "shunt" effect by limiting flow in the side-branches off the preferential channel (ZWEIFACH 1977a).

Sphincter control is thought to be determined by local metabolic needs (KESSLER et al 1977; SILBERBERG 1979; VANHOUTE 1981) though the central nervous system is presumed to intervene in situations where the injury has marked systemic effects (see 2.3.6). Other modifying factors

include local tissue temperature (VANHOUTE 1981) and vasoactive agents, for example, adrenaline, histamine (BURROWS 1981; VANHOUTE 1981). Some studies suggest that adrenal corticoids may maintain the "tone" of the smooth muscle elements (ZWEIFACH et al 1953; VANHOUTE 1981).

This autoregulatory mechanism ultimately provides a relatively consistent blood flow across the particular organ vascular bed, significantly eliminating the effect of changes in systemic arterial and venous pressures (ZWEIFACH 1977b).

2.3.5 Endothelial Contractility.

Contractility has been postulated to be one of the functional capacities of endothelial cells for many decades. It was suggested that this proposed contractility of capillary endothelium enabled the capillary to independently alter its diameter, so adjusting flow to metabolic needs (HAMMERSEN 1976). This concept was supported by the early observation of Krogh that capillary flow was intermittent (cited by ZWEIFACH 1977b).

The advent of in vivo microscopy of the microcirculation seemed to provide experimental evidence for rejecting the possibility of contractility until MAJNO and PALADE (1961) reported the occurence of interendothelial gaps in response to local applications of histamine.

The idea of endothelial contractility has since been supported by the discovery of microfibrils (both thick and thin) within the endothelial cells (HAMMERSEN 1976; HAMMERSEN et al 1983) and also by the vital-microscopic demonstration of significant bulging of endothelial nuclei into the lumen following electrical stimulation. Such bulging reduced capillary diameter and thus the local blood flow (LUBBERS et al 1979).

The concept of endothelial contractility is thus used to explain two entirely different processes:

i) Under pathological conditions contractility is suggested to offer mechanisms of regulation of transendothelial exchange via the intercellular junctions (LUBBERS et al 1979). This opinion is held by most investigators (HAMMERSEN et al 1983).

ii) Endothelial contractility is also offered as a mechanism controlling flow distribution in the microvascular network. Considerable debate continues on this issue since the spatial arrangement and function of the "terminal vascular bed" (2.3.4) offers different mechanisms for such local control. Further, the majority of vital microscopists have never observed contractility in a true sense (HAMMERSEN et al 1983). Most would now appear to hold the view of ZWEIFACH (1973a) that "endothelium cannot be considered, in the true sense of the term, contractile". This is not to deny that "endothelial cells can alter their shape and outline under certain influences" (HAMMERSEN 1976). Such alterations are almost certainly significant in the alterations in vascular permeability seen in inflammation.

2.3.6 Systemic Control of the Microcirculation.

In addition to the influence of the metabolic activity of parenchymal cells, the blood supply to any given area is controlled by nervous impulses and humoral agents (SELTZER & BENDER 1975; ASCHHEIM 1977). Adjustment of the arteriole and precapillary sphincter calibre regulates the blood flow through this area. The venous circulation functions as a capacitance system (BISHOP & DORMAN 1968; ASCHHEIM 1977). The work of BERNAUD (1949) and CANNON (1939, 1953) (cited by

BISHOP & DORMAN 1968) "established the important role of the autonomous nervous system in vascular homeostasis" via modifications to both blood flow and the capacity of venous reservoirs (BISHOP & DORMAN 1968). Such nervous regulation is mediated by smooth muscle cells in the vessel wall - stimulation generally producing contraction. Vessel lumen is thereby increased or decreased according to the level of stimulation (SELTZER & BENDER 1975).

