

2.5.1.3 Initial lymphatics in function.

Although the thoracic duct was probably seen by some members of the Alexandrian school in the 1st and 2nd century B.C., the lymphatic system was not described as such until the independent work of RUDBECK and BARTHOLI in 1652 (cited by GROITE 1979). More than 300 years later, the mechanisms of lymph formation are still, to a large degree, an enigma to researchers of the microcirculatory system (GROITE 1979).

Some of the earliest suggestions were that the terminal lymphatic endothelium was contractile. However, this ability appears to be restricted to lymphatic trunks which have smooth muscle in their walls (PULLINGER & FLOREY 1935). It is not observed in vessels where such cells are absent (RHODIN & SUE 1973).

Early observations suggested that there was a slight pressure gradient from the tissues into the lymphatics (McMASTER 1946). However, multiple later studies have suggested that the techniques used in these experiments were inaccurate in normal tissues since most required fluid pools within the tissue, a feature not normally present, except in oedematous states. (Pressure gradients from tissue to lymphatic are readily demonstrable in oedematous tissue) (CASLEY-SMITH 1973).

However, more recent measurement techniques (see 4.2.2.1) have suggested that in fact the gradient of tissue pressure would be more likely to remove fluid from the lymphatics than to cause it to enter them (GUYTON et al 1971). (In these studies the interstitial fluid pressure was measured as slightly negative (that is, subatmospheric) in most tissues whereas intralymphatic pressure is usually measured as approximately zero (FOLDI & CASLEY-SMITH 1978)). Thus, the question of lymphatic filling remains.

One force which seems to offer a solution to the entry of fluid to lymphatics has been offered by CASLEY-SMITH (1973) and by ELHAY & CASLEY-SMITH (1976), namely the osmotic pressure gradient since the protein concentration within the lumen of the initial lymphatics is approximately 3 times that of the concentration within the tissue space. On the basis of this gradient, CASLEY-SMITH has defined three phases of functioning of the initial lymphatics: a filling phase, a resting, intermediate phase and an emptying phase. (See FIGURE 2.13).

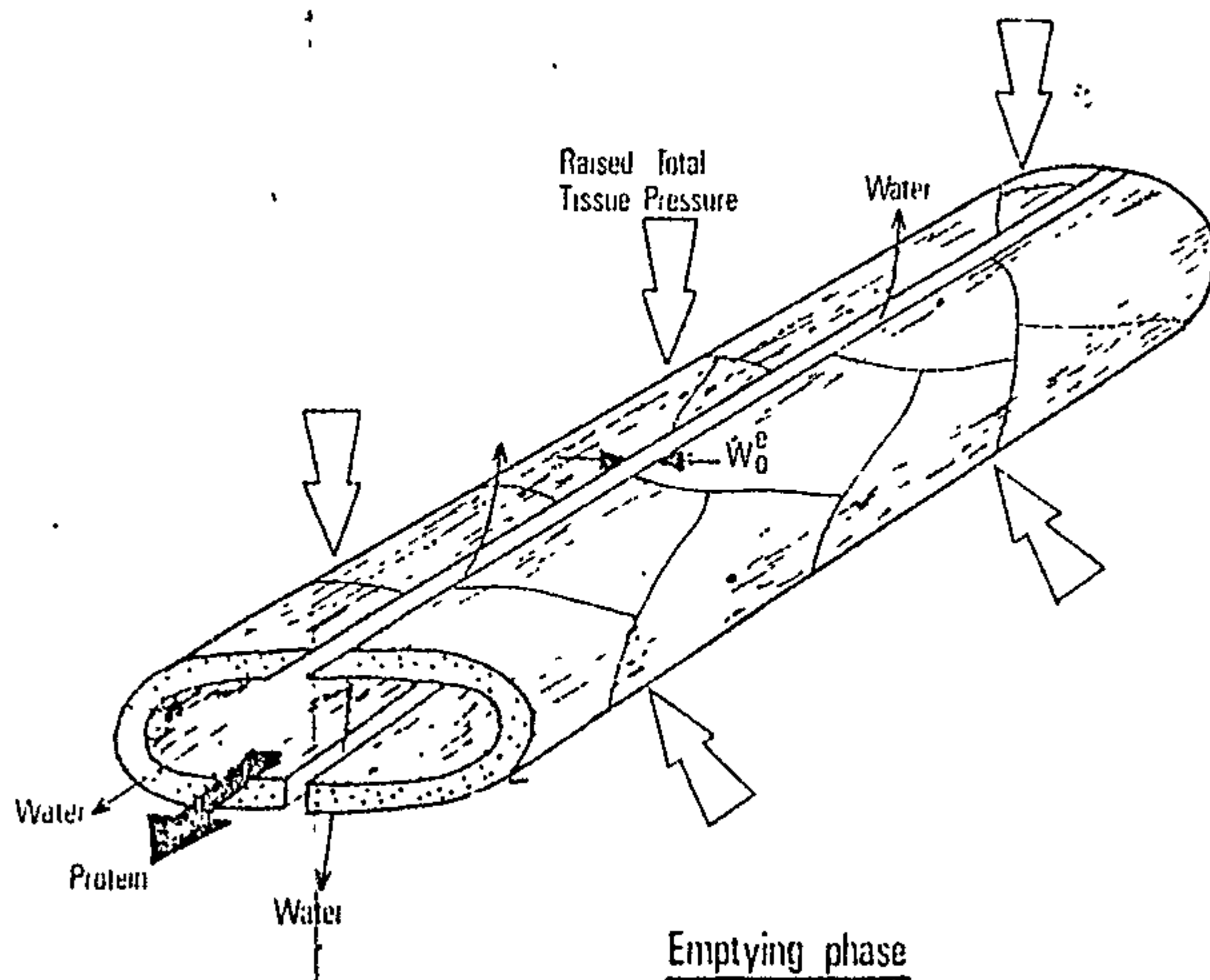


FIGURE 2.13a The emptying phase of the initial lymphatic cycle. This commences when the total tissue pressure rises and compresses the vessels. Water is forced out of the "close" junctions - these are sealed to protein at this time. Remaining fluid and small and large molecules are pumped into the remote collecting lymphatics (lower left) (from FOLDI & CASLEY-SMITH 1978, p82).

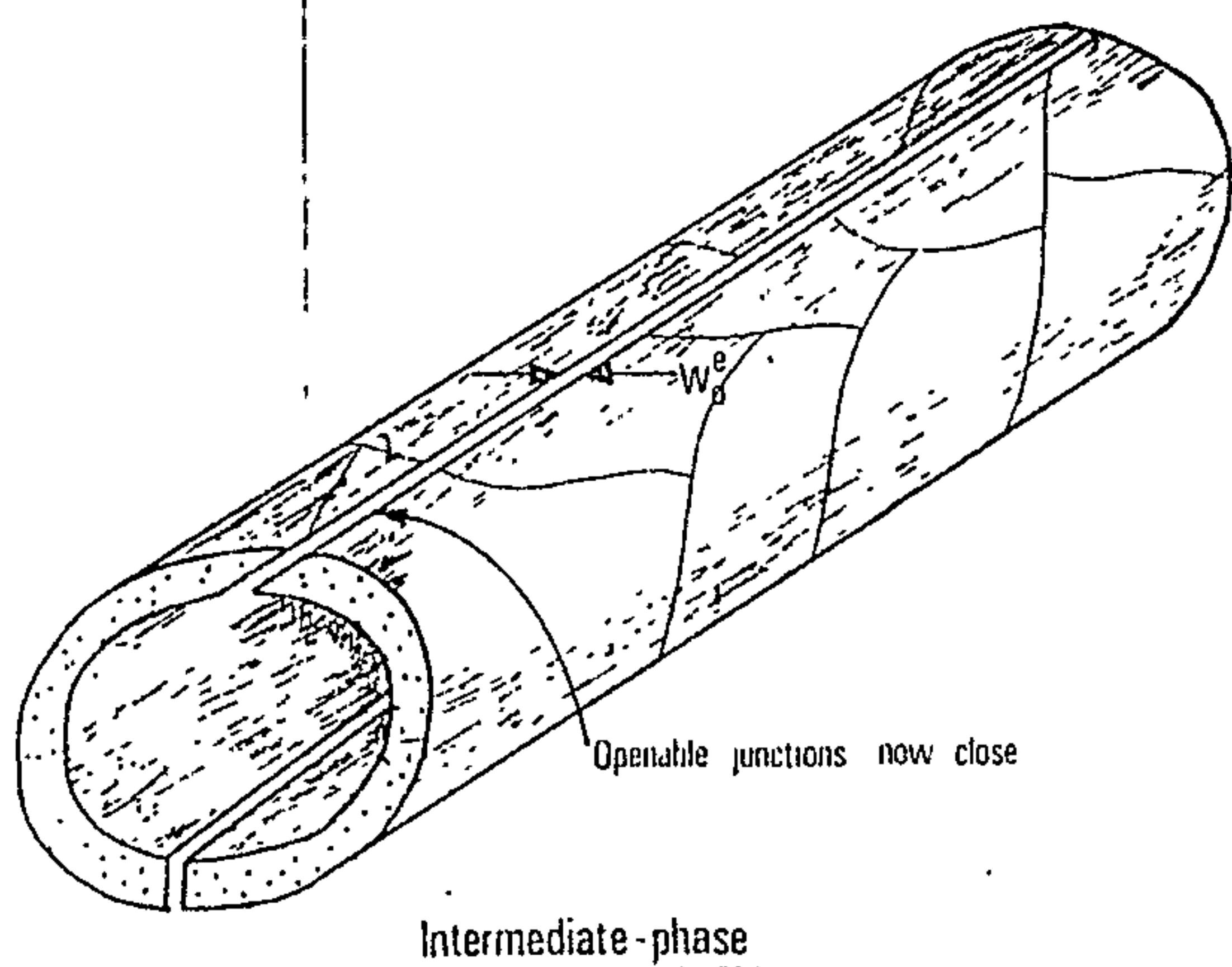


FIGURE 2.13b The filling phase of the initial lymphatic cycle. This commences when the total tissue pressure falls (for example when an adjacent muscle relaxes) and water and proteins enter the vessel via the open junctions (from FOLDI & CASLEY-SMITH 1978, p83).

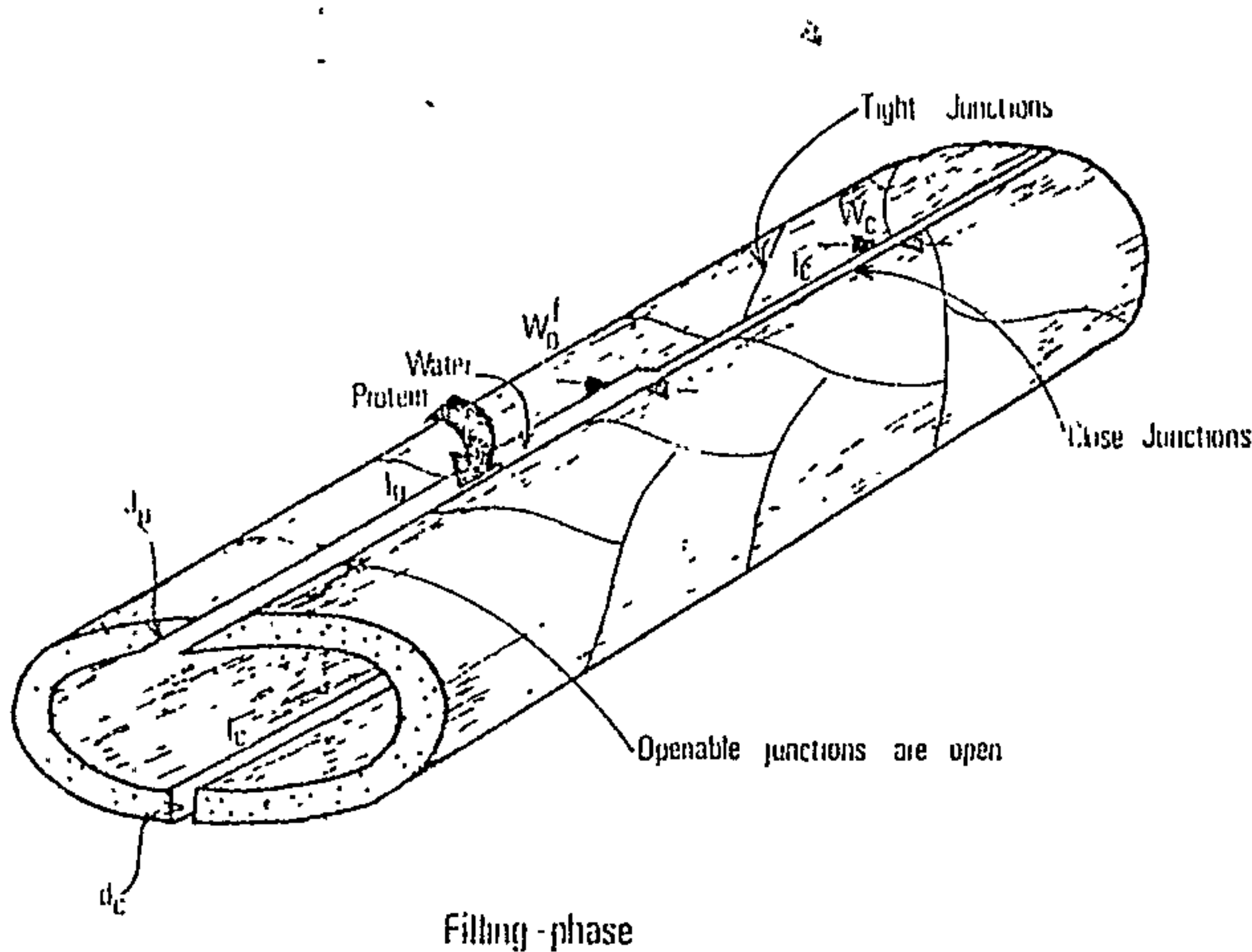


FIGURE 2.13c The intermediate phase of the initial lymphatic cycle. This occurs when the net inwards and outwards forces balance. Then the openable junctions become close and a static situation prevails until the next increase in tissue pressure causes a new "emptying phase" (from FOLDI & CASLEY-SMITH 1978, p84).

The period when tissue compression causes fluid loss via the "closed" junctions (and thereby concentration of intralymphatic protein) (described above) is defined as the emptying phase. This compression (due to the pulse or adjacent muscle contraction for example (RHODIN & SUE 1973)) is associated with "pumping" of lymph from the initial to the collecting lymphatics. Note: any molecules small enough to pass through the "closed" junctions are also expressed along with the fluid.

Upon tissue relaxation, the higher intra-lymphatic protein concentration is suggested to cause fluid influx via the openable junctions. This fluid carries additional protein with it, though at a lower concentration, and thus it dilutes the lymph (the filling phase).

When the net pressure across the junctions is zero, the intermediate phase occurs. The vessels remain filled with closed junctions (see "flap valves", 2.5.1.1) until a new phase of raised tissue pressure commences the next emptying phase. This cycle is outlined in FIGURE 2.14.

These postulations were initially based upon mathematical calculations from physiological laws and data. They remain to a certain extent hypothetical though the calculations are in harmony with observed measurements (ELHAY & CASLEY-SMITH 1976; CASLEY-SMITH 1977c). Several factors are in their favour (CASLEY-SMITH 1976b, 1977b; PERL 1975 & others) including demonstration of cyclic variations in intralymphatic protein concentration (CASLEY-SMITH 1976b, 1977c). This hypothesis is also in harmony with earlier suggestions by other authors (RHODIN & SUE 1973).

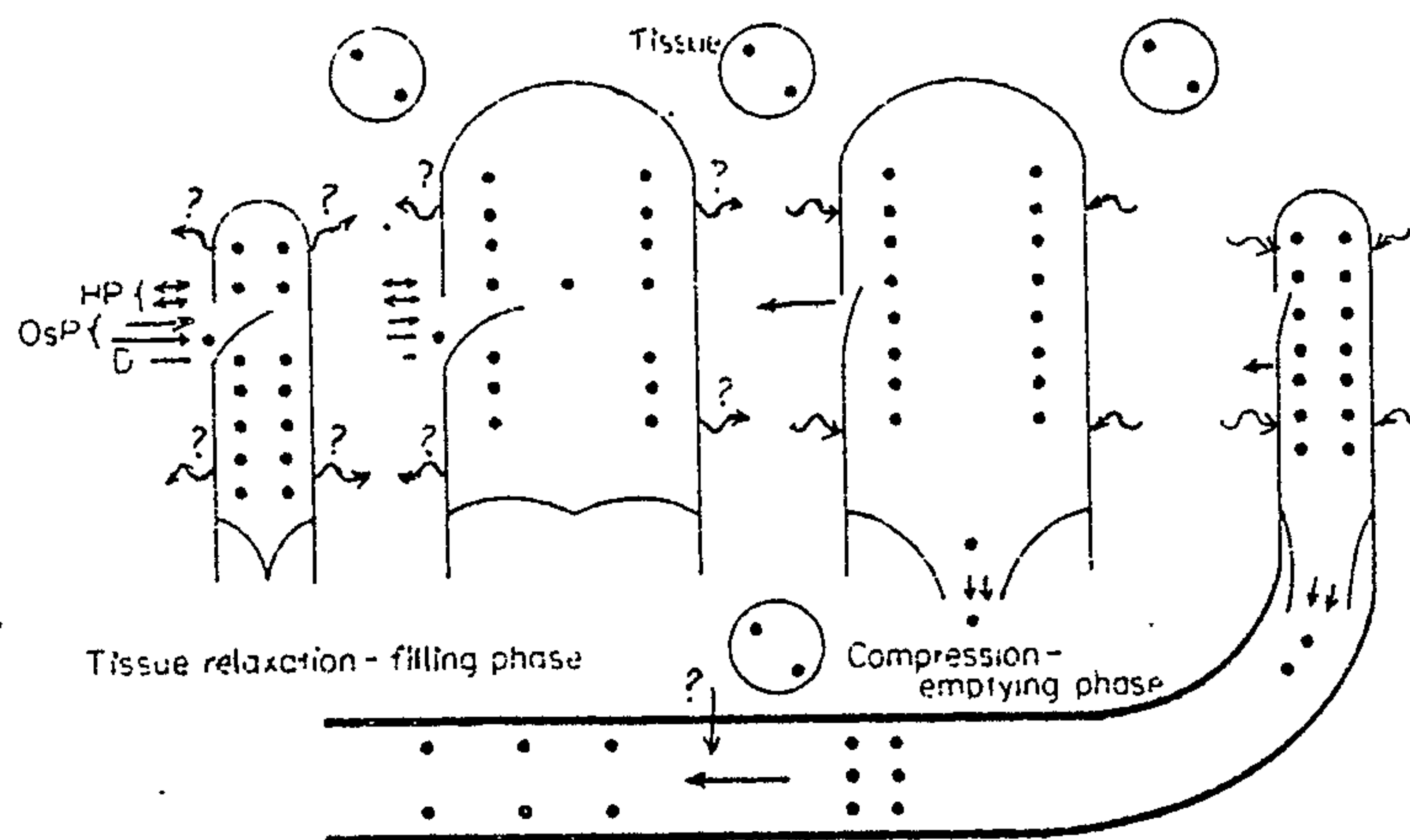


FIGURE 2.14 A diagram of the initial lymphatic cycle. The dilation, filling, and compression and telescoping during emptying are shown. The thick arrows indicate protein movement; the thin ones indicate fluid flow. Their lengths show the presumed variations in flow rates during the cycle. The wavy arrows indicate the solid tissue pressure or the pull of the filaments which may not be important in normal tissue, although they are in inflammation, (hence the "?"). Proteins (dots) are represented in their presumed relative concentrations in the tissues (circles), in the initial lymphatics during the cycle, and in the (thick-walled) collecting lymphatics before and after their hypothesised dilution by fluid inflow through its wall. During filling, material enters the initial lymphatics via the open junctions. The hydrostatic pressure difference (HP) is shown as either inwards or outwards, according to the circumstances, although it is probably only directed inward during oedema (see Chapter 4). The hypothesised osmotic pressure difference (OSP) due to the differences in protein concentration causes fluid to enter the vessels. It varies in force with variations in the concentrations of the intralymphatic proteins. There is also similarly variable outward diffusion (D) of proteins. During compression, the junctions become closed; the net hydrostatic-osmotic pressure difference forces only fluid out of them. This flow presumably decreases as the contents become more concentrated. A relatively small amount of lymph fluid and protein is passed to the collecting lymphatics, leaving the concentrated remaining protein to provide the osmotic pressure needed to cause the cycle to continue (from CASLEY-SMITH 1973, pl87).

The postulated model demonstrates a series of self-regulating feedback mechanisms which are suggested to allow modulation of each of the phases according to prevailing local conditions. Casley-Smith suggests that this system is part of an integrated system in which the whole blood-vascular-tissue-lymphatic complex forms an integrated homeostatic mechanism (FOLDI & CASLEY-SMITH 1978). Tissue oedema is postulated to partly be the result of overstraining this lymphatic drainage system (4.8).

2.5.2. The Collecting Lymphatics.

The collecting lymphatics form the second link in the lymphatic system. They basically function to transmit the material taken up by the initial lymphatics and pass it into the blood circulation (usually in the neck).

Thus, examination of the lymphatics from initial to the larger collecting lymphatics reveals decreasing numbers of open junctions until all junctions are tight or close. Wall thickness also increases, further decreasing permeability. More significant is the increasing presence of smooth muscle allowing the active transport of lymph (FOLDI & CASLEY-SMITH 1978). This is aided by the presence of intralymphatic valves, however, movement arises not only from contractions of their walls, but also from varying tissue pressures resulting from adjacent muscle contractions, respiratory movements^{and} the pulse. (CASLEY-SMITH 1977c).

Here also the vessel functioning has a homeostatic basis: if segments are more distended than usual by an increased flow of lymph, the contractions will be more vigorous and more frequent, thus removing the excess (FOLDI & CASLEY-SMITH 1978).

The lack of open junctions in the collecting lymphatics results in retention of most of the protein molecules with only a small loss occurring in the lymph nodes. However, water and small molecules may still easily escape and as the walls become more and more muscular, thus raising the intralymphatic pressures, the lymph is further concentrated (FOLDI & CASLEY-SMITH 1978). FIGURE 2.15 outlines the overall functioning of the lymphatic system.

Other functions

In addition to these functions of fluid and protein transport, the lymphatic system also serves as a route for the migration of lymphocytes throughout the body. Interchange is particularly prominent in association with the lymph nodes (LEAK & BURKE 1973; FOLDI & CASLEY-SMITH 1978).

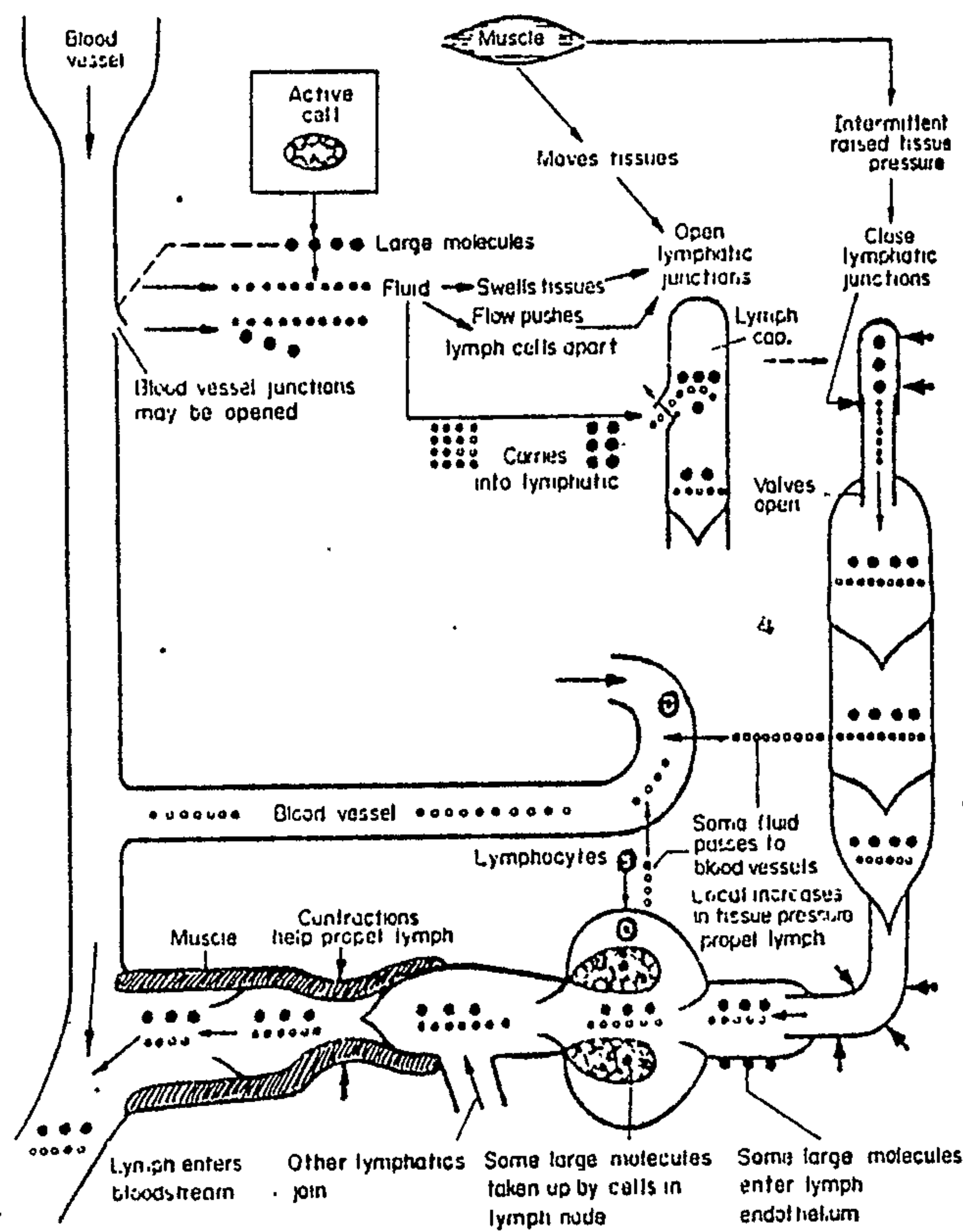


FIGURE 2.15 A summary of the functioning of the whole lymphatic system (from FOLDI & CASLEY-SMITH 1978, p96).

2.5.3 Role of the Lymphatics in Exchange in Normal Tissue.

In the discussion of the role of the lymphatics in tissue homeostasis, it is appropriate to differentiate two classes of substances. The first is that of the small molecules (up to 2,000 M.W) of which water is chief; water may be used to exemplifying all small molecules. The second is that of the macromolecules - of between 20,000 and 2,000,000 M.W. These are perhaps more important since by definition, they are too large to be able to pass through the blood capillary intercellular junctions in significant amounts, yet they are small enough to exert significant osmotic pressures when in reasonable concentrations (CASLEY-SMITH 1977c).

There is no question that the reabsorptive transport of small molecules occurs primarily via the blood vessels, particularly in fenestrated endothelia. Nonetheless, the lymphatics fill a crucial role, qualitatively (though not quantitatively) in the fluid and protein homeostasis of normal tissue (TAYLOR et al 1981).

With respect to macromolecules, the lymphatics are reported to transport up to 50% of the total plasma protein pool per day. Since the concentration gradient of such molecules strongly favours movement out of the blood vessels (4.2), there is a tendency for their accumulation within the tissues. This tendency is balanced by lymphatic transport and catabolism of the protein molecules within the tissues along with some uptake by the venules (CASLEY-SMITH 1977c).

2.6 RHEOLOGY OF THE NORMAL CIRCULATION.

"Rheology is the science concerned with the flow and deformation of matter". It is applied to the study of the flow properties of blood and its cellular elements since it was found that circulatory flow was chiefly governed by rheologic factors (SCHMID-SCHONBEIN & WELLS 1971).

The rheologic changes of blood and tissue fluid are central to the inflammatory response. Changes involving the blood vessels are primary in this response, with the reactions involving the lymph and white cells flowing secondarily to and depending upon the initial reactions of the blood and its flow. A resumé of the rheology of the normal microcirculation is *àpropos* here. The changes seen in association with inflammation will be discussed in Chapter 3.

The flow properties of blood are determined both by its physical characteristics (the cells and plasma proteins) and by the dynamics of

their interactions during flow (WELLS-1973). These factors are outlined below, though it must be emphasised that in the determination of blood rheology, each factor frequently shows considerable dynamic interaction with the others.

The article by SCHMID-SCHONBEIN & WELLS (1971) provides an excellent historical review of the developments in the field of haemorheology along with a lucid presentation of the principles and terminology of microrheology.

2.6.1 Haematocrit.

Clinically, the single most significant factor influencing blood viscosity is the volume concentration of cells (the haematocrit). This is primarily associated with erythrocytes which comprise most of the haematocrit: increased cell concentration is associated with increased viscosity.

2.6.2 Relative Rates of Flow.

Also significant are the relative rates of flow of fluid elements within the blood stream, and the shear stress between them, that is, the force of one fluid element or layer rubbing or shearing across its neighbour.

The shear rate is an expression of the gradients of velocity between the stationary vessel wall and the fluid element most distant from the wall (namely the central flow) (WELLS 1973). Blood viscosity shows marked changes in response to changes in the shear rate; with ² falls below its normal minimum range (approximately 4 dynes/cm), stasis is likely to develop, particularly if other factors such as vasoconstriction and arterial hypotension are present (ZWEIFACH 1972).

In the functioning microcirculation, periodic vasomotion could theoretically cause marked increases in haematocrit with subsequent sharp increases in viscosity. However, the slow periodicity of these vasoconstrictions is seen to lead to gradual haemodilution with "screening out" of cells. On the other hand, the return of vasodilatation leads progressively to haemoconcentration. This does not impair flow since the pressure gradient which accompanies vasodilatation is sufficient to deform and align the cells. High fluidity of microcirculatory flow is thus maintained, principally because of the slow rates of vasomotor change and the high erythrocyte deformability (2.6.4.1) (SCHMID-SCHONBEIN et al 1981).

2.6.3 Plasma Proteins.

The total concentration of the plasma proteins, the relative concentrations of each of the major protein fractions and their physical properties are also important in influencing whole blood viscosity. These effects may develop through interactions between the plasma proteins and the red cells (WELLS 1973). Globulin and fibrinogen are particularly important because of their cohesive action which enhances the attraction of red cells to each other. Increasing either the globulin or fibrinogen levels markedly increases red cell aggregation and whole blood viscosity, particularly at low shear rates. Albumin, on the other hand, reduces aggregation and lowers blood viscosity by virtue of its relatively smaller size and more negatively charged surface (WELLS 1973).

These features adopt increased significance as a result of plasma protein extravasation in inflammation (3.2.1.6).

2.6.4 Cells.

In addition to the volume concentration of cells, the rheologic character of the suspended cells is also an important factor in determining flow characteristics of whole blood. As has been alluded, the erythrocyte is paramount in functioning micro-haemorheology.

2.6.4.1 Erythrocytes.

Though normally described as biconcave discs, erythrocytes have been found to be extremely deformable. They are seen to assume a wide variety of (sometimes odd) shapes in flowing blood as a response to the hydrodynamic stresses to which they are subjected under normal circumstances (FUNG 1977a). Through such deformations, relatively fluid flow may be maintained, even in haematocrits up to 60% (WELLS 1973).

With respect to microcirculatory flow, the deformability of red cells is a critical variable. Without some degree of flexibility, erythrocytes would be incapable of passing through a normal capillary. Several studies have concluded that the normal red cell is able to squeeze through a pore of about 3 microns diameter without injury at physiological pressures. (See WELLS 1973).

In flowing blood, red cells (RC) never contact the vascular endothelium; they are separated from it by a relatively constant "lubrication layer" of plasma, which thus reduces the shear strain on the vessel wall, so minimising friction and viscous flow. The constancy of this lubrication layer is also chiefly enabled by the red cell deformation (FUNG 1977b).

In addition to these individual cell deformations, groups of red cells deform and aggregate in various configurations in response to altered intravascular stresses (SCHMID-SCHONBEIN et al 1972). This also leads to significant changes in vessel flow. Such changes, outlined by ZWEIFACH (1972), are described below.

At stasis, red cells are attracted to each other forming long chains or rouleaux. (See FIGURE 3.4). As flow begins, shearing forces tend to pull these rouleaux apart until they are fully dispersed. As flow rate further increases, the velocity gradient rises and the cells begin to align themselves in streamlines, further reducing their resistance to flow and decreasing blood viscosity. At high rates of shear, normal red cells undergo ellipsoid-type deformation, thus further reducing cell-drag (WELLS 1973).

Each of these observations is highly dependent on the normal predicted deformability of these cells, a feature which may be altered, for example, by changes in pH and osmolality. (These alter the amount of free membrane available for flexion). Various disease states, for example sickle cell anaemia, may alter cell deformability. Similarly, pathologically increased aggregation is seen following acute myocardial infarctions and major surgical trauma (SCHMID-SCHONBEIN & WELLS 1971).

2.6.4.2 Leucocytes.

While less studies have been conducted on the rheology of leucocytes, it is apparent that the normal white cell demonstrates considerably more viscosity than does the erythrocyte. Individual cells also demonstrate reduced deformability (compared with erythrocytes) being unable to pass through pores of 5- or 8- microns at physiological pressures (WELLS 1973).

Direct microscopic observations of white cells flowing in vivo within the microcirculation have demonstrated that they are more frequently seen near the walls, exhibiting more of a rolling motion than the streamline flow of a simple particle (which the red cell more closely resembles) (WELLS 1973).

The rheological differences noted between the various leucocytes (for example, polymorphonuclear leucocytes behave in a more viscous fashion than do lymphocytes) result primarily from the biochemical, humoral and biophysical differences between their cell membranes, and the manner in which endothelium reacts to them (WELLS 1973).

2.6.5 Vessel Architecture.

The ultrastructure and gross anatomy of the various segments of the microvasculature are different; accordingly, the rheology of the blood in each is different. The following outline is adapted from the review by WELLS (1973). See also SCHMID-SCHONBEIN & WELLS (1971).

The small arteries and arterioles in general represent a bulk flow condition with high rates of shear and consequently low viscosity. When the precapillary arterioles decrease to an internal diameter of 25-30 microns, the total cross-sectional area could only include about 15 cells.

Below this diameter, the blood does not flow as a heterogenous mixture of small particles, but rather a collection of relatively large (when compared to vessel size) deformable masses. It is then that the flow properties of the cells and their suspending medium determines blood viscosity.

At the level of the capillary, flow is reduced to the level of the single cell. Here the flow of the individual cell and the shape and rigidity of the vessel dominate.

Beyond the capillary into the venules, cells gather into vessels of increasing diameter, but with slower flow rates than arterioles. Subsequent to this low velocity, the low rate of shear becomes the predominant variable (termed "creep flow"). Normal creep flow is commonly accompanied by red cell aggregation which gradually disperses as the rate of shear rises in the larger veins.

2.7 THE ORAL VASCULATURE.

2.7.1 Blood Vasculature.

The supply of blood to all the structures related to the oral cavity is via three major arteries - the lingual, facial and maxillary - all of which are branches of the external carotid artery. The following summary is adapted from VIDIC & MELLONI (1979).

The facial artery, having run upwards deep to digastric and stylohyoid muscles and submandibular gland, gives off two branches which supply the tonsillar region. It then traverses behind the submandibular gland, giving off branches to this gland and the associated lymph nodes, and also to the submental region.

After the artery loops around the base of the mandible, just anterior to the attachment of masseter, it ascends into the face, supplying both upper and lower lips. Lip vascular supply is supplemented by terminal branches of the infraorbital artery and the mental branch of the inferior alveolar artery, respectively.

The maxillary artery, a terminal branch of the external carotid, vascularises all the muscles of mastication from the infratemporal fossa. The inferior alveolar branch follows the nerve of the same name to supply the mandible, lower teeth, and associated periodontium and overlying mucosa.

In the pterygopalatine fossa, the maxillary artery becomes the infraorbital to supply the maxilla, upper teeth, associated periodontium and overlying mucosa via the anterior, middle and posterior superior alveolar branches. It supplies the palate via the nasopalatine, greater palatine and lesser palatine branches.

The lingual artery supplies blood to the tongue by two terminal branches, the dorsal lingual and the sublingual. The latter branch also vascularises the lingual gingival mucosa, the floor of mouth and the sublingual salivary gland. The parotid gland is supplied with blood from the terminal segment of the external carotid artery.

In general, the venous return from these regions accompanies the path of the major arteries. This does not occur in all situations. For example, the structures supplied by the maxillary artery are drained into the pterygoid plexus situated between the temporalis, and medial and lateral pterygoid muscles. The plexus then converges into a short maxillary vein running adjacent to the maxillary artery. Venous communications exist between the plexus and the facial vein (via the deep facial vein) and the cavernous sinus (via veins of the sphenoidal emissary foramen and foramen ovale). See also SELTZER & BENDER (1975).

Significant anatomical variations have been described in the course of the above vessels, particularly whilst vessels are outside bony confines. Remembering that the face is not a discrete organ, but

represents a limited area in the general arterial blood supply to the head and neck, it is not surprising that anastomotic channels are also observed (in angiographic investigations) between the above arteries (LASJAUNIAS et al 1979).

2.7.2 Lymphatics.

The lymphatic system of the head and neck is primarily of interest because of its relationship to metastatic spread of carcinoma arising in this region (LARSON et al 1965 & 1967; VIDIC & MELLONI 1979). Lymphatics are also critical in the progression of infective processes and in the drainage of excess fluid and protein from the tissues of the mouth, face and neck both in normal function and in post-surgical inflammation.

A general feature of the lymphatic system is that it is subject to considerable developmental variations and thus description of its regional distribution must be regarded with certain reservation. Further, lymphatic capillaries frequently overlap. Thus, lymphatic drainage of the same tissue region may flow to more than one group of nodes (VIDIC & MELLONI 1979).

SCHWEITZER (1907, cited by SHAPIRO & RUBEN 1971) was the first to describe the lymphatic supply of the gingiva, identifying that these vessels were situated in the submucosa and drained both facially and lingually along the periosteum (SHAPIRO & RUBEN 1971). Lymphatic afferents from the periosteum follow the neighbouring mucosal vessels. Midline cross-over is frequently seen.