By and large, vascular nerves are adrenergic (part of the sympathetic nervous system) (BAEZ 1977a). They are reported to maintain a level of contraction ("tone") as a result of continuous impulse discharge; tone may thus be enhanced by increasing the frequency of discharge, or reduced by decreasing it (ZWEIFACH et al 1953; BISHOP & DORMAN 1968). These effects are obviously important since investigations by KOZNIEWSKA et al (1980) demonstrated that blood flow, vasodilatation and increased permeability to albumin in the rabbit hind limb were reduced following sciatic nerve transection.

Vascular nerves may also be cholinergic (both sympathetic and parasympathetic) leading to vasodilatation (BAEZ 1977a). The differing response between these types of innervation is explained by the presence of different receptors. Thus, two are described for the sympathetic system (alpha and beta) and one for the parasympathetic (gamma). The alpha receptor is associated with vasoconstriction, pupillary dilation and intestinal relaxation in response to adrenaline: adrenergic stimulation of the beta receptor produces vasodilatation. The gamma receptor responds to acetylcholine and also produces vasodilatation (BISHOP & DORMAN 1968). See FIGURE 2.10.

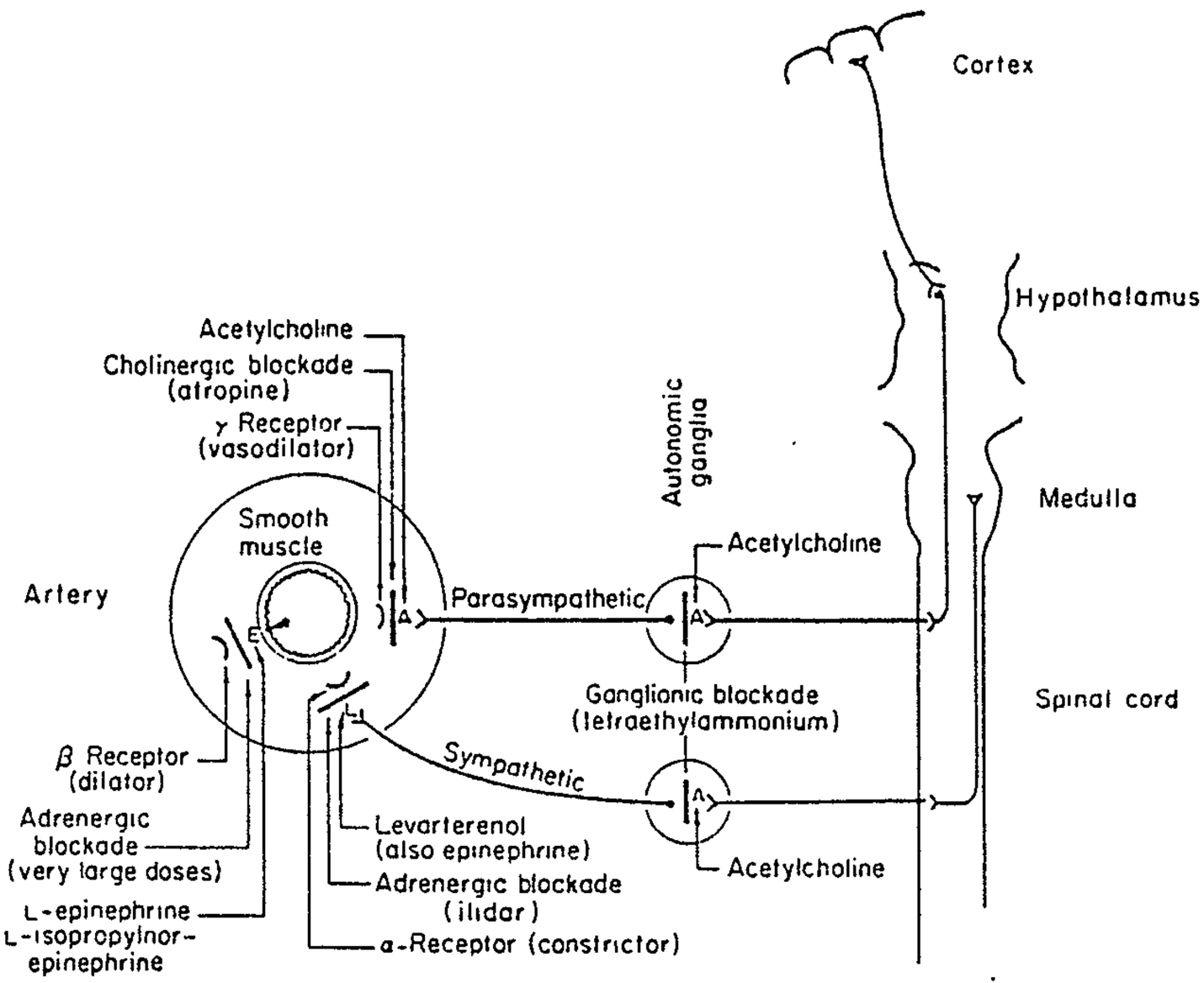

FIGURE 2.10 Schematic diagram of the various receptors in the smooth muscle of the wall of a blood vessel (α, β, γ) of the autonomic ganglionic synapses, and the sites at which adrenergic and ganglionic blocking drugs act (from BISHOP & DORMANN 1968, p34).

The location of alpha and beta receptors in various tissues is obviously critical since they produce opposite effects. Alpha-receptors are generally described in association with vessel smooth muscle and beta receptors chiefly restricted to coronary and skeletal muscle beds. There may be some intra-wall positioning differences also, with alpha receptors located in the medioadventitial region of the wall (adjacent to adrenergic nerve terminals) and beta receptors further inwards towards the lumen (being more receptive to circulating factors) (BAEZ 1977a). Further work is required to clarify these factors.