2.7.3 Microcirculation in the Oral Cavity.

Whilst the oral tissues are no exception to the homeostatic functions described above for the vasculature in general, many treatment regimens are largely determined by empirical judgements of the dental surgeon. However, were the normal cardiovascular involvement in oral problems understood, treatment could be adapted to allow the optimum contribution of this system (BISHOP & DORMAN 1968).

There is a relative paucity of understanding in the literature as to how the oral microvasculature compares both structurally and functionally with the vasculature of other body tissues. (Note that the vascular supply to the dental pulp is considered to be outside the scope of this review).

As a result of the work of KING (1947, cited by BISHOP & DORMAN 1968), it is possible to microscopically examine the blood supply of the gingivae and periodontal membranes in vivo. While experimental situations concentrate on direct visualisation of the cheek pouch, (BISHOP & DORMAN 1968) clinical investigations generally survey the anterior labial gingivae (CALLENDER 1972; MORMANN et al 1979). CALLENDER (1972) provides an excellent description of the technique of intravital microscopy as related to the gingival microcirculation. Unfortunately, the majority of such studies have been concerned with the changes seen in diabetes rather than with describing the normal oral microvasculature.

Morphology.

Vessel dimensions would seem to be similar to the dimensions described in the foregoing discussion (LIN et al 1975). Capillaries of both continuous and fenestrated types are described to exist, though

the continuous type is predominant (LISTGARTEN et al 1974). No mention is made of any site variation in such occurrence.

Considerable similarity is apparent between descriptions of skin and gingival microcirculations. In both, a great deal of morphological variation is observed; capillaries in both sites are significantly more complex and varied than capillaries in muscle (LISTGARTEN et al 1974). This variability relates also to the ultrastructure of the basement membrane, in which the lamina rara is apparently absent in some oral sites (FRANTZIS et al 1971).

The gross morphology of the oral microcirculation is apparently similar to the dermal microcirculation, being formed into loops with frequent arteriovenous anastomoses. These loops extend superficially between epithelial rete pegs (CIMASONI 1974), as in skin (BISHOP & DORMAN 1968; SIMPSON 1977).

This is compared with vessels of the crevicular epithelium which does not form epithelial ridges. Here the vessels immediately deep to the epithelium form a flat sheet. This suggests that the vascular pattern of the marginal gingiva is dictated by the configuration of the connective tissue-epithelium interface.

MARUYAMA et al (1976) identify three morphological types of capillary loops (FIGURE 2.16), namely: i) normal type - "hairpin" with or without clubbing, ii) mixed type - both hairpin and complex types, and iii) complex types - waving, crossing, branching and intercommunication types. In normal mucosa, this study found that 53.8% were of the normal pattern and 46.2% mixed. This situation was seen to dramatically alter in inflamed states and in other pathological conditions.

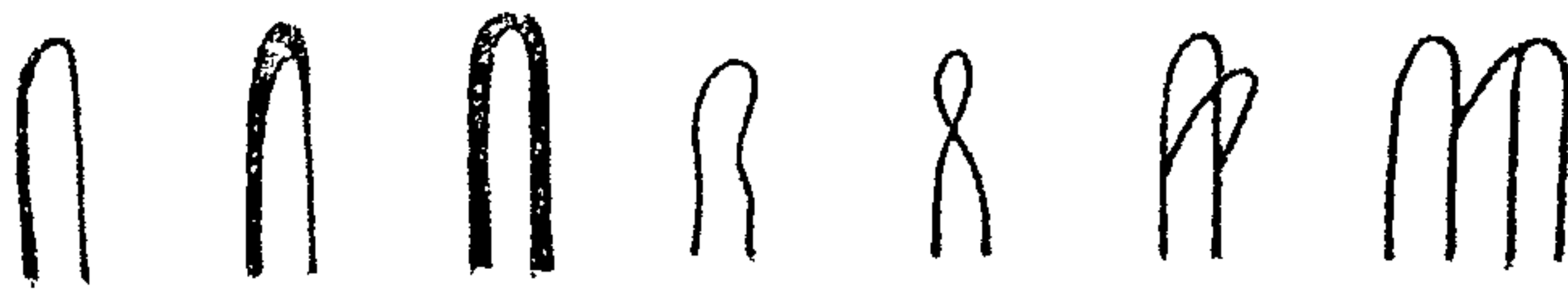


FIGURE 2.16 The form of capillary loops in oral tissues (left to right): normal, clubbing, dilatation, waving, crossing, branching, and intercommunicating (from MARUYAMA et al 1976, p181).

Vessel ultrastructure in oral tissues also appears highly similar to that of skin. This was supported in a small pilot study using light and electron microscope examination. In this study, a small wedge of gingival tissue was removed from the corner of mucoperiosteal flaps raised for the removal of mandibular third molars (the specimen was taken just prior to closure, that is, after 15-20 minutes).

The results of these examinations (shown in PLATES 2.1, 3.1), support the assumption that most of the data presented earlier relating to the ultrastructure of skin microvessels applies also to their oral counterpart. Obviously, there are some functional differences (see below), and more detailed investigation is required to verify this assumption fully.

Rate of flow.

One study (BISHOP & DORMAN 1968) described most gingival capillaries to be "dilated and carrying blood at a maximal rate". Only a small number demonstrated stationary erythrocytes, and none were seen to be devoid of red cells. STALLARD (1968) also observed high flow in oral vessels stating that, in the gingival circulation, a nearly constant nutrient supply is maintained even under conditions of greatly altered arterial pressure.

This liberal vascularisation is suggested to represent (in part) the basis of local tissue resistance to infection, and to the

constant thermal and mechanical stresses to which the gingiva is subjected (BISHOP & DORMAN 1968).

A definitive examination of regional variations in blood flow is reported by GORDON et al (1978). This demonstrated that, of the oral tissues sampled, glandular tissue showed the highest flow rates, whilst skeletal tissue (mandible), the lowest. Components of the oral mucosa (gingival and palatal tissue) indicated flow rates which were close to those of glandular tissue.

In comparison with other body tissues, this study tends to confirm the views of BISHOP and DORMAN (above) that oral tissues have considerably higher volume flow than skin. However, the oral blood flow was generally less than half that observed in the caecal sack, which has one of the highest flow rates of body tissues.

Flow rates show gradual diminution with progressive ageing and that the absence of microbial flora in experimental situations also led to reduced flow. Both such factors will be considered further in Chapters 5 and 6 respectively.

Other features.

In another area of comparison, KOZAM (1968) demonstrated that the resistance of the microvessels of the lower labial mucosa was significantly higher to "stretch stress" than similar vessels in the skin of the antecubital fossa.

An important surgical consideration is that the vessels are preferentially orientated in an apicocoronal direction (MORMANN et al 1979). Further, the potential for collateral circulation to the marginal gingiva from the periodontal ligament is greatest at the interdental papilla. Vertical incisions along the mid-axis of the teeth

resulted in greater ischaemia, suggesting decreased potential for such collaterals. (See 6.3.1).

Little discussion is made in the literature regarding the ultrastructure of the oral lymphatics.

2.7.4 Blood Supply to the Facial Skin.

Skin as an organ plays a central role in the dissipation and conservation of metabolic heat and the regulation of blood volume. It is also important in the maintenance of osmotic pressure because of its role in water and salt balance (BISHOP & DORMAN 1968).

With respect to these functions, various features may be observed. Firstly, blood flow shows marked temperature-dependent variations between different parts of the body and even between different parts of the same region. The amount of this variation in flow may be considerable.

Secondly, since skin blood flow is chiefly determined by metabolic needs, particularly maintenance of thermal balance, such flow may, under certain conditions, greatly exceed local metabolic needs (BISHOP & DORMAN 1968). This is the basis for the classification of skin as an "operant" organ (See SOBIN & TREMER 1977).

It appears that blood flow to much of the facial skin demonstrates little constriction in response to cold stimuli, even when such stimuli are generalised and not just related to the head. Thus, in the forehead, cheek, chin, submandibular region and anterior neck, active vasodilatation is the predominant feature. Other portions including lips, parts of the ears and nose are however, dominated by vasoconstrictive tone (BISHOP & DORMAN 1968)...

2.8 CONCLUSION.

A broad overview of the microvasculature is described with discussion of the functioning of various ultrastructural features. Many of the subjects considered remain strongly debated enigmas despite major technological advances in microvascular methodology.

Central to microcirculatory functioning is the three-dimensional organisation of the component vessels - the "terminal vascular bed" - and the control of molecular and fluid exchange across the walls of such vessels. The interstitium and cellular parenchyma of the various tissues are the objects of this exchange; they also exert some effect on permeability itself, particularly to large molecules such as plasma proteins.

The lymphatic system acts as a homeostatic mechanism restoring lost protein and fluid to the internal environment. As will be seen (Chapter 4), the elimination of some transudated protein by tissue lysis is also important to homeostasis.

Each of these components shows tissue-dependent variation, and hence, oral tissues are compared with the general schema. Significant in this comparison is the suggestion that the level of perfusion in the mouth is apparently higher and less fluctuating than that of skin.

With the normal functioning of the vasculature as a base, Chapter 3 is directed towards the alterations which follow surgical injury.

CHAPTER 3 ACUTE INFLAMMATION.

OUTLINE.

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3.1 INTRODUCTION.

Inflammation may be defined as the local response of living tissue to injury. It is manifested both by the formation of a protein-rich fluid-exudate and by an infiltration of cells (CACI & GLUCK 1976). As such, inflammation represents a collection of complex biological responses to a disturbance of the local or general homeostasis; it aims at the elimination of injurious agents and restitution of the homeostatic balance (HAUCK 1979). It should be noted at this point that the early concepts of inflammation as being a purposive and useful process (HUNTER 1794, cited by HURLEY 1981) have been questioned. This question is by-passed here, being considered in Chapter 9.

Since the tissue response to injury is not a single event, "but a collection of distinct yet interrelated mechanisms", it is often difficult to separate between the direct effects of the injury and the tissue reaction (HURLEY 1981). The mechanisms of this reaction are, in themselves, non-specific; a variety of injurious stimuli may lead to similar tissue responses (HURLEY 1972). Their expression, however, as an inflammatory response is varied, being dependent upon the type of injury and the tissue affected. All peripheral structures in the area of injury - the microvasculature, the connective tissue, the ground substance - may be affected (HAUCK 1979).

In acute inflammation, the reaction is primarily vascular in nature, whereas in chronic inflammation, the extravascular compartment predominates (SILBERBERG 1979). Chronic inflammation, by definition, does not fall within the scope of this discussion. It should therefore be noted that the term "inflammation" and its derivatives will, in this thesis, generally refer only to the acute reaction unless otherwise qualified.

The vascular events of acute inflammation have been intensively studied, particularly as relating to the observed fluctuations of vascular permeability. While it is true to say that such vascular events are central to the acute inflammatory reaction, it cannot be said that inflammation is solely a vascular phenomenon. In fact, it is "the sum of individual events taking place in ground substance, interstitial fluid, connective tissue, and non-vascular cells ... and (the) lymphatic system as well as in the blood vascular system itself. In short, inflammation is a tissue response" (LEAK & BURKE 1973).

Resulting from the extent of the experimental focus on the blood vasculature, inflammatory changes are conveniently catalogued with the phases of vascular permeability as the time scale. The time course of these interrelationships is plotted in FIGURE 3.1.

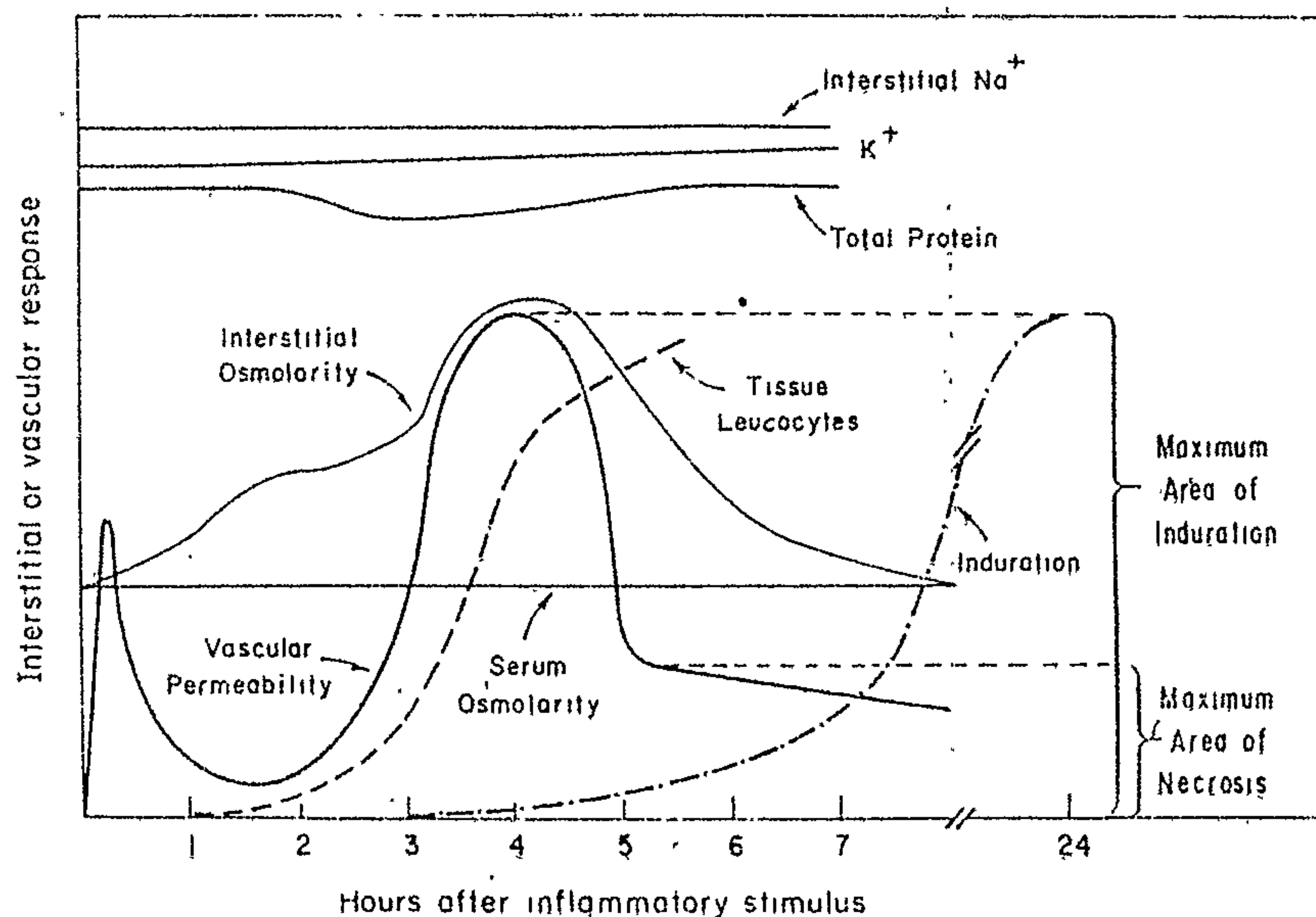


FIGURE 3.1 A comparison of the time of occurrence of events in early inflammation. Changes in interstitial composition (osmolarity, electrolyte, and protein concentration) are compared with changes in vascular permeability and development of induration and tissue leucocytosis (from LEAK & BURKE 1973, p201).

There remains however, little understanding of the cause/effect interrelationships between these tissue components, and hence the following discussion relates chiefly to microcirculatory events. Where possible however, factors pertaining to the generalised tissue response are also included. Since such tissue changes focus around the

inflammatory exudate, discussion of many of these aspects is included in Chapter 4 in relation to the formation of oedema.

Macroscopically, the primary reaction pattern seen in acute inflammation is characterised by erythema, fever, oedema, pain and loss of function - the cardinal signs (Chapter 1). Microscopically, inflamed tissue exhibits vasodilatation (frequently with associated hyperaemia or stasis), thrombosis (with possible tissue infarction), increased vascular permeability (with accompanying oedema) and cellular infiltration (GILMORE 1976). At the molecular level, the inflammatory process may be seen to have two components - fluid-phase and cellular phase (involving both blood and tissue cells). The following discussion is divided according to this outline.

It should also be remembered that accompanying any significant injury, a vast number of systemic and metabolic changes occur - the "acute phase response" (KUSHNER 1982). Such changes include fever, leucocytosis, and increased production of various hormones such as insulin, cortisol and adrenal catecholamines (RYAN & MAJNO 1977; KUSHNER 1982).

3.2 MICROSCOPIC EVENTS IN ACUTE INFLAMMATION.

In reviewing the microscopic events seen in inflamed tissues, the author is primarily concerned here with the description of the observed morphological changes and the consequences of such changes. The mediation of tissue changes is considered under the discussion of the "molecular events" - 3.3.

3.2.1 Vascular Events.

Two sets of vascular events take place in inflammation: changes in flow rate and vessel calibre, and changes in permeability (RYAN & MAJNO 1977). These vascular events are closely associated with the movement of leucocytes from the circulating blood into the extravascular tissues (considered in 3.2.2).

3.2.1.1 Changes in flow rate and vessel calibre.

The examination of the vascular events associated with inflammation is chiefly performed in living transparent tissues. From such investigations, COHNHEIM (1882) provided the classic description of the vascular events of inflammation from his work on the tongue and mesentery of the frog. Quoting from his "Lectures on General Pathology" (cited by EBERT & GRANT 1973):

"The first thing you notice in the exposed vessels is a dilatation which occurs chiefly in the arteries, then in the veins and least of all in the capillaries. With the dilatation which is gradually developed, . . . there immediately sets in . . . an acceleration of the blood stream, most striking again in the arteries, but very apparent in the veins and capillaries also. Yet this acceleration never lasts long: after half an hour or an hour, . . . it invariably gives place to a decided retardation, the velocity of the stream falling . . . below the normal standard".

Cohnheim also noted that, concurrent with vessel dilatation, many capillaries which had previously been carrying little or no blood opened up to full flow. Further, he observed that the rapidly flowing blood is divided into two zones: "a peripheral zone of almost cell-free plasma and an axial stream containing closely packed red and white corpuscles" (HURLEY 1972; 1981; EBERT & GRANT 1973).

Since Cohnheim's day, the in vivo study of inflammation has been considerably refined through the introduction of technological

advances. However, such advances have led to little modification of Cohnheim's description, even though some of the conclusions which he drew have been proven incorrect (EBERT & GRANT 1973).

The changes which occur in the blood vasculature may be summarised as follows (adapted from HURLEY 1972; 1981; BOHM 1977): The vascular events of acute inflammation generally commence with a short-lived vasomotor reflex of arteriolar vasoconstriction (associated with the processes of haemostasis). Concurrent with this vasoconstriction (which may last up to five minutes), the injured tissue liberates vasoactive compounds so that the constriction is succeeded by vasodilatation (chiefly of venules and arterioles) and increased blood flow. The opening up of many previously "closed" capillaries is also observed.

These inflammatory agents and/or the inflammatory response cause excitation of the adjacent sensory nerve endings producing an antidromic reflex (FIGURE 3.2) causing arteriolar dilatation in the neighbourhood of the damaged area. In addition to this positive vasodilatation, the precapillary sphincters become refractory to vasoconstrictor stimuli (ZWEIFACH 1973a).

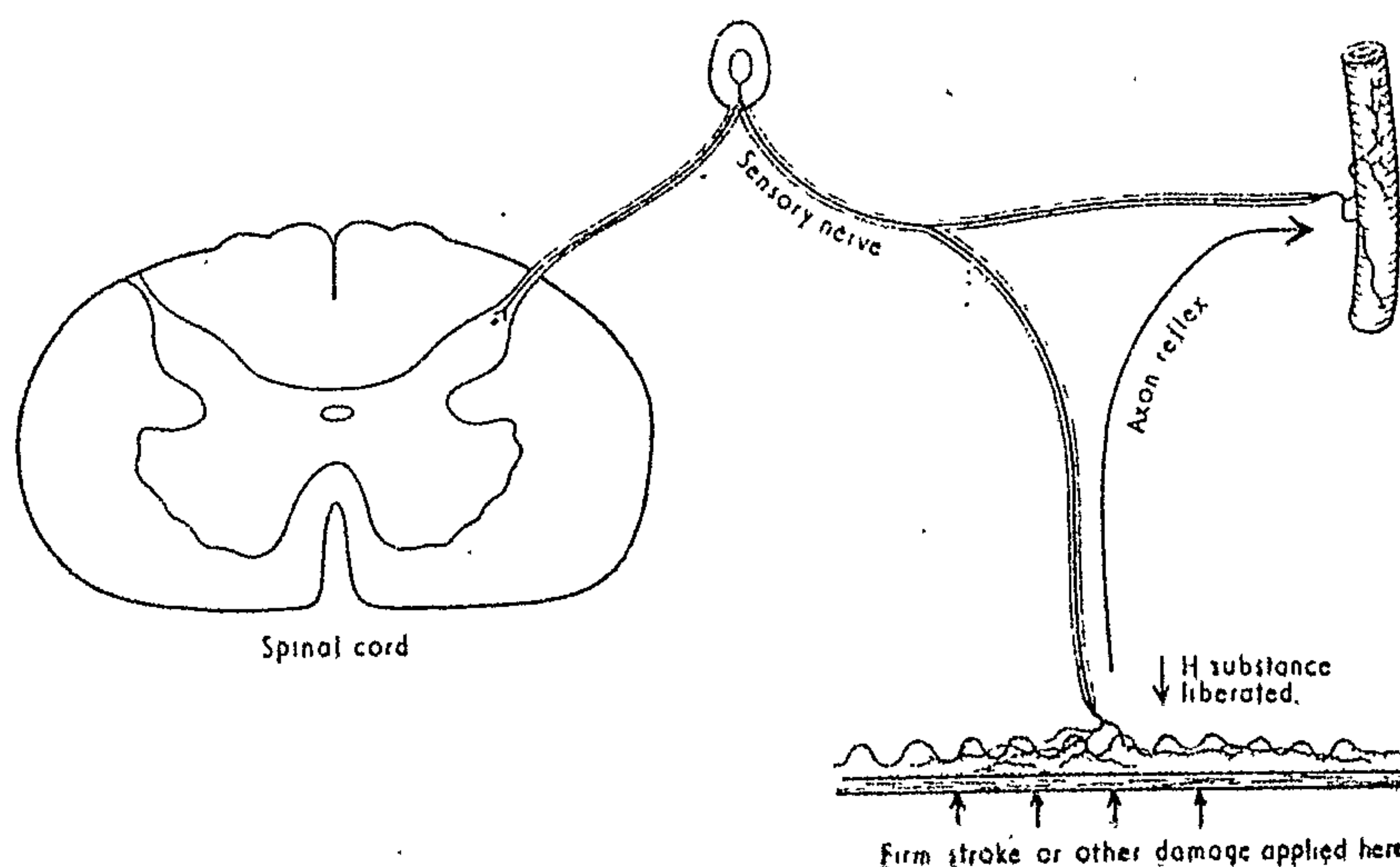


FIGURE 3.2 The mechanism of the axon reflex which dilates neighbouring arterioles when a stimulus is applied to the skin. The nerve impulse passes centrally along the axon to its place of division and then peripherally to the arteriole. This phenomenon occurs even after division of the sensory nerve distal to the spinal root ganglion, provided that the peripheral nerve has not had time to degenerate (from FLOREY 1970a p26).

The duration of this increased flow is variable, depending chiefly upon the severity of the injury; it may last hours or days. As the flow rate begins to retard, the central axial column of cells widens, diminishing the peripheral plasma zone until flow may eventually cease in some vessels.

Such stasis may progress to permanent thrombosis and thus ischaemia of the regional tissue. Normally however, flow gradually resumes. Further discussion of the haemorrheology in inflammation is given in 3.2.1.6.

3.2.1.2 Alterations in permeability.

Also occurring during the period of increased flow, several modifications of the vascular wall are observed. These changes, occurring principally in the post-capillary venules (MAJNO & PALADE 1961), result in an increased permeability of the vessel wall to fluids and large molecules (SIMON 1979).

Loosely speaking, this phenomenon of increased permeability involves two distinct events: i) increased filtration, and ii) increased permeability. "Increased filtration" refers to the enhanced passage of the fluid and small molecules which normally traverse the capillary barrier. This filtration is partly responsible for the initial oedema which is clinically observed in association with inflammation (SIMON 1979). On the other hand, "increased permeability" (by definition), refers to the passage of macromolecules across the vessel wall; under normal conditions, most such molecules have little transendothelial access (SIMON 1979).

These changes are suggested to be due to an intensification of the physiological mechanisms of transendothelial transport see 2.3.2),

or to the institution of completely different mechanisms (FLOREY 1970b). It is noted that the "vasodilatation itself does not (necessarily) cause increased leakage of protein from the dilated vessels". Increased permeability and vasodilatation may occur as separate phenomena (HURLEY 1981).

Changes in vascular permeability were first observed in 1867 by COHNHEIM (RYAN & MAJNO 1977). He however, did not perceive the significance of his observations until later, concentrating instead on leucocyte diapedesis and changes in vessel calibre and flow. It was ARNOLD, using injections of colloidal suspensions of pigment, who shortly later (1876) observed depositions of pigment in the vascular wall, interpreting these as indications of local leakiness (cited by RYAN & MAJNO 1977).

This technique - now termed vascular labelling - has been one of the most significant devices in allowing understanding of vascular leakage. It allows identification, under light or electron microscope, of vessels which have become leaky without being totally disrupted.

In such a vessel, flow still occurs, blood cells floating past without escaping. Protein-rich plasma, however, seeps across the basal lamina which acts (as in normal vessels - 2.3.1.3) as a coarse filter, delaying or blocking passage of larger molecules (especially lipoproteins) and particles. Colloidal materials (such as colloidal carbon black), injected intravenously, will also be retained if of appropriate size (that is, about 30nm diameter). The collection of such tracers at the site of leakage allows microscopic identification of these sites (RYAN & MAJNO 1977; SIMON 1979).

Various patterns of leakage are discussed below and in 3.2.1.5.

3.2.1.3 Direct and indirect vascular injury.

Vascular leakage following local injury can occur by at least two mechanisms: i) directly as an effect of the injurious agent itself (mechanical trauma, heat, irradiation and so on), or ii) indirectly as an effect of chemical substances that appear in and around the site of injury (RYAN & MAJNO 1977).

Using the technique of vascular labelling, it has been shown that direct injury can effect all types of vessels - arterioles, capillaries, venules and lymphatics. Indirect injury, by contrast, demonstrates high specificity for venules.

The question as to how the walls of small blood vessels react after both direct and indirect injury is intriguing. Those mechanisms which are involved in indirect injury continue to be exhaustively investigated and are discussed in 3.3. Those relating to the expression of direct injury have received considerably less attention.

Direct injury is reported to cause separation of the endothelial cells at their junctions. This is accompanied by cell damage in varying degrees (see below). Severe direct injury, such as surgery, may lead to complete destruction and loss of endothelial cells (HURLEY 1972). Generally however, many injuries are reported to cause little visible ultrastructural cell damage other than opening of the junctions (MARCHESI 1970; SMITH et al 1970; HURLEY 1972).

Cellular changes in response to injury.

Several factors are important in determining the degree of cell damage. These include the severity and duration of the injury, and the availability of nutrients. It is important to note that all cell types are not equally susceptible to injury; resting connective tissue cells

such as fibroblasts for example, are generally considered fairly resistant when compared with specialised cells, or those with active or elaborate metabolic functions (WRIGHT 1955).

When a cell is injured, it undergoes several biochemical alterations which may lead to histologically-apparent structural changes if the injury is sufficiently severe and if it does not cause rapid death. These structural changes may become progressive and irreversible (leading to necrosis), or they may resolve (WRIGHT 1955; BOYD 1970).

Since the tissues of mammals possess a very high oxidative metabolism, yet have no capacity for storing oxygen, they are completely dependent upon a continuous circulation of adequately oxygenated blood. Any restriction of this supply inevitably leads to injury to the cells by interfering with their energy-releasing reactions.

The first changes generally involve the mitochondria, leading to decreased production of ATP. Thus the sodium/potassium pump is weakened, allowing water to accumulate within intracellular organelles and the cytoplasm generally; these structures thus become engorged, and later disrupted if damage is sufficiently severe (WRIGHT 1955; BOYD 1970; CAPPELL & ANDERSON 1972). Vacuoles then form within the cytoplasm. In addition to accumulated water, these also enclose lipoids which are absorbed by the cell, but are unable to be digested (WRIGHT 1955).

Up to this point, early changes may be reversible if the agent causing the injury is removed. However, once degeneration has reached a poorly-defined critical level, irreversible alterations progressively develop.

The actual point of cell death is almost impossible to determine since many of the changes by which necrosis is recognised only occur after cell death. After death, cells undergo a process known as autolysis. This results from the degeneration of intracellular enzyme systems, and their indiscriminate action on the cells themselves. Autolysis occurs much more rapidly in tissue which has been removed from the body than in necrotic areas (such as wound edges and infarcts) since plasma contains inhibitors of these enzymes. The breakdown products provide nutrients for the cells still living in the periphery of the area of necrosis (WRIGHT 1955; BOYD 1970; CAPPELL & ANDERSON 1972; HESLOP 1981).

It is significant that 24 to 48 hours following return of the vessel to normal permeability, the only evidence of the preceding injury is a cytoplasm whose ultrastructural appearance suggests "unusually active metabolism" (HURLEY 1981). Thus it is clear that less severe direct injuries do not generally lead to endothelial destruction (HURLEY 1981).

The effects of these changes are discussed in 9.3.

Vascular response to direct injury.

In situations where tissue damage is mild, the vascular response is of the "delayed-prolonged" type (see 3.2.1.5). While this response is characteristically defined as resulting from mild direct rather than indirect injury (RYAN & MAJNO 1977), the factors which lead to such permeability changes have not been elucidated. It is suggested that this form of direct injury induces some disturbance of the cell metabolism which causes cell contraction, or shape alterations with loosening of the intercellular junctions once the disturbance reaches a critical level (HURLEY 1972). Obviously such a metabolic disturbance

must be reversible up to a point, in view of the soon restored vessel integrity (described above). It is commonly preceded by an "immediate-transient" vascular permeability response, which is readily explained by recognition of the damage occurring to mast cells in the vicinity leading to the release of permeability factors (see 3.2.2.5).

The surgical injury.

In the context of surgery, severe direct injury will normally only be present at the incision line, or where tissues are torn through retraction (for example, periosteum). Crush injuries, resulting from the application of haemostat forceps, or soft tissue abrasion, associated with inadequate protection during the use of a surgical bur, could also be considered as severe direct injuries (see also LEAK & BURKE 1973). Leading as they do to local endothelial destruction, such severe direct injuries initiate permeability responses of the "immediate-prolonged" form (3.2.1.5).

Conventional retraction too causes direct injury, though to a lesser degree. This is unlikely to be sufficiently severe to produce marked endothelial cell destruction and is thus likely to produce a delayed permeability response as addressed above.

This distinction between direct and indirect injury assumes clinical significance since the pharmacological means to affect one or the other is unlikely to be the same. However, although this distinction is theoretically simple, it is not always sharp, since some bacteria, for example, (normally described as causing direct injury) may produce some agents which are similar, if not identical, to tissue mediators, and thereby are responsible for development of an indirect response (RYAN & MAJNO 1977).

3.2.1.4 Ultrastructure of vascular leakage.

If it is accepted that the vascular microcirculation can be induced to "leak" either by direct or indirect injury, the sites and mechanisms whereby this occurs must next be considered. Several possible mechanisms for leakage of the endothelial layer have been suggested following studies with tracer molecules. They are briefly outlined as follows. Those mechanisms which have the greatest support are discussed in more detail.

Endothelial destruction.

In some situations, the cells are disrupted or destroyed leaving the basement membrane intact (it being relatively resistant to injury, HURLEY 1981). Filtration and molecular sieving according to size may then proceed across this membrane. Platelets, which adhere to this damaged surface, may cause some interference (usually incomplete) with such molecular passage (RYAN & MAJNO 1977).

Formation of intercellular gaps.

It is now fairly clear that, while there is some variation in their expression (3.2.1.5), all inflammatory stimulæ may produce opening of the intercellular junctions (CASLEY-SMITH 1979a; CASLEY-SMITH & CARTER 1979; HURLEY 1981; SIMON 1979). (See PLATE 3.1). This followed the decisive studies of MAJNO & PALADE (1961) which first demonstrated that the leakage seen in inflammation was associated with open endothelial cell junctions. (See also JOYNER et al 1979).

The mechanism of such opening continues to be hotly debated. Some authors support the idea of endothelial contractility (2.3.6) in response to inflammatory stimuli, as originally demonstrated by MAJNO et al (1969) (BOHM 1977; ADDICKS et al 1979; HURLEY 1981). The ability

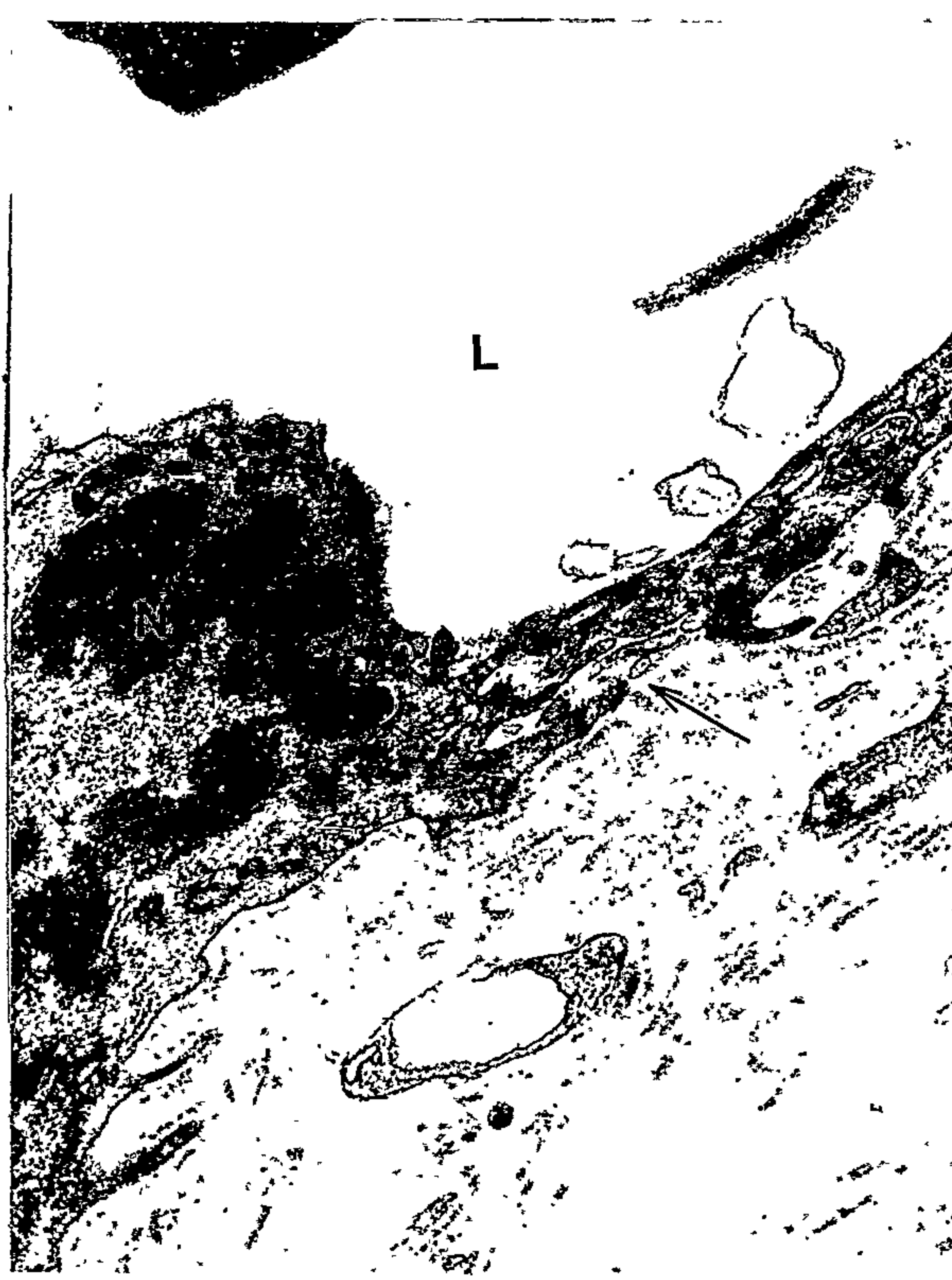


PLATE 3.1 A. An electronmicrograph from human attached gingiva (see PLATE 1.1 for description) showing a small vessel (? venule). The open junction shown (arrow) is possibly evidence of early changes associated with inflammation. However, few studies have been undertaken on gingival vessels, and as tracer molecules were not used in this investigation, it is possible that this appearance may represent a normal variation of oral vessels, perhaps a fenestrated endothelial configuration. (Magnification x39,245).

B and C demonstrate similar features in a larger vessel. Marked interdigitation of junction is noted in C. (Magnification x20,160). (Other symbols as with PLATE 1.1).

of endothelial cells to contract depends upon the presence of contractile protein filaments within the cells. Several different sizes and types of filaments are described within these cells. Some probably relate to a semi-rigid "cyto-skeleton" (RYAN & MAJNO 1977), but recent immunocytochemical studies appear to confirm that others are actually contractile (DRENCKHAHN 1983; HAMMERSEN et al 1983).

The observed focal distribution of leakage is suggested to result from the scattered distribution of cells containing such "contractile" filaments (MAJNO et al 1969; ADDICKS et al 1979). An alternative, perhaps complementary explanation, particularly for the propensity of venular junctions to open, is that such opening of intercellular junctions is facilitated by the lack of junctional complexes in these sites (2.3.1.2) (BOHM 1977; SIMON 1979; HAMMERSEN et al 1983). Intercellular gaps might therefore be produced in venules merely by mild injuries, without any active cell involvement (RYAN & MAJNO 1977).

It should also be noted that recent studies have detected histamine receptors on the luminal endothelial cell membrane, particularly of venules. These receptors appear to be concentrated in regions rich in filaments and close to cell junctions suggesting some active involvement in the opening response (HAMMERSEN et al 1983).

In summary, it must be restated that "the mechanism of gap formation (remains) unknown" (WILLOUGHBY 1973). Nonetheless, it cannot be denied that endothelial cells do separate at their junctions in association with inflammatory stimuli, and current research would suggest some degree of endothelial cell contraction in response to appropriate stimuli. Such a response is generally reversible with the cells coming together again after a varying period, to reform a normal

junction (HURLEY 1981).