Humoral mechanisms are also involved in the control of blood flow. Some suggest that such mechanisms are perhaps even more significant than nervous control in small vessels (SELTZER & BENDER 1975). Hormones produced by the adrenal cortex are central to this control, particularly adrenaline, which produces marked

vasoconstriction via contraction of smooth muscle (ZWEIFACH et al 1953).

Acetylcholine is also significant, producing smooth muscle relaxation and hence vasodilatation. However, its action is blocked when adrenaline or noradrenaline is present (SELTZER & BENDER 1975).

A large number of other biological substances are also capable of modifying vascular tone (BURROWS 1981). Dramatic alterations in local blood flow may occur through vasodilatation, or intense vasoconstriction, in response to nervous stimuli or blood-borne agents (WESTWICK-1979). This will be expanded further in Chapter 3.

2.4 THE EXTRAVASCULAR SPACE.

Tissues consist largely of cells embedded in an interstitial matrix composed of complex mucopolysaccharides ("glycosaminoglycans", HARRIS 1979). Most authors recognise two phases in loose connective tissue, (CATCHPOLE 1973; CASLEY-SMITH 1977a; MEYER & SILBERBERG 1977; CASLEY-SMITH & VINCENT 1978; FOLDI & CASLEY-SMITH 1978; CASLEY-SMITH 1979a). The first, a fixed system consisting essentially of a system of interconnected fibres (largely collagen) to which immobile proteins and glycosaminoglycans are somehow attached (MEYER & SILBERBERG 1977). The other, a labile, diffusible system ("gel phase") which is essentially an aqueous solution of salts, metabolites and polymers filling the interstitium (MEYER & SILBERBERG 1977).

Repeated studies have emphasised that the relationships between these two phases are very labile. In normal circumstances, it would appear that the fluid phase is "in equilibrium with or in a steady state with the gel phase" (ZWEIFACH 1973a) and with the blood (CATCHPOLE 1973). This equilibrium may be profoundly affected by a

variety of conditions and substances (CATCHPOLE 1966; CASLEY-SMITH & VINCENT 1978) - see 3.3.

In the "fixed" system, fibres criss-cross the tissue forming a network which gives tissue structural shape. They also give the tissue its strength as a result of the high elastic modulus of the fibres in extension (MEYER & SILBERBERG 1977). This becomes of particular significance in situations of oedema (4.4).