Increased transport by endothelial vesicles.

Whilst many authors (MARCHESI 1970; ZWEIFACH 1973a; JOYNER et al 1979; HURLEY 1981) agree with SIMON 1979 that "the main pathway by which large molecules and fluids escape during the acute phase of inflammation is between the endothelial cells", some suggest that the system of vesicles is also highly significant (ARTURSON 1979). BOHM (1977) and KALIN & BRUNE (1979) report the presence of increased numbers of vesicles in the cells of inflamed vessels, and they claim, thereby, a role for such vesicles in the increased vascular permeability of the inflammatory reaction.

These views are qualified by macrophysiological studies which have shown that although injured blood vessels are much more permeable than usual to macromolecules, their permeability to small molecules is only slightly increased (CASLEY-SMITH & WINDOW 1976, cited by CASLEY-SMITH 1979a; CASLEY-SMITH & CARTER 1979). However, although the small vesicles appeared little altered by injury, CASLEY-SMITH & WINDOW (1976) found that many large (approximately 200 nm) vesicles were seen to arise in the injured capillary cells and that such large "vacuoles" persisted long after closure of the open junctions (cited by CASLEY-SMITH 1979a).

These large vacuoles are thus postulated to account for the persistently increased permeability to macromolecules (CASLEY-SMITH 1979a). Their origin is however unknown (CASLEY-SMITH 1979a), and ARTURSON (1979) reports that there is as yet no proof that vacuoles are involved in the transportation of material. The role of vesicles and vacuoles is further questioned since their function under physiological circumstances is far from clear (2.3.1.1).

Note: the relative quantitative importance of these various morphological changes in injury-related increased vascular permeability is not known. It would appear that whilst such features (especially the open junctions) may permit easy access of blood, transendothelial flow is still greatly limited by the low hydraulic conductivity of the interstitial tissue (ARTURSON 1979; CASLEY-SMITH 1979a).

Transcellular leakage.

It is unlikely, in theory, that a living cell would give up its milieu interieur to become freely permeable (RYAN & MAJNO 1977). However, the reported observation of transcellular canals in normal epithelium (SIMIONESCU et al 1975b), raises the possibility of their increased functioning in pathology (through increases in size and/or number) (RYAN & MAJNO 1977) and this may explain apparent leakage. See also 2.3.2.

Other suggested sources of inflammatory exudate.

Firstly, the increased capillary hydrostatic pressure which is subsequent to the arteriolar dilatation seen in inflammation causes raising of the pressure within the microcirculation. This in turn causes increased ultrafiltration across the endothelial surfaces remaining intact (HADDY et al 1976; RYAN & MAJNO 1977). This filtration is related to increased fluid transfer to the interstitium, but it does not account for increased macromolecular transport.

Secondly, it is reported that the normal secretory mechanism of cells may be considerably altered in the presence of toxic agents resulting in secretion of excessive amounts of fluid. Since secretion is a prominent function of connective tissue cells (especially fibroblasts) such increased secretion may be significant where there is injury to this tissue (RYAN & MAJNO 1977).

3.2.1.5 Functional patterns of vascular leakage.

Turning from consideration of the various cellular mechanisms which contribute to the development of increased vessel permeability, we must now look at vascular leakage from a functional or clinical viewpoint as it develops with time. There is now considerable evidence that "the topography of vascular leakage . . . is more complex than the simple pattern of venular labelling caused by histamine-type mediators" (HURLEY 1981). Leakage is seen to occur in capillaries, veins, venules and, less commonly, arterioles. The pattern of vessel leakage varies with the nature of the damaging agent, the time span after injury when examination is performed, the site of tissue injury and the intensity of the stimulus (CASLEY-SMITH 1979a; HURLEY 1981).

Several authors have developed classifications for these patterns. In a pioneering work, COTRAN & MAJNO (1964) described four types, all relating to those patterns seen in skin and skeletal muscle. Their criteria related to the topography of the injury. In an updated review, WILHELM (1973) modified this classification, listing three types based on consideration of the time-sequence of the permeability reactions. This remains the basis for description of patterns of vascular leakage, though some modification of terminology has occurred.

The following summary is primarily a review of Ryan and Majno's discussion and it uses terminology as proposed by these researchers as based on Wilhem's work (RYAN & MAJNO 1977). The patterns of leakage are illustrated in FIGURE 3.3.

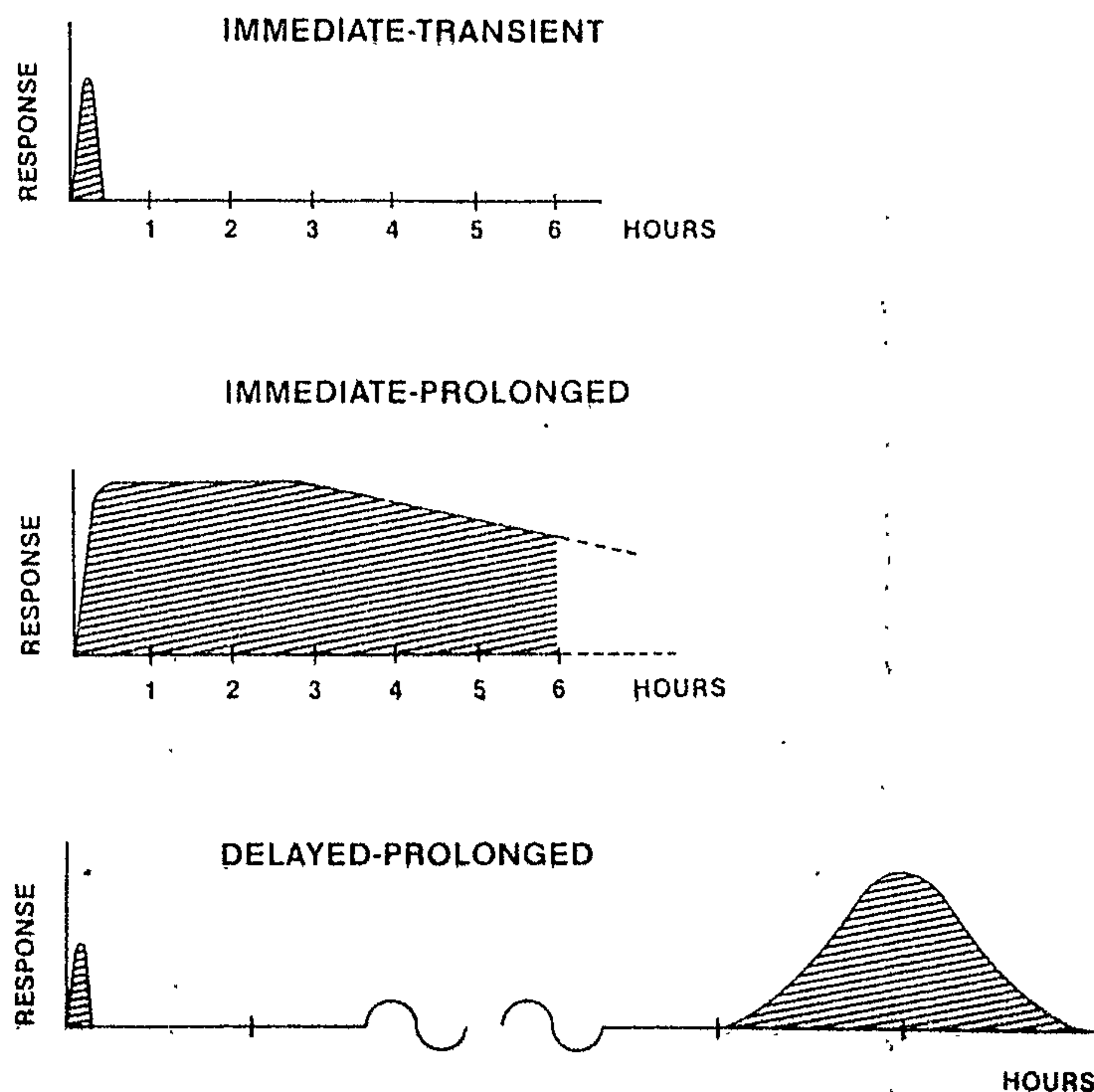


FIGURE 3.3 Types of vascular leakage that may occur in a focus of injury classified according to their course in time. The delayed-prolonged variety (bottom) is often preceded by a short burst of leakage of the immediate-transient variety (from RYAN & MAJNO 1977, p197).

Immediate-transient leakage.

Immediate-transient leakage is a short-lived burst of leakage commencing immediately after exposure to the injurious agents. Its average duration is approximately 15 - 30 minutes. Where the damage is slight, it will occur within the traumatised area (as seen in the wheal of the triple response described in 3.3). Where tissue destruction has occurred, this response occurs around the area of damage. Structurally, it is characterised by specific small venule involvement (up to 100 μ in size, and therefore not surrounded by smooth muscle).

This response is mediated by chemical substances released from injured tissues; it can be reproduced by the local injection of histamine-type mediators (histamine, serotonin, bradykinin). This pattern is most frequently seen in mild kinds of injury.

Immediate-prolonged leakage.

Immediate-prolonged leakage is typically the result of severe direct injury. It commences immediately and continues until the damaged vessels are repaired or plugged (even up to 48 hours). This type of leakage is commonly seen in the case of severe burns (RYAN & MAJNO 1977) and it is also described in association with surgical incisions (WILHELM 1973). As severe direct injury is involved, all vessels in the area are affected. Mediated leakage is again apparent in the venules adjacent to the area of injury.

Delayed-prolonged leakage.

Several features of the delayed - prolonged leakage are well described - namely the functional events and the stimuli eliciting this type of response. However, its pathogenesis remains the subject of controversy. Two main patterns are described by WILHELM (1973) : either beginning 1/2 - 1 hour after injury and reaching a peak in about 4 hours, or beginning 2 - 10 hours after injury, and reaching a peak at 12 - 24 hours, though occasionally as late as 48 hours or more. Commonly, leakage patterns of this type are preceded by an initial short burst of permeability (as in immediate-transient). The response is then termed "biphasic" (BOHM 1977). Injurious agents known to cause this form of response include thermal injury, X-ray injury, some bacterial products and a variety of chemicals (HURLEY 1981).

Current opinion is that the delayed-prolonged response is, to a large extent, a special type of direct injury or more specifically, a result of mild direct injury in which both capillaries and venules are thus frequently involved depending upon the areas receiving injury. The severity of the stimulus is also critical since increased severity is seen to shorten the delay of onset until the response becomes

monophasic and thus identical to the immediate-prolonged response (RYAN & MAJNO 1977).

There is still some doubt as to whether mediators (either long-acting or continuously released short-acting agents) are involved (RYAN & MAJNO 1977).

Note: these are only patterns developed for clarification of understanding. In clinical practice, combinations and variations of these are also seen. Further, since variations of vascular responses have also been observed in vessels of the same type, one should be aware of the limitations of describing "standard inflammatory responses" (BOHM 1977).

3.2.1.6 Rheology in inflammation.

When considered from a rheologic standpoint, three phases may be identified in inflammation, although considerable variation may again be observed.

There is firstly a high flow phase with vasodilatation and increased volume flow up to 10 times that of normal vessels (WELLS 1973; HURLEY 1981). Blood viscosity and red cell aggregation are minimised during this phase (WELLS 1973). This is followed by the second, a low flow phase.

This phase commences with the leakage of plasma from the vessels. It is likely this leakage involves albumin more than the larger plasma protein molecules such as fibrinogen and globulin. This results in relatively increased concentration of these components within the vasculature. Hence, blood viscosity and red cell aggregation increase (WELLS 1973). More importantly, the simple loss of plasma

itself results in haemoconcentration with raised haematocrit and viscosity (WELLS 1973; HURLEY 1981).

Together, these factors cause resistance to outflow from the capillaries to reach critical levels. With the persistence of high inflow, marked leakage and subsequent stasis soon follow (WELLS 1973). In these circumstances, shunting of some flow via collateral vessels aids in maintaining tissue perfusion at the border of the area of inflammation. Shunting however, never completely corrects the problem and stasis develops (WELLS 1973).

Most inflammatory lesions however only infrequently reach the static phase for any length of time (HURLEY 1981). Stasis has long been regarded as a major cause of intravascular thrombosis (RODMAN 1973). However, RODMAN (1973) and HURLEY (1981) state that, in most cases, flow will gradually resume, unless it is stopped for very long periods. The presence of activated coagulation factors (particularly Factors IX and XI), in association with stasis is likely to trigger thrombus formation (RODMAN 1973). Erythrocyte petechiae are also common when stasis is complete (WELLS 1973).

Such alterations in flow rate are critical in two additional areas. Firstly, they are, to a large extent, critical in determining the amount of oedema which develops. If, for example, local blood flow is restricted or temporarily stopped, exudate will be reduced or non-existent. Studies on hypovolemic rabbits in a state of shock (defined as a state of inadequate tissue perfusion) demonstrated grossly abnormal permeability reactions to histamine-type substances; accompanying oedema was markedly diminished (see RYAN & MAJNO 1977).

Secondly, it appears that a certain level of flow and hydraulic pressure is necessary for leucocyte diapedesis. As described by Cohnheim, emigration ceases when flow becomes static (HURLEY 1981).

These phases are outlined diagrammatically in FIGURE 3.4. The reader is referred to section 2.6 for discussion of the principles of rheology in the normal circulation.

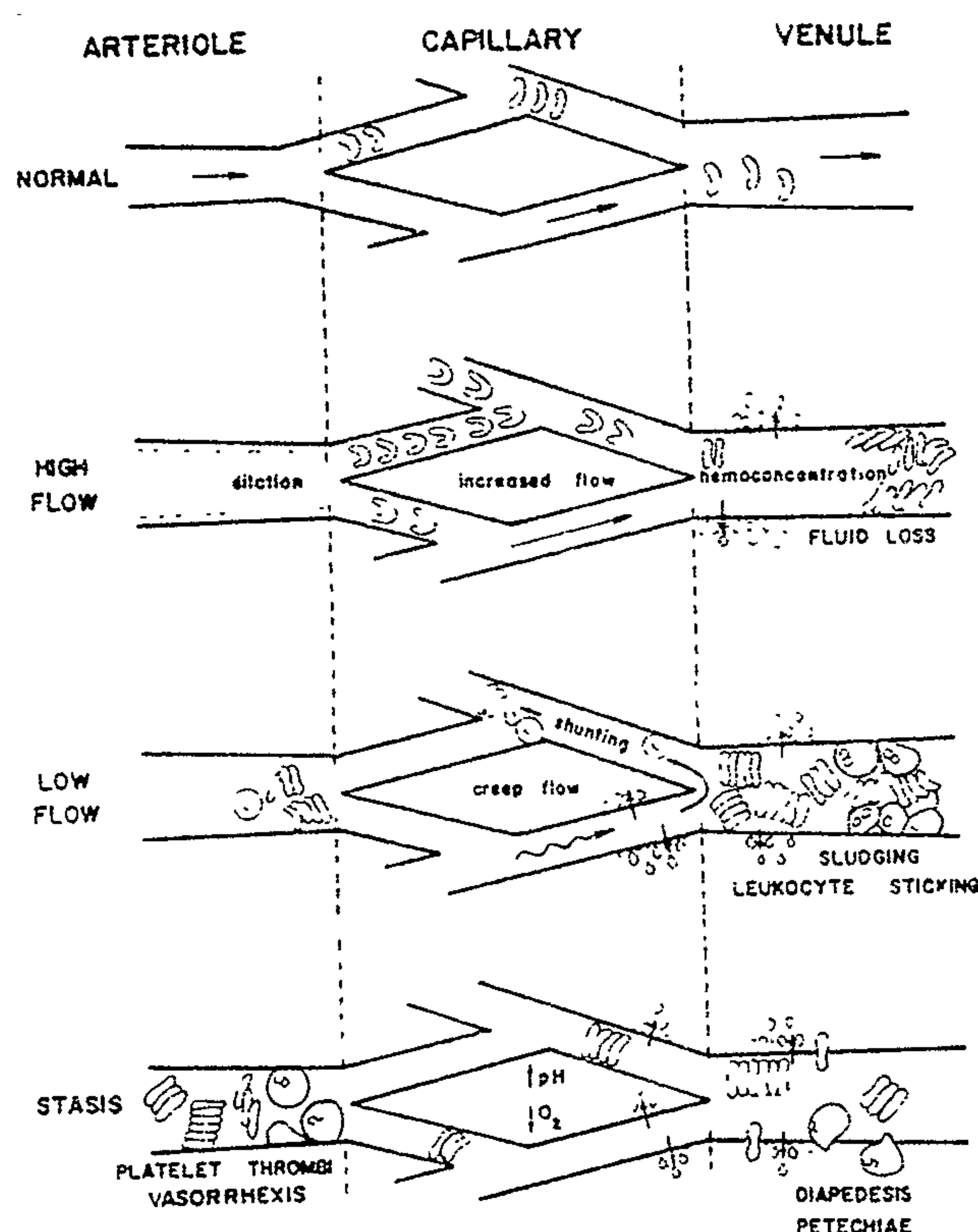


FIGURE 3.4 The rheologic phases of the inflammatory process (from WELLS 1973, p156).

3.2.1.7 Lymphatics in acute inflammation.

It has been becoming increasingly obvious that the initial lymphatics represent an important aspect of exchange between the tissue and vascular components, both in normal function and in inflammation. Cohnheim was also aware of the lymphatics, conducting experiments to discover whether swelling was due to transudation of fluid or to blocking of lymphatic flow. He discovered that there was no obstruction

of lymphatic flow; rather, such flow was considerably increased (EBERT & GRANT 1973).

The mechanisms by which initial lymphatics could actually transport fluid in conditions of raised tissue pressure remained a mystery for some time. Since they appeared to be little more than endothelial tubes, it might be supposed that they would collapse when tissue pressure increased. However, PULLINGER and FLOREY (1935) demonstrated that under oedematous conditions, lymphatics became dilated.

These investigators observed that fibres, continuous with the surrounding connective tissue, were attached to the abluminal surface of the lymphatic vessels. The attachment was such that the greater the interstitial swelling, the stronger the pull of these fibres on the vessel walls, and the greater the distension of the vessel (EBERT & GRANT 1973).

Ultrastructural studies (described in 2.5.1) have demonstrated that dilatation is further enabled by the absence of "tight" junctions in these vessels and the presence of considerable overlap of the endothelial cells at their junction. The expansion of these vessels with the subsequent opening of the intercellular junctions provides an uninterrupted passage for the free movement of fluids, particles and cells into the lymphatic lumen (LEAK & BURKE 1973).

Such lymphatic drainage occurs "by processes which are merely an accelerated form of normal lymphatic function". Oedema is only seen to result from inflammatory processes if the rate of exudate formation exceeds the capacity of the lymphatics to remove such fluid from the tissues (HURLEY 1981). See Chapter 4.

Since it is the removal of the plasma proteins from the tissues for which the lymphatics are chiefly responsible, failure of the lymphatics to remove all this protein rapidly leads to the accumulation of oedema (CASLEY-SMITH 1973). This is the basis of "primary" lymphatic failure. Various types of lymphatic failure and consequent oedema are described. These classifications are dependent upon the protein content of the oedema and the rate of fluid flow (CASLEY-SMITH 1973; FOLDI & CASLEY-SMITH 1978) and are outlined in Chapter 4.

3.2.2 Cellular Events.

A primary pathognomonic histological feature of inflammation is the associated leucocytic infiltration. Initially, and particularly in association with bacterial contamination, the polymorphonuclear leucocyte predominates. Normally within 48 hours, most of the polymorphs disappear and the cellular infiltrate is transformed almost entirely to an accumulation of mononuclear phagocytes (RYAN & MAJNO 1977; HURLEY 1981).

Studies of living transparent tissues have demonstrated three stages in the behaviour of leucocytes in early inflammation - pavementing, emigration through the vascular wall and their subsequent movement in the extravascular tissues (HURLEY 1981).

Following consideration of these stages, attention is turned to the various cells which are involved; their origins and their functions in inflammation. Various defects of leucocyte function may exert significant effects on the inflammatory process. These defects are covered briefly in Chapter 5; they are well reviewed by RYAN & MAJNO (1977).

3.2.2.1 Leucocyte sticking.

Concurrent with the early stages of flow retardation and well prior to stasis, leucocytes appear in the marginal "plasmatic zone" (COHNHEIM 1882), on occasions momentarily sticking to the endothelium. With injuries of moderate or greater intensity, progressively more and more leucocytes leave the the central stream and begin adhering to the wall for increasing periods (see PLATE 3.2) until the luminal surface of the venules in the inflamed area becomes covered with a layer of adherent leucocytes (described as "pavementing" by Cohnheim).

Such pavementing or sticking of leucocytes against the vessel wall can only be studied in living tissues and many aspects remain obscure (EBERT & GRANT 1973). It is reported to occur chiefly in venules and small veins (HURLEY 1972; RYAN & MAJNO 1977) though this response may occur in any injured vessel if the injury is sufficiently severe (MARCHESI 1970).

Recent research has indicated that leucocyte sticking is dependent upon some change in the vascular wall and not upon alteration of the adhering leucocytes (HURLEY 1981). Electron micrographs demonstrated that such adhering leucocytes were indeed in close apposition to the endothelial cells (less than 10nm). However, such studies revealed "no distinctive ultrastructural features of the surfaces of the endothelial cells or the adherent leucocytes which could explain, or even suggest, the mechanism by which the adhesion occurred" (MARCHESI 1970).

Some suggested that sticking was always in association with an endothelial gap. However, although it is certainly apparent that such endothelial gaps may trap all kinds of cells, the phenomenon of

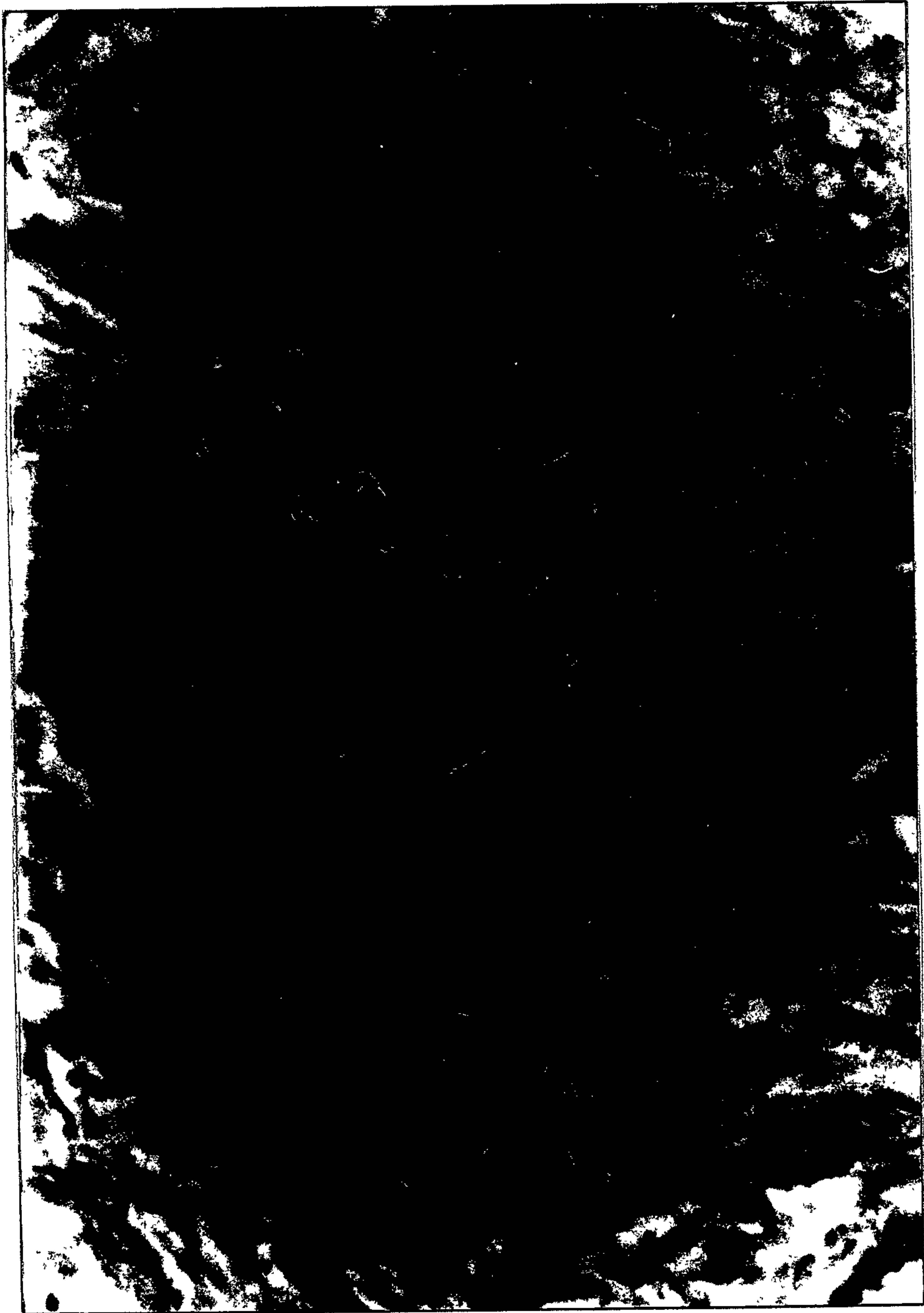


PLATE 3.2 This plate shows the appearance, under light microscope (H & E stain), of submucosal fibrous tissue from human attached gingiva (specimen taken from similar site and at similar time as PLATE 1.1). Early "pavementation" (3.2.2.1) of neutrophil polymorphs is seen, presumably in venules (transverse and longitudinal sections). (Magnification x150).

sticking appears to involve totally different effects (RYAN & MAJNO 1977).

It is also suggested that leucocyte adhesion involved the endothelial "coat" of CHAMBERS & ZWEIFACH (1940) - the glycocalyx of modern literature (see 2.3.2). It is suggested that this glycocalyx is responsible for electrophysiological interactions of the membrane (BARNHART & BAECHLER 1978) and though no morphological alterations can be identified in this structure during inflammation (SIMON 1979), it is unlikely that any alteration in "stickiness" of this layer would be microscopically apparent (RYAN & MAJNO 1977). Current investigations appear to support the involvement of this structure in endothelial-leucocyte interactions (U. RYAN 1983).

Other work has also pointed to local molecular changes to explain the mechanisms whereby sticking occurs - perhaps some calcium bridging between endothelium and leucocyte. This is supported by studies which demonstrated that chelation with EDTA in vivo prevented pavementing in the rabbit ear chamber, in the hamster cheek pouch and in mouse mesentery (see RYAN & MAJNO 1977).

SIMON (1979) states that the suggested involvement of divalent cations (such as calcium) in leucocyte adherence is the "only acceptable theory today". Such cations are suggested to act via their masking of the normally negative surface charge on cell membranes (ZWEIFACH 1973a). At this stage, however, all that can be said is that "divalent cations may be somehow implicated" (RYAN & MAJNO 1977).

Placing these two aspects of research together, it would seem plausible that electrophysiological changes in the endothelial glycocalyx (probably involving divalent cations) allow adhesion between

the leucocyte and endothelium at the site of inflammation. (See also GORDON & PEARSON 1980).

3.2.2.2 Leucocyte emigration.

As surmised by ARNOLD (1875, cited by RYAN & MAJNO 1977) and COHNHEIM (1882), leucocytes have been seen via electron microscope studies to migrate across the vessel wall via the interendothelial junctions (RYAN & MAJNO 1977). Serial section micrographs demonstrate polymorphs, monocytes, and eosinophils "dissecting" their way (perhaps by enzymatic means - SIMON 1979) down the junctions. These junctions would appear to re-form seemingly unaltered behind the escaping cell (HURLEY 1981). The adjacent endothelial cells also appear to actively participate in this migration (HURLEY 1972).

It might be anticipated that open endothelial junctions might facilitate leucocyte emigration, and that increased vascular permeability would follow cell movement. However, HURLEY (1972) and others have shown that increased vascular permeability and leucocyte emigration are two separate phenomena. Thus emigration does not necessarily induce vascular leakage (BOHM 1977; RYAN & MAJNO 1977) and conversely, histamine-induced increased vascular permeability (easily demonstrated with colloidal carbon) does not enhance leucocyte diapedesis (BOHM 1977).

These two phenomena however, frequently operate concurrently. In fact, in some experimental models they appear to be dependent one upon the other (ISSEKUTZ 1981; WEDMORE & WILLIAMS 1981; ISSEKUTZ & MOVAT 1982; WILLIAMS et al 1983). These different observations might be explained by considering the state of activation of the PMN; highly activated cells might discharge lysosomes indiscriminately, thus

temporarily damaging the cell junction (ARFORS 1983). The oxygen radicals also released by such lysosomal discharge have been shown to produce both reversible and irreversible endothelial alterations, and it is likely that they are involved in these changes (BJORK et al 1980).

Under these circumstances where leucocyte emigration and increased permeability are associated, some suggest that the basement membrane exerts a significant barrier function with more cells retained by this membrane than are migrating between endothelial cells (HURLEY 1972; 1981; ZWEIFACH 1973b). However, these observations are disputed (SIMON 1979) and while it may appear reasonable to attribute some temporary barrier function to the basement membrane, there is no consensus on this point and the mechanisms whereby cells and macromolecules penetrate it have not been elucidated (see BOHM 1977).

3.2.2.3 Time course of leucocytic infiltration.

In the generally described picture of leucocyte emigration, as exemplified in studies using relatively mild irritants, maximum neutrophil accumulation is seen after approximately 4 hours, following which their migration rapidly declines (RYAN & MAJNO 1977). By contrast, no rise in the total number of mononuclear cells is seen until after 4 hours; a substantial peak is seen only after 18-24 hours (RYAN & MAJNO 1977).

This picture (particularly as related to neutrophils) however, appears to be dependent upon the inflammatory stimulus. Significant bacterial contamination, for example, leads to massive neutrophil infiltration with delayed monocyte infiltration. Thus, in general, it appears that the emigration of mononuclears into the exudate commences

as neutrophil emigration declines, and continues for a much longer period (RYAN & MAJNO 1977).

Early reports (SPECTOR 1969) ascribed these differences merely to the longevity of monocytes when compared with neutrophils in the wound (see 3.2.2.5) or to the relative rates of accumulation (DALE & WOLFF 1971). Thus although both cells were believed to emigrate together, monocytes were initially "overwhelmed" by the numbers of neutrophils present in the early response, but which soon (within 48 hours) disappeared "thus exposing the less numerous but more long-lived monocytes" (RYAN & MAJNO 1977).

More current thought however, suggests that the different migratory responses may partly be a result of variations in chemotactic responsiveness between the two cells (RYAN & MAJNO 1977). However, the lower motility of monocytes and their lower numbers, as well as the short life-span of neutrophils in the wound are probably also contributory (HURLEY 1981).

It is interesting that neutrophils themselves are reported to elaborate substances which both attract and inhibit monocyte migration (DALE & WOLFF 1971; RYAN & MAJNO 1977). The literature however, contains conflicting reports regarding the accumulation of monocytes in the wounds of patients with neutropenia: both a normal response (DALE & WOLFF 1971) and a depleted response (BAMBARA et al 1979) are reported. It can only be assumed that any neutrophil-derived factors are not essential mediators for the monocyte response in vivo (DALE & WOLFF 1971).

3.2.2.4 Chemotaxis.

The movement of living cells under the influence of chemical substances was first described by Stahl (1884) (cited by HURLEY 1981). Such chemotaxis ("the attraction of cells towards chemical substances" - RYAN & MAJNO 1977), is generally believed to be responsible for the attraction of circulating leucocytes to the site of injury.

The mediation of this leucocyte emigration is uncertain. The factors responsible for it may be derived from injured tissues or from neutrophils stimulated by a serum substrate. It is suggested that these chemotactic factors may leak out of the injured tissue and into the interendothelial junctions. This however, remains speculation (RYAN & MAJNO 1977).

Whatever the mechanisms, it is clear that "inflammatory cells can migrate unidirectionally along a concentration gradient of an attractant substance". This migration is characteristic of the normal inflammatory response and its disruption consequently leads to disruption of healing (DIEGELMANN et al 1981).

As was pointed out by HARRIS (1953; 1954), initial investigations into identification of chemotactic factors were very vague, particularly since they often failed to distinguish cellular accumulations due to true chemotactic attraction from those which were merely a result of the trapping of randomly moving leucocytes. Harris therefore devised a system which allowed direct observation and continuous photographic recording of leucocyte locomotion. The cells were incorporated in a film of clotted plasma between a slide and coverslip. Dark-field microscopy and long photographic exposures were used to demonstrate the paths traced by the cells (seen as white tracks

on a dark background) (RYAN & MAJNO 1977).

Harris's photographic trace method was supplemented in 1962 by the development of a millipore assay system by BOYDEN (cited by RYAN & MAJNO 1977). Boyden's system consists essentially of two compartments separated by a horizontal filter membrane. Leucocytes are placed in the upper compartment and the solution under test in the lower. The number of leucocytes found in the lower compartment after a given time is a measure of the chemotactic activity of the test fluid (RYAN & MAJNO 1977).

This system has the advantage of being able to assess the chemotactic activity of substances in solution. Under most circumstances however, it is difficult or impossible to distinguish between stimulated random locomotion and true chemotaxis in the Millipore (Boyden) system. It is therefore suggested that such indirect techniques should be confirmed by direct microscopic observation using the slide-coverslip technique as described by Harris (RYAN & MAJNO 1977).

Discussion of the various proposed chemotactic factors (including bacteria and complement fragments) is included in discussion of inflammatory mediators (3.3).

3.2.2.5 Origin and function of cells seen in acute inflammation.

Polymorphonuclear leucocytes.

(Synonyms: polymorph, neutrophil, granulocyte, PMN leucocyte, PMN).

Polymorphs are end cells of the myeloid series which arise from a specific precursor in the bone marrow (VAN FURTH & WILLEMZE 1979; HURLEY 1981). Whilst immature, neutrophils synthesize and store a variety of substances (see below). Once mature, however, they lose this

capacity for synthesis (VAN FURTH & WILLEMZE 1979). Once released into the circulation, their lifespan is only seven to ten days, and probably less when in an area of inflammation (HURLEY 1981).

In the tissues, PMNs are amoeboid and actively phagocytic. They participate in the inflammatory reaction in two ways:

Firstly, either by active secretion, or following cell death and disintegration (see 3.2.2.6); neutrophils may discharge enzymes and other active substances into surrounding tissues. Such enzymes include lysosomal enzymes, collagenase, elastase, fibrinolysins, myeloperoxidase and tissue damaging proteins (notably cationic proteases and acid hydrolases) (see 3.3.2.3).

Thus, polymorphs accelerate autolysis of necrotic tissues and breakdown of extravascular fibrin. This is perhaps their most significant function, especially in sterile injuries, although they also demonstrate phagocytic activity (ROSS & ODLAND 1969) (see below).

Polymorphs may also release pyrogens, permeability increasing factors and factors chemotactic for additional leucocytes (HURLEY 1981). (For a detailed discussion of lysosomal enzymes see 3.3.2.3).

The second function of polymorphs in inflammation - phagocytosis - was first described by METCHNIKOFF (1891). He regarded this phenomenon as central to the inflammatory response stating that "inflammation generally must be regarded as a phagocytic reaction on the part of the organism against irritants" (page 189). The work of LEWIS (1931) was also significant in this field.

Phagocytosis is "the ingestion and disposal of foreign or effete particulate matter" (RYAN & MAJNO 1977). It can be subdivided into the

following phases: a) attachment of particles to the cell surface (called opsonisation, this involves both contact and recognition), b) ingestion of particles by the cell (including formation of the phagocytic vacuole (phagosome) and degranulation), and c) breakdown of particles within the cell (including microbiocidal effects) (RYAN & MAJNO 1977).

A lucid discussion of the mechanics of phagocytosis is given by RYAN & MAJNO (1977) and VAN FURTH (1977). It is noted that polymorphs are generally not considered to be able to ingest whole cells (VAN FURTH & WILLEMZE 1979).

Destruction of the phagosomal contents will normally occur upon release of the lysosomal enzymes into the phagosome. Such intracellular kill is aided by the formation of oxygen radicals (both by NADPH-oxidase and as by-products of arachidonic acid metabolism) and particularly of a complex consisting of the enzyme, myeloperoxidase (MPO), hydrogen peroxide and iodine (NAIDORF 1977; FANTOME & WARD 1982). This MPO system is however, somewhat weakened in the anaerobic environment of wounds due to diminished production of hydrogen peroxide and other oxygen radicals (NAIDORF 1977; SILVER 1982). Mild hypoxia is however, reported to enhance its functioning (HOHN et al 1976). The role of oxygen-derived free radicals in phagocytosis is extensively discussed by FANTOME & WARD (1982) and ARFORS (1983). It has recently been proposed that these radicals are responsible for some of the increase in microvascular permeability seen during inflammation (BJORK et al 1980).

Once extensive phagocytosis has occurred, the PMN phagocyte is unable to ingest further particles, suggesting that phagocytic receptor sites may be lost from the surface, perhaps being internalised within

the phagocytic vacuoles (RYAN & MAJNO 1977).

Because most neutrophils migrating into an area of injury disintegrate in that area, releasing their enzymal contents, the number of these cells present may significantly alter the degree of cell-mediated tissue lysis which occurs. In the presence of large numbers of PMN's, marked lysis may occur leading to permanent tissue destruction. There would therefore appear to be an "optimal" PMN infiltration above which cell-mediated tissue damage may add to the surgical insult. Crush injuries, for example from the application of haemostats, are suggested to produce marked infiltration (HURLEY 1983). There is little information regarding the size of the PMN infiltrate in response to other aspects of the surgical insult.

Macrophages.

Mononuclear phagocytes are first identified in the bone marrow as promonocytes (RYAN & MAJNO 1977). They enter the blood as monocytes and eventually become tissue macrophages. In inflammation, monocytes enter the tissues where they rapidly enlarge and mature to form typical inflammatory macrophages. As such, these cells have a potential lifespan of months (perhaps longer) and they retain their capacity for mitotic division and most of their synthetic capabilities (VAN FURTH & WILLEMZE 1979; HURLEY 1981).