The aqueous gel filling the space between the fixed components is composed of salt and metabolites and also serum and other freely diffusable proteins (MEYER & SILBERBERG 1977). The nature of its composition allows both diffusion and convection depending upon the concentration gradients. It is however, primarily the "fixed" yet somehow labile proteoglycans which contribute most of the viscous and osmotic elements (MEYER & SILBERBERG 1977). This will be discussed further in Chapter 4.

Both the fibrous and glycosaminoglycan components of the interstitial connective tissue are implicated in the observed exclusion of protein molecules from the extravascular space (LAURENT et al 1977; WATSON 1980). This is partially related to dimensions; it is also because this tissue is not a simple aqueous fluid, but a charged macromolecular gel (CATCHPOLE 1973). (See Chapter 4).

Interstitial channels

Early electron micrographs of interstitial tissue demonstrated spaces, apparently within the water-rich phase, which were occupied by neither cells, fibres, nor proteoglycans (GERSCH & CATCHPOLE 1960; CASLEY-SMITH & VINCENT 1978). Many studies described the appearence of these "vacuoles"; more recent studies have shown them to be continuous

channels randomly traversing the interstitial matrix (CASLEY-SMITH & VINCENT 1978).

Having a diameter of between 50 and 200nm, these channels are normally only a few microns long, though in tissues where true lymphatics are absent (for example, brain, bone marrow and retina), they may reach several centimeters, then being termed "prelymphatics" (FOLDI & CASLEY-SMITH 1978).

As a result of the labile nature of this two-phase system, various changes occurring in a tissue may lead to considerable changes in both size and number of the channels (CASLEY-SMITH 1979a). The quantity of fluid flowing through a region is postulated to be a significant governing factor in this, as partly evidenced by the increase in numbers of channels seen adjacent to lymphatic and blood vessels (particularly post-capillary venules) (CASLEY-SMITH 1977a; CASLEY-SMITH & VINCENT 1978).

CASLEY-SMITH has postulated that as a result of tissue functioning (especially fluid movement), suitably oriented channels become enlarged at the expense of others which receive less and less water. These flow characteristics, and hence channel diameter, appear to be related to a dynamic balance between deposition on, and erosion of, the channel walls. Increased functional needs associated with, for example, increased fluid load and injury, appear to result in marked alterations in this balance with the result that both the number and diameter of the channels are increased (CASLEY-SMITH & VINCENT 1978; CASLEY-SMITH 1979b).

Tissue channels are critical to the flux of material through the tissues. In many respects, the channels are identical in structure and function to the "circulatory system" of primitive animals in which the entire transfer of nutrients and wastes is via such channels (CASLEY-SMITH & VINCENT 1978). In harmony with these nutrient distribution/waste removal functions, fluid flow and channel contents show dynamic change related to tissue function. Although the channels themselves are thus very labile and ill-defined, the network they form facilitates much higher fluxes of water and macromolecules between the blood vessels, and between the blood vessels and lymphatics (CASLEY-SMITH & VINCENT 1978).

In some situations it is further suggested that the interstitium is a significant factor in the permeability of the vessels to macromolecules, particularly in fenestrated endothelium (CASLEY-SMITH & VINCENT 1978). Hence, the importance of the interstitial channels in facilitating macromolecule passage through such tissues (CASLEY-SMITH 1976a, b).

It is thus obvious that the functional changes apparent in these channels are intimately associated with the functioning of the adjacent blood and lymphatic microcirculations. This aspect is further discussed in association with the effect of osmotic and hydrostatic pressures on fluid and protein movement within the tissues (4.2).

2.5 THE LYMPHATICS: MORPHOLOGY AND FUNCTION.

As a result of normal functioning, there is a net transfer of protein molecules into the extravascular tissues. If this accumulation were maintained, the circulation would be deprived of plasma colloids and disruption of the forces of permeability homeostasis would occur.