Their chief function is phagocytosis which occurs in a similar way to polymorph phagocytosis. Unlike polymorphs, these cells are able to ingest effete erythrocytes and other cells, tissue debris and masses of fibrin. It is in this scavenging action that macrophages are prominent in areas of resolving inflammation (HURLEY 1981). (See also 1.5.1.1).

Macrophages also have a secretory function which is similar to that of neutrophils; however, due to their greater longevity, secretory activity in macrophages is relatively more significant (HURLEY 1981).

Other important functions include roles in immune responses, macrophages being the effector cells in cellular immunity. Macrophages may also be transformed into epithelioid or foreign body giant cells. As such they are a prominent feature of chronic inflammation (HURLEY 1981). Recent studies have also demonstrated these cells to have significant roles in fibrogenesis and neovascularisation in wounds (CLARK et al 1976), as described in 1.5.1.1.

The functional potential of these cells is apparently closely linked to their stage of differentiation and activation. Macrophage activation follows contact with products of activated lymphocytes (lymphokines), immune complexes and the complement cleavage product C3b. It results in a series of specialised responses including enhanced lysosomal enzyme activity, increased secretion of proteinases (including collagenase, plasminogen activator and elastase), release of complement components, formation of tissue thromboplastin, and the synthesis of prostaglandins. Activated macrophages also appear to contribute towards much of the negative regulation of cell proliferation, perhaps as a result of their synthesis of prostaglandins (DIEGELMANN et al 1981).

Current research is thus consolidating the central role of these cells in multiple aspects of the tissue response to injury, and the related fields of neoplasia and the immune response (DIEGELMANN et al 1981).

Platelets. (discussed in detail in 1.3)

Platelets have been known for some time to play a central role in haemostasis. More recently, it has become apparent that they also elaborate mediators of inflammation. Some suggest that platelets may actually initiate inflammation since in some experimental situations, polymorphs only appear following the aggregation and degranulation of platelets (SIMON 1979).

Studies of comparative pathophysiology have led some to suggest that the platelet is in fact an inflammatory cell. This concept carries some weight since these cell fragments perform similar functions to neutrophils (including phagocytosis) and they release granules which are similar to PMN lysosomes in both content and function. Their accumulation in the area of injury is also similar to leucocyte chemotaxis, although it probably occurs in response to different mediators. Platelets are also described as interacting with bacteria, viruses and antigen-antibody complexes (SIMON 1979; SMITH & BOLAM 1979).

Though such comments may only be speculation, modern research has emphasised that platelets are much more significant in repair of injury than merely to effect primary haemostasis. However, while not denying the above observations, the conclusions drawn do not have unanimous support. In fact, one series of investigations appears to demonstrate that platelets are actually anti-inflammatory in function, perhaps through their formation of microthrombi which limit vascular flow. In these studies, thrombocytopenic animals demonstrated enhanced responses in some experimental models of acute inflammation in which complement activation is significant (SMITH & BOLAM 1979). Thus significant discrepancies are evident amongst researchers.

In any case, these findings emphasise that primary haemostasis must be considered to be the initial phase of inflammation and repair rather than an isolated series of reactions (NACHMAN & POLLEY 1978).

Lymphocytes.

Lymphocytes are described as the effector cells of the immune system. In this role they are not phagocytic or pinocytic, do not adhere to foreign surfaces and do not respond to chemotactic stimuli (HURLEY 1981). They also do not contain peroxidase or lysozyme (VAN FURTH & WILLEMZE 1979) and are thus not directly concerned with the destruction of microorganisms or effete/ necrotic tissue.

After sensitised, thymus-derived ("T-") lymphocytes contact the sensitising antigen, they release soluble substances - lymphokines - which are thought to be significant in vascular permeability alterations and possibly in lymphocyte chemotaxis. (see 3.3.2). Sensitised bone-marrow ("B-") lymphocytes on the other hand transform into plasma cells which provide the major source of humoral antibodies.

In these roles, lymphocytes are not significantly involved in acute non-immune inflammation. However, research in the late 1960's demonstrated that heterologous anti-lymphocyte sera have both immunosuppressive and anti-inflammatory properties, and that the anti-inflammatory components could be separated from the immunosuppressive component (GARCIA-LEME et al 1976). Anti-lymphocyte sera are also reported to curtail acute inflammatory reactions provoked by thermal and chemical injury, though these sera were not seen to antagonise any of the substances which are considered to be the pharmacological mediators of the acute inflammatory response. (See GARCIA-LEME et al 1976; 1977).

These authors went further, suggesting that lymphocytes might play a direct role in the development of non-immunological acute inflammation. This conclusion followed observations that the intact lymphocyte (and also products of its lysis) either partially reversed previously inhibited inflammatory reactions or potentiated responses to mild irritant chemical stimuli in leucopenic rats. Granulocytes however, were ineffective in either situation.

These researchers therefore proposed that a pro-inflammatory principle (which they termed "lymphocyte pro-inflammatory factor" or LpIF) is present within lymphocytes and that it may be involved in modulation of the inflammatory response. This concept is supported by the discovery that there is good correlation between the intensity of the oedema developing in leucopenic rats and the number of circulating leucocytes present (or more particularly, the number of circulating lymphocytes).

More recent investigations (GARCIA-LEME 1981a) raise the possibility of an additional subclass of lymphoid cells (termed "non-B") which are capable of producing LpIF and are thus associated with the development of non-immune inflammation. This hypothesis was developed following the discovery that a variety of steroidal and non-steroidal anti-inflammatory drugs blocked this response.

The clinical significance of these discoveries remains unclear. It is to be noted that most research in this field has been performed on rats in which the percentage proportion of neutrophils and lymphocytes is roughly reversed when compared with human peripheral blood (that is, approximately 22% neutrophils and 73% lymphocytes in rat peripheral blood). It is however, also reported (GARCIA-LEME et al 1977) that filtrates of lysed lymphocytes from dogs are also active in

correcting inhibited inflammatory responses in leucopenic rats.

Mast cells.

The presence of mast cells within the connective tissue has been described for several years. These cells are normally located in close vicinity to blood vessels (SIMON 1979) where they reappear soon after vessel regeneration in the healing wound (NEWCOMBE 1972).

These cells are characterised by their large, metachromatically-staining cytoplasmic granules which contain heparin, histamine, 5-hydroxytryptamine (5-HT) (NEWCOMBE 1972). They are said to be amongst the most fragile cells in mammals. Thus, minimal injurious stimuli may induce granule discharge, though the degree of release depends upon the type and intensity of the injury. The release of histamine and 5-HT from such cells is one of the mechanisms leading to increased venular permeability in the initial inflammatory response (SIMON 1979).

Fibroblasts.

These cells are discussed in section 1.5.1.1.

3.2.2.6 Mechanisms of lysosomal enzyme release.

First described in rat liver, lysosomes are a group of subcellular organelles containing various hydrolytic enzymes, predominately with an acid pH optima, bound in latent form within a relatively impermeable membrane (GOLDSTEIN 1977). The release of these substances is suggested to be significant in tissue and cell lysis; the mechanisms of their release are discussed here whilst their postulated functions are discussed in 3.3.2.3 in association with other chemical mediators of inflammation. The following précis is adapted from GOLDSTEIN (1977). See also WEISSMAN et al (1971).

Firstly, following exposure to a variety of toxins, polymorph plasma membranes may be disrupted, releasing all intracellular materials - "cell death". In these circumstances, cytoplasmic enzymes, potassium and cellular constituents along with lysosomal hydrolases are released into the tissues.

In other situations, materials may gain access to the interior of the cells' vacuolar system wherein they cause rupture of the lysosomal membranes from within, allowing their contents firstly to disrupt the cell plasma membranes and subsequently pass into the surrounding tissues - the "suicide sac" hypothesis. This circumstance arises when crystalline substances such as silica are ingested by phagocytes.

A third mechanism - termed "regurgitation during feeding" - involves the release of lysosomal hydrolases into the surrounding tissues by intact cells during endocytosis. There is no accompanying leakage of cytoplasmic enzymes. This mechanism is postulated to occur when lysosomes discharge their contents into incompletely closed phagosomes. The cell engaging in phagocytosis remains viable - its released lysosomal contents are free to act on surrounding tissues.

The final mechanism postulated for lysosomal enzyme release is termed "reverse endocytosis" or "frustrated phagocytosis". This is seen to occur where polymorphs encounter immune complexes deposited on solid surfaces such as collagen fibres and membranes. The cells then adhere to these surfaces and selectively release their lysosomal contents.

Study of reverse endocytosis has been facilitated by the use of cytochalasin B which interferes with polymorph cytoplasmic filaments, preventing normal phagocytosis. These polymorphs nevertheless release

(or secrete) lysosomal enzymes when they encounter particles or other appropriate stimuli (eg. complement component C5a). These postulated mechanisms of lysosomal release are illustrated in FIGURE 3.5.

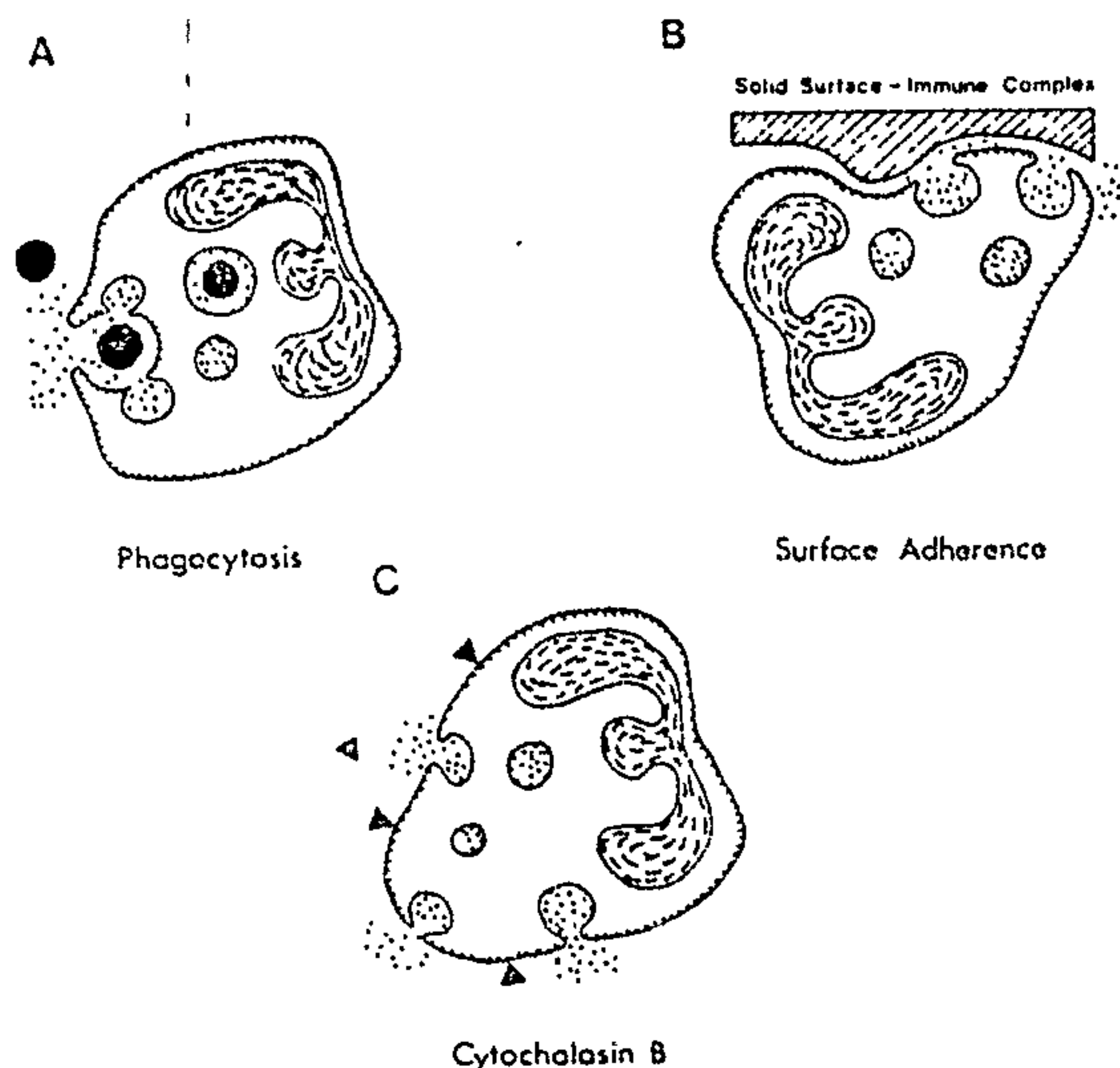


FIGURE 3.5 Mechanisms of lysosomal enzyme release. **A:** "Regurgitation during feeding" (incomplete closure of the phagosome). **B:** "Reverse endocytosis" as a consequence of adherence to an immunologically-prepared, non-phagocytosable surface. **C:** cytochalasin B-treated PMN leucocyte; exocytosis as a consequence of external stimulation.

Recently, a complex sequence of steps leading to lysosomal hydrolase activation and secretion has been described in neutrophils by WEISSMANN et al (1980). This is outlined below.

When a ligand (that is a molecule bound to a protein surface, for example, an opsonised particle) binds to appropriate surface receptors, enhanced membrane fluidity develops. This enhanced fluidity is followed, or directly associated with, a change in membrane surface potential. Concurrently, calcium is lost from the plasma membrane. Careful measurement demonstrated that these membrane changes are quite local, occurring only in regions where ligand binding has occurred.

It is only after these events have occurred, and after a significant time-lag, that the cells generate superoxide anion, arachidonic acid metabolites and active forms of the lysosomal hydrolases with their subsequent secretion according to one of the above patterns. These biochemical events are associated with condensation of subplasmalemmal microfilaments and the assembly of

developed at the area of injury. This was later surrounded by a bright red area, with the central area becoming swollen about 1 minute later still. This he called the "triple reaction" (cited by FLOREY 1970a).

Lewis and colleagues demonstrated that superficial injection of histamine reproduced this triple response and hence they postulated that this amine or some similar "H" substance was a mediator of the reaction to injury (see RATNOFF 1969 and FLOREY 1970a).

Since Lewis' day, many potential mediators of the inflammatory reaction have been postulated, all of which are suggested to initiate, mediate or somehow modulate various aspects of this process.

Several observations suggest that endogenous mediators might be significant in the inflammatory response:

- i) The response is seen to be a reproducible stereotyped sequence of events evoked by a variety of stimuli.
- ii) A latent period is noted between the injury and the development of a response.
- iii) Despite limitation of injury to a defined area, the vascular alterations extend well into the adjacent, undamaged tissues.
- iv) Injured tissues have been demonstrated to contain a number of endogenous factors which are seen in vitro to produce effects similar to those seen following injury (HURLEY 1981). See also WILHELM (1973).

Proof that a particular substance mediates microvascular changes would require demonstration of its ability to induce some of the phenomena of inflammation when injected. The substance would also have to be detected in its active form and appropriate concentration at the

relevant time. Further, evidence is required that the response which it is reputed to evoke is suppressed by prior administration of a specific antagonist (JOHNSTON et al 1979; MOVAT 1979; GARCIA-LEME 1981b; HURLEY 1981).

These criteria have been met in only one instance; that of histamine, which has been shown to be responsible for the brief and relatively unimportant initial phase of vascular leakage (HURLEY 1981).

Current researchers have however, proposed a number of potential mediators of the inflammatory response. Nowhere is it more apparent than here that the various components and mediator sequences are intimately related. These interactions are partly suggested to involve cross activations between mediator groups in which Hageman Factor (Factor XII in the clotting sequence) is central (3.3.1.4). Modification of the levels of intracellular cyclic nucleotides by various mediators is also apparent; this modification is suggested to provide feedback mechanisms to the inflammatory process (3.3.2.6).

Inflammation must therefore be considered a "multimediated process". It would seem in many instances that several agents contribute to a single aspect of this process and that the same agent may be active in different events (GARCIA-LEME 1981a).

These proposed mediators may be separated arbitrarily into two groups:

i) those arising from the fluid-phase (plasma, extracellular fluid):

3.3.1.

ii) those arising from cells - both blood cells and tissue: 3.3.2.

Each of the major mediators is discussed independently according to this arbitrary schema and some attempt is then made to demonstrate their

interrelationships. They are summarised in TABLE 3.2.

TABLE 3.2 Classification of Endogenous Mediators of Inflammation.

Origin	Major groups	Major mediators
Plasma	Kinin system	Bradykinin Kallikrein Plasminogen activator
	Complement system	C567 complex C3 fragments C5 fragments C kinin
	Clotting system	Fibrinopeptides Fibrin degradation products
Cells	Vasoactive amines	Histamine 5-Hydroxytryptamine
	Acidic lipids	Prostaglandins Leukotrienes
	Lysosomal components	Cationic proteins Acid proteases Neutral proteases
	Others	Cyclic AMP Oxygen radicals ?Altered tissue proteins

(adapted from RYAN & MAJNO 1977, p224).

3.3.1 Fluid-Phase Components.

The fluid-phase components of acute inflammation are chiefly plasma proteins which normally exist as inactive precursors. These proteins are activated by enzymatic cleavage to release biologically active fragment(s), the enzyme being either released from within a cell, or itself activated in another sequence (WARD 1980).

They may be grouped into three broad classifications based on their in vitro functions: vascular alterations (the kinins), coagulation/fibrinolysis, and cell lysis (complement - whose components

are identified as C1 to C9). These three mediator systems are interrelated in a number of ways - the interaction may activate, increase or decrease the activity of the other (See 3.3.1.4).

3.3.1.1 Kinin system.

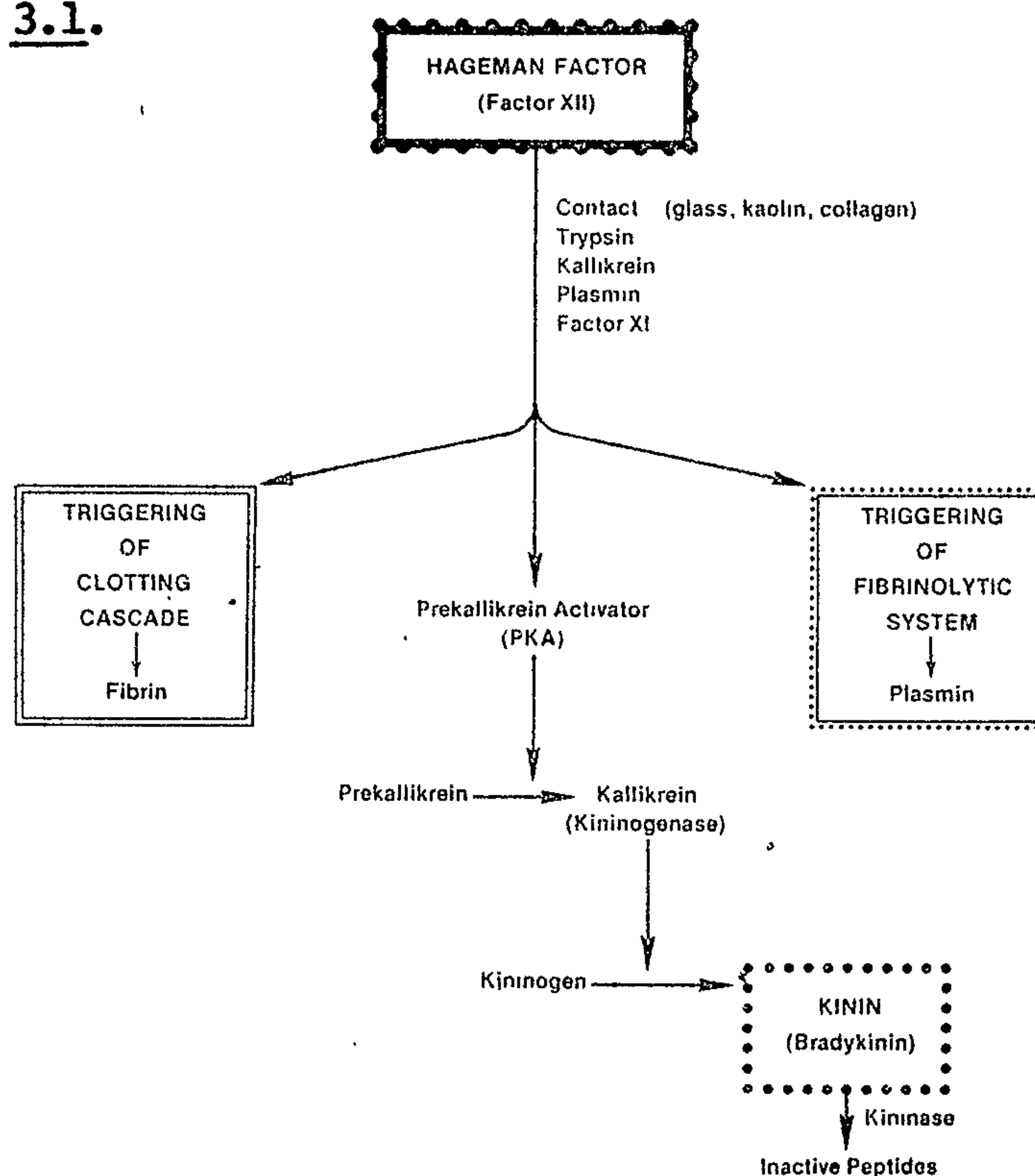
The kinins are a group of vasoactive polypeptides capable of eliciting pain, and of increasing vascular permeability and the rate of macromolecule and fluid efflux. They are also reported to induce contraction of intestinal and venular smooth muscle, and to cause arteriolar and precapillary sphincter dilatation producing increased flow (RYAN & MAJNO 1977; HURLEY 1981).

The major effector agent of the kinin system is bradykinin. On the basis of molar concentration, bradykinin is the most potent known permeability factor (RYAN & MAJNO 1977). This nonapeptide is formed by the digestion of a plasma glycoprotein (kininogen) by a proteolytic enzyme (kallikrein). Kallikrein is found in plasma as an inactive precursor (prekallikrein) (HURLEY 1981), and it too is suggested to mediate increased vascular permeability (RATNOFF 1969) and to be chemotactic for neutrophils and monocytes (RYAN & MAJNO 1977), though less so than bradykinin (MOVAT 1979).

The effects of the kinins are believed to be amplified by products of acidic lipid metabolism (HURLEY 1981), (see 3.3.2.2).

Hageman Factor (Clotting Factor XII) is critical to the activation of these and other related vasoactive peptides, and for the initiation of coagulation and fibrinolysis. The activation and interactions of Hageman Factor are discussed in 3.3.1.4. (See also 1.3.4).

The work of Miles and co-workers in the 1950's, of Cochrane and Wuepper, and Kaplan and Austen in the early 1970's, in isolating, purifying and characterising the components of the kinin system, has been chiefly responsible for the elucidation of this system (cited by RYAN & MAJNO 1977). A review of the history of the kinins is recorded by MILES (1969). Its components are represented diagrammatically in TEXT FIGURE 3.1.



TEXT-FIGURE 3.1 The plasma kinin system (from RYAN & MAJNO 1977, p225).

Bradykinin has a short life-span in the circulation since it is inactivated by another plasma proteolytic enzyme (kininase I). In addition, the kinin system is held under control by the action of kallikrein antagonists in plasma and tissues, and by the inhibitory action of C1 esterase inhibitor on activated Hageman factor, kallikrein and plasmin (RYAN & MAJNO 1977; KAPLAN et al 1982). It has thus been impossible both to isolate kinins within the inflammatory exudate and to deplete the plasma of one or more components of the kinin system. Thus, kinins remain "potential" (not proven) mediators of inflammation (MOVAT 1979).

3.3.1.2 Coagulation/Fibrinolytic systems.

In addition to its activity in initiating kinin-generation, Hageman factor (Factor XII) may also initiate coagulation by activation of Factor XI, leading to the intrinsic pathway (GILMORE 1976). The conversion of soluble fibrinogen to insoluble fibrin is the end step of this pathway. Initiated by thrombin, this reaction results in the formation of fibrinopeptides A and B as by-products. These agents are also thought to increase vascular permeability, though the mechanisms whereby they achieve this are unclear (HURLEY 1981).

The procoagulant activity of Hageman factor is restricted by both plasmin and kallikrein which may cause cleavage of Hageman factor into fragments. These biologically active peptides are also identified as potential inflammatory mediators. Their reported effects include enhancing of the effect of bradykinin on smooth muscle, inducing vascular leakage, and chemotactic attraction of neutrophils. See RYAN and MAJNO (1977).

Once fibrin has polymerised, its removal can only be accomplished by phagocytosis or enzymatic cleavage of the polymer. The latter is the proposed in vivo function of the proteolytic enzyme, plasmin. Plasmin is normally present in plasma in inactive form as plasminogen. Other than its cleavage of fibrin and Hageman factor, this enzyme, having broad proteolytic specificity, has been shown in vitro to be able to activate prekallikrein, release bradykinin from kininogens, and to activate the complement system via activation of C1 esterase (the classic pathway) and cleavage of C3 and C5 (GILMORE 1976; RYAN & MAJNO 1977). (See 3.3.1.3).

Cleavage of fibrin by plasmin also results in the formation of biologically active fragments. To such fibrin degradation products (FDP's) have recently been attributed the capabilities of enhancing vascular permeability in skin (BARNHARDT et al 1971; RYAN & MAJNO 1977) and of neutrophil chemotaxis (RYAN & MAJNO 1977; HURLEY 1981). Their enhancement of vascular permeability is suggested to be a direct action on the endothelium rather than a synergistic effect on other mediators (GERDIN & SALDEEN 1978).

3.3.1.3 Complement system.

In 1910, FRIEDBERGER (cited by RYAN & MAJNO 1977) reported that serum incubated with immune precipitates and then injected into the circulation of an animal causes a severe reaction resembling anaphylactic shock. Later studies reported that such serum also caused in vitro contraction of smooth muscle, local induction of increased vascular permeability in skin, and leucocyte chemotaxis (seen in the Boyden system). Such properties have now been identified as resulting from the activated complement system (RYAN & MAJNO 1977).

Complement is another plasma protein cascade consisting of "nine distinct serum proteins (labelled C1 to C9) which interact sequentially to mediate certain of the effects of antigen-antibody interactions" (AUSTEN 1971) The final action of this sequence is cell lysis (GILMORE 1976). Though this sequence obviously has profound influence on immunologic inflammation, it is also believed to contribute to non-immune inflammation.

Two pathways for the initiation of the complement system have been outlined. The 'classical' pathway is activated by antigen-antibody complexes as well as by non-immunologic agents such as plasmin and

trypsin (that is, by most hydrolytic cell enzymes). The 'alternative' pathway (also called the Properdin system or the C3-Activator (C3-a) system), by-passes C1, C4 and C2. It is activated by antigen-antibody complexes, various polysaccharides and bacterial lipopolysaccharides (RYAN & MAJNO 1977). Both pathways are controlled by the inherent instability of certain complement enzymes and by the presence of serum inactivators and inhibitors, for example C1 esterase inhibitor (RYAN & MAJNO 1977).

The significant role of the complement system in the context of this thesis is the formation of potential mediators of inflammation in the form of various biologically active by-products. Fragments of C3 and C5 appear to be the main permeability factors generated (HURLEY 1981). Other factors (particularly C5a) also have chemotactic or chemokinetic effects on leucocytes, especially PMN's.

The relationship of these complement by-products to non-immune inflammation has been studied in complement-depleted animals. These studies demonstrated that no delay occurred in the migration of both neutrophils and monocytes to the wound. However, there was a 50% reduction in the volume of neutrophils present in the wound. A lesser reduction in monocyte volume was also evident (WAHL et al 1974).

Thus, the complement sequence would appear to play some role in attracting neutrophils to the wound. It obviously is not, however, the sole mediator of this cellular response to non-immunological stimuli (WAHL et al 1974). These results are reported to confirm those of other researchers (WAHL et al 1974; MOVAT 1979). However, other authors (ISSEKUTZ 1981; WEDMORE & WILLIAMS 1981; ISSEKUTZ & MOVAT 1982) found a critical dependance between neutrophil infiltration and complement component C5a in one experimental model. (See 3.4). This sequence may

Activated Hageman factor (previously known as permeability factor/dilute - PF/dil), is described as having three effects:

- a) triggering of the clotting cascade (by activating Factor XI),
- b) triggering of the fibrinolytic system (by activating plasminogen proactivator to produce plasminogen activator), and c) prekallikrein activator (PKA) activity (PKA is thought to be a fragment of Hageman factor which activates prekallikrein to kallikrein) (RYAN & MAJNO 1977). Thus the kinin, coagulation, and fibrinolytic systems are known as the "Hageman factor-dependent systems". These systems interact in various ways with the complement system, the interactions being described by RATNOFF (1971) as a "tangled web". The activation of the fibrinolytic enzyme, plasmin, is a key link in these interactions (see 3.3.1.2).

In many of the fluid-phase mediator sequences, the presence of the activated enzyme feeds back to accelerate its own activation reaction or that of other components of these HF- dependent systems (KAPLAN et al 1982). Thus, plasmin is suggested to act in sequence with HF to convert prekallikrein to kallikrein (AUSTEN 1971). Kinin-free kininogen (the fragment remaining after bradykinin is released) is another example, since it acts as a cofactor with HF in the coagulation sequence and other reactions (KAPLAN et al 1982).

The activation of Hageman factor itself is a further example of such positive feedback. Such activation (depicted in FIGURE 3.6) commences with the relatively slow autoactivation of HF, the activated form of which (HFa) both accelerates its own reaction and leads to the activation of prekallikrein. A more rapid burst of HFa formation occurs following the digestion of surface-bound HF by the kallikrein so formed (KAPLAN et al 1982). The effect of this interaction is seen in Fletcher

Factor Deficiency in which prekallikrein is deficient. Here HF activation does not proceed at the normal rate and some clotting defect is present (RYAN & MAJNO 1977).

It is further significant that the "classical complement pathway shares with the Hageman factor-dependent pathways a control protein active at the early stages of each cascade" (KAPLAN et al 1982). This protein, C1 esterase inhibitor, as well as inhibiting activation of the first complement component, is also the major plasma inhibitor of HFa and HFf (fragment) and it is one of two inhibitors of plasma kallikrein (RYAN & MAJNO 1977; KAPLAN et al 1982).

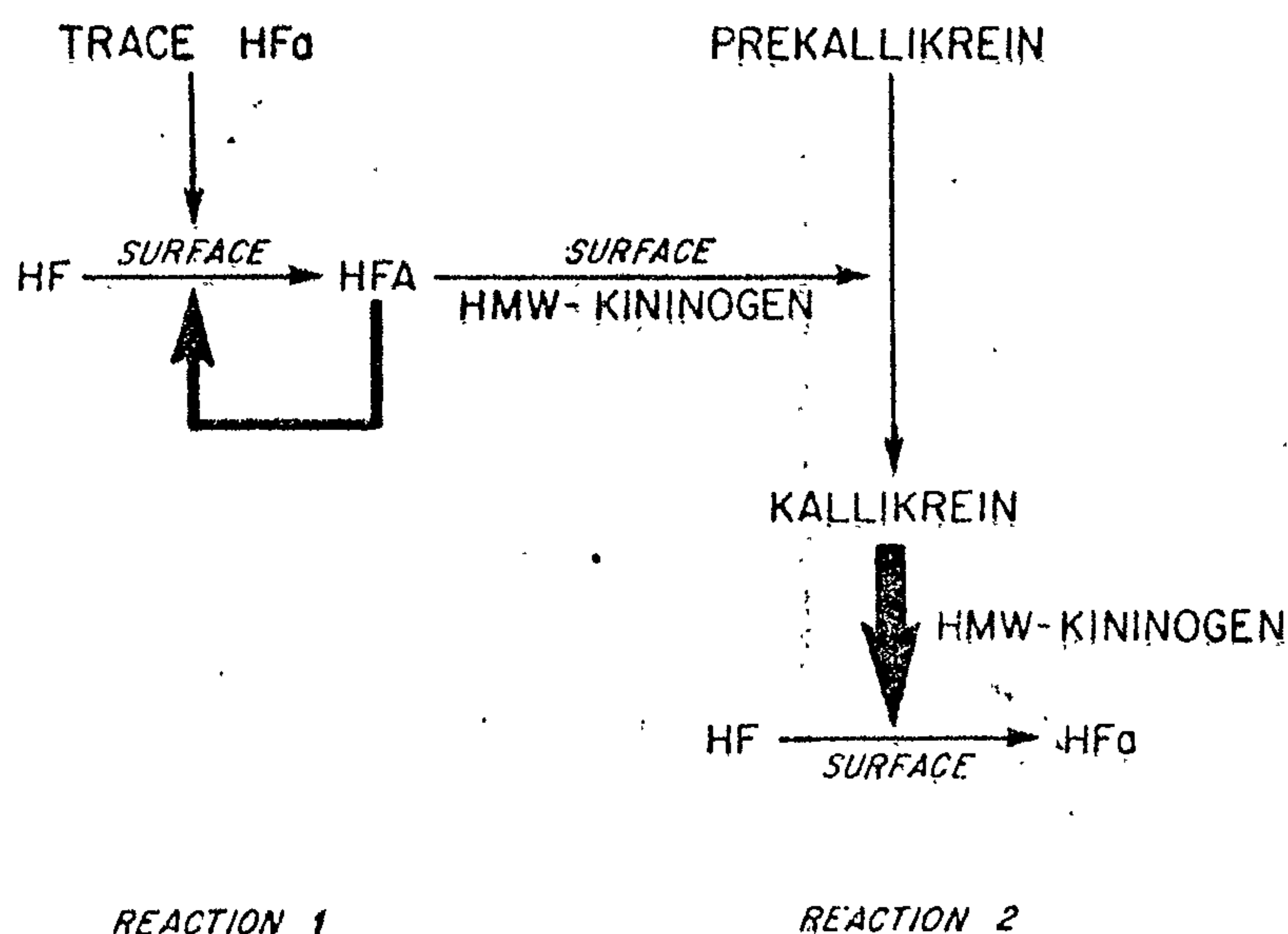


FIGURE 3.6 Hageman Factor activation. Separation of the initiating events of contact activation into two steps. The first consists of HF autoactivation. The second is the reciprocal interaction in which HFa (activated HF) converts prekallikrein to kallikrein which digests HF to form additional HFa (from KAPLAN et al 1982, p30).

Several other complex interactions are described within these pathways. For detailed analysis of these, the reader is referred to the excellent review articles of AUSTEN (1974), COCHRANE et al (1974), MOVAT (1979), KAPLAN et al (1982). The historical reviews of RATNOFF (1969; 1971) are also interesting. The major interrelationships of this "tangled web" are illustrated in TEXT-FIGURE 3.3.

3.3.2 Cellular-Phase Components.

In addition to the 'physical' contribution of cells involved in the inflammatory response (in such functions as phagocytosis - 3.2.2), these cells elaborate various highly significant pharmacologically-active substances potentially capable of causing a wide cross-section of actions associated with inflammation. These actions include cell lysis, alterations in vascular permeability, pain, leucocyte chemotaxis and the degradation of various tissue proteins. RYAN & MAJNO (1977) identify several different groups of potential mediators: a) vasoactive amines, b) acidic lipids, c) lysosomal components, and d) others.

3.3.2.1 Vasoactive amines (Histamine, 5-hydroxytryptamine).

The vasoactive amines, histamine and 5-hydroxytryptamine (synonyms: serotonin, 5-HT) are comparatively simple chemical substances which are widely distributed in plant and animal tissues (WILHELM 1973). Histamine is found in inactive form in the granules of mast cells, in eosinophil and basophil leucocytes and in platelets. Serotonin is also found in large concentrations in mast cells and to a lesser extent in platelets and many tissue cells (RYAN & MAJNO 1977).

As has been mentioned, histamine was the first substance to be proposed as a mediator of inflammation; it remains as the only such substance to have satisfied the criteria for such mediators (EBERT & GRANT 1973; HURLEY 1981) - see 3.3. This amine is known to cause active vasodilatation and a transient phase of increased vascular permeability which commences at injury and lasts for only 10-15 minutes (HURLEY 1981). The extent of these alterations, however, is quite marked (HADDY et al 1976).

Recent studies have suggested that histamine may also be involved with later aspects of wound healing. Low doses of histamine are suggested to stimulate, and high doses to inhibit, collagen formation (DABROWSKI & MASLINSKI 1981). Perhaps relating to the same property, histamine is also reported to inhibit granulation tissue formation by increasing the intracellular levels of cyclic AMP (YOKOYAMA et al 1980) - 3.3.2.6. These areas require further investigation before their in vivo relevance can be determined.

The effects of histamine in inflammation are apparently the result of stimulation of both the H1 and H2 receptor sites in venular endothelium: the oedema which is produced is suggested to result chiefly from increased vascular permeability with a lesser effect produced by increased capillary hydrostatic pressure and surface area. It is also noted that in some experimental models of inflammation, histamine continues to be released throughout the inflammatory response. In most situations, however, it is believed that there is a decrease in histamine release following the initial burst (OWEN & WOODWARD 1980).

Serotonin is reported to increase vascular permeability in rats and mice in a similar way to histamine (WILLOUGHBY & DI ROSA 1971; HADDY et al 1976). HURLEY (1981) states however, that although 5-HT is a potent vasoconstrictor, it does not induce increased vascular permeability in man and most other species. This would seem to be confirmed in studies on a variety of animals in which serotonin produced arteriolar dilatation and venous vasoconstriction with subsequent increased filtration, however, only in rats did it produce increased vascular permeability (HADDY et al 1976). It would seem that in rats and mice the roles of these amines is reversed - a relatively

minor role being attributed to histamine (OWEN & WOODWARD 1980).

Amine release from mast cells occurs in response to: a) physical injury (for example, mechanical trauma, irradiation, heat) b) various chemical agents (for example, snake venoms, trypsin, toxins, ATP, and a neutrophil cationic protein), and c) immunological processes (for example, exposure to anaphylatoxins - C3a and C5a) (3.2.2.5) (RYAN & MAJNO 1977). Platelets may be stimulated to release amines by stimuli such as thrombin, trypsin, collagen, adrenaline, and ADP (HARLAN & HARKER 1981).

It is generally considered that these vasoactive amines play a relatively minor role in the development of the inflammatory response, particularly that following surgical injury (WILHELM 1973; HURLEY 1981).

3.3.2.2 Acidic lipids.

(Prostaglandins, thromboxanes, prostacyclins, leukotrienes ...)

The discovery of Vane (1971) and Smith and Willis (1971, cited by VAN DE STADT 1982) that nonsteroidal