Thus the "primary function of the lymphatics is to drain the interstitium of (such) plasma proteins ... and return these to the circulation" (LEAK & BURKE 1973) (See Chapter 4). Lymphatics are also important in the return of fluid to the vascular circulation (CASLEY-SMITH 1979c). Discussion of these functional considerations will follow an outline of lymphatic structure. The initial and collecting lymphatic vessels are the most important to this discussion.

2.5.1 The Initial Lymphatics.

Following passage through the tissues, interstitial fluid is taken up by the smallest and most permeable lymphatic vessels, the initial lymphatics (LEAK & BURKE 1973). These are usually between 100 and 500 micrometres long and very irregular in shape, usually appearing flattened. They form a network of interconnecting vessels which usually lie in close proximity to the venules.

Their structure is similar to that of venous capillaries but certain significant differences have been identified (see LEAK & BURKE 1973; CASLEY-SMITH 1977c):

i) the basement membrane is considerably less well developed and may appear to be absent in some sites.

ii) lymphatic endothelium is generally thicker than blood endothelium though it usually appears thinner since it has a wider and more irregular lumen than the accompanying blood capillary.

iii) No fenestrae are seen in the initial lymphatics.

iv) Many fine, probably non-contractile fibrils are seen attached to the ablumenal surface of the endothelial cells connecting them to the interstitial tissue. These fibrils function to maintain the patency of the lymphatics in situations of raised tissue pressure (FOLDI & CASLEY-SMITH 1978). The existence and function of these fibrils was

first postulated in 1933 by DRINKER & FIELD (LEAK & BURKE 1973). See FIGURE 2.11.

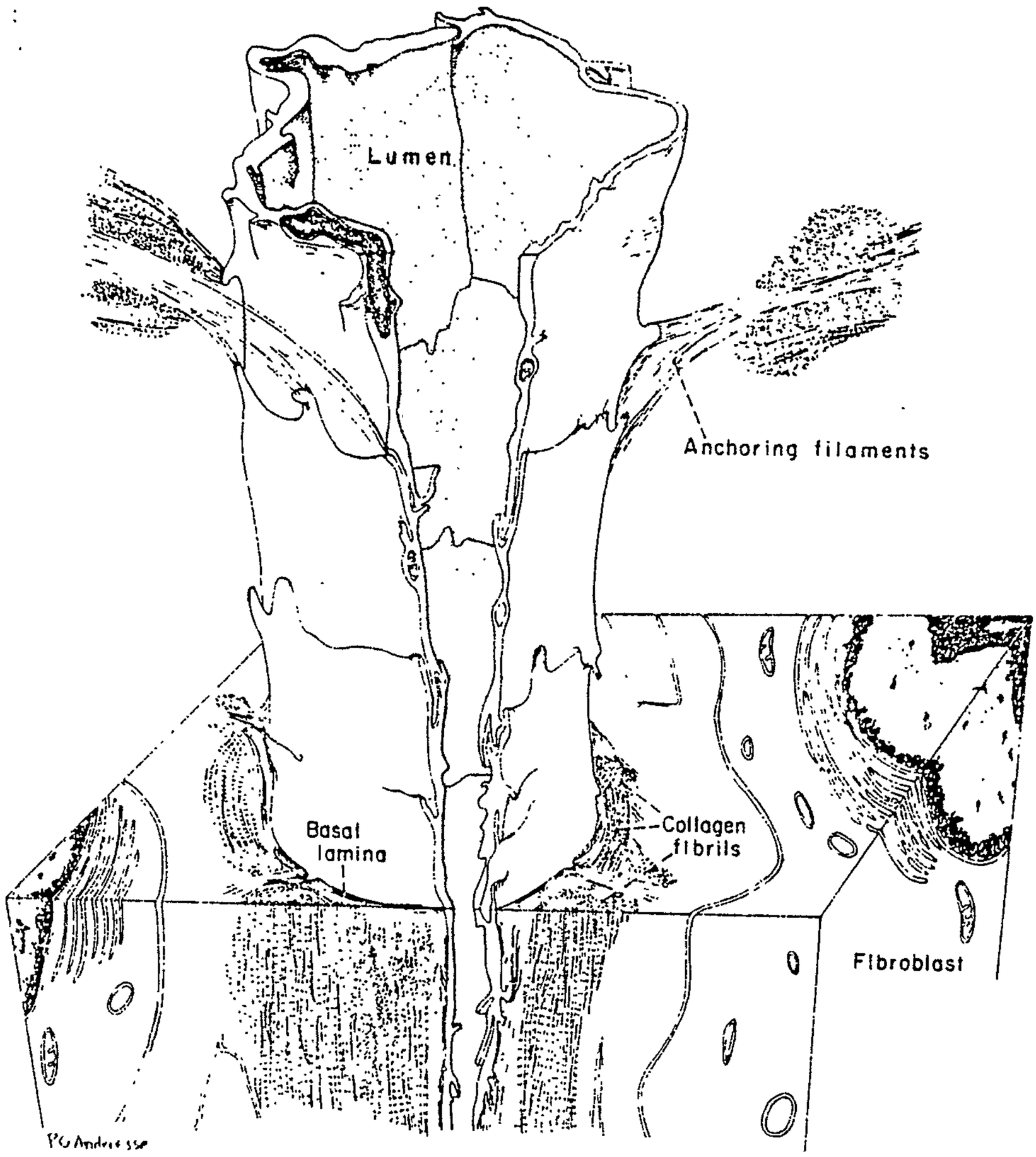

FIGURE 2.11 Three-dimensional interpretive diagram of an initial lymphatic, reconstructed from collated electron micrographs. The three-dimensional relationship of the lymphatic capillary to the surrounding interstitium is illustrated, including the anchoring filaments, irregular basal lamina and collagen fibres (from LEAK & BURKE 1973, p218).

2.5.1.1 Intercellular junctions.

Perhaps the single most important morphological feature of initial lymphatic endothelium is its intercellular junctions (FOLDI & CASLEY-SMITH 1978), since the lymphatic system is chiefly dependent on these junctions both for uptake and retention of material (LEAK 1971; CASLEY-SMITH 1977c). These junctions range in morphological

configuration from cells that simply overlap each other (often for long distances), to terminal margins with extensive interdigitations. Some sites also demonstrate specialised regions of adhesion as represented by macular adherentes and desmosomes. However, these sites rarely cause complete obliteration of the intercellular clefts (being approximately 20nm wide, CASLEY-SMITH 1977c), and hence passage of fluid and particles is not prevented when the initial lymphatic is at rest (LEAK & BURKE 1973).

Where overlapping margins are present, the specialised regions of adhesion are frequently absent. The cells are therefore able to freely slide over each other as a result of pressure changes between the interstitial tissues and the lymphatic lumen. The overlap thus serves as a reserve of lymphatic wall allowing easy and rapid expansion of the vessel.

Since the inner cell margin of such junctions is within the lumen, it may act as a one-way "flap-valve". The valve opens the intercellular cleft when interstitial pressure is higher than lymphatic intraluminal pressure, and closes when intraluminal pressure equals or exceeds interstitial pressure (LEAK & BURKE 1973). See FIGURE 2.12.

FOLDI and CASLEY-SMITH (1978) state that these open junctions may show considerable alteration under differing circumstances. Great increases in their number and permeability occur in regions of high metabolic activity and particularly in association with the presence of oedema. Even mild injury such as scratching the skin has been shown to cause an increase in the number of these junctions (CASLEY-SMITH 1973; FOLDI & CASLEY-SMITH 1978).

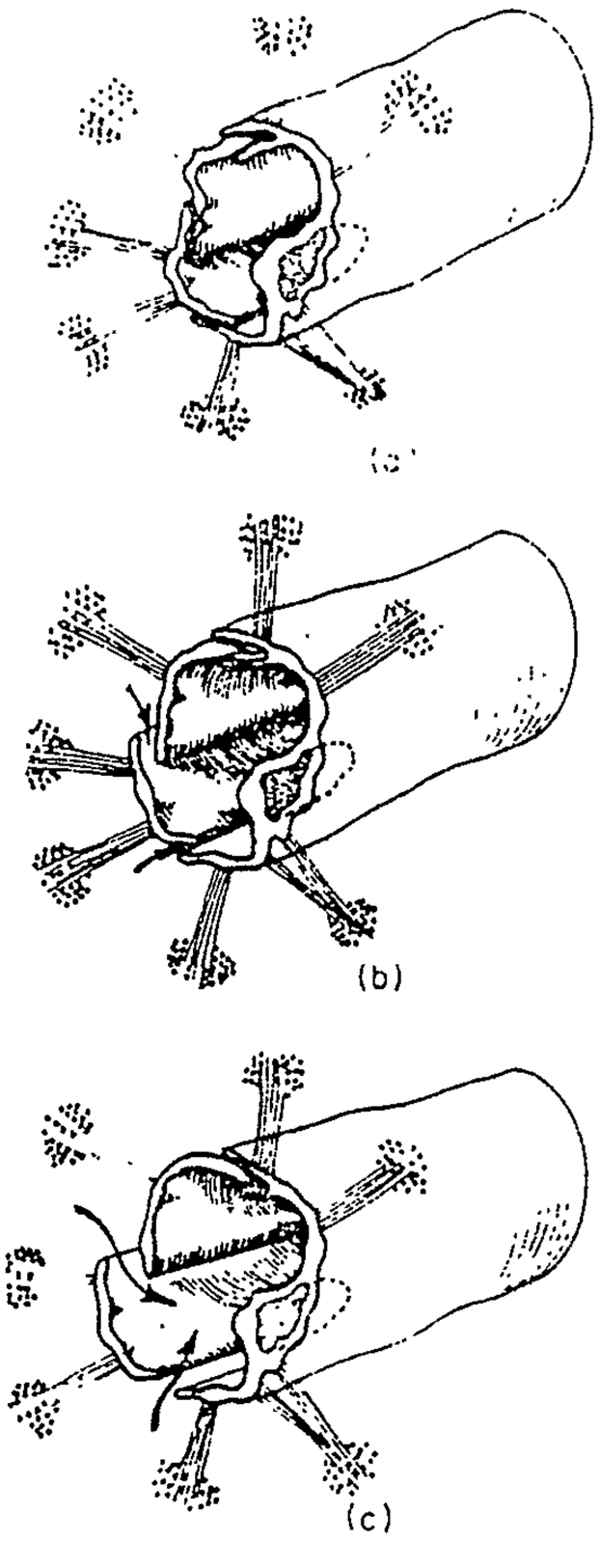

FIGURE 2.12 The lymphatic capillary tissue interface is depicted in three figures in this diagram. The anchoring filaments provide a firm connection between the lymphatic wall and surrounding interstitium as depicted in (b) and (c). The lack of adhesion devices between the opposing endothelial cells that overlap each other for long distances facilitate the ease with which adjacent cells separate (arrows) to allow for expansion of the lymphatic lumen and also increase the number of patent junctions. The anchoring filaments and the extensive overlap of adjacent endothelial cells provide a very simple, but effective, mechanism for regulating the direction of bulk flow from the interstitial area into the lumen of the initial lymphatic, the unstabilised, overlapped portion of endothelial cell within the lumen at each junction acting as a "one-way valve" (from LEAK & BURKE 1973, p235).

2.5.1.2 Lymphatic endothelial vesicles.

Cytoplasmic vesicles of similar dimensions to those described in capillaries and veins, are also apparent within the lymphatic endothelium. Whereas those in capillaries and venules have been proposed to account for the slow leakage of macromolecules from the blood to the tissues, in lymphatics they are suggested to be partly responsible for uptake of such molecules by the lymphatic system. However, passage in lymphatics is chiefly associated with the intercellular junctions (CASLEY-SMITH 1973; CASLEY-SMITH 1977c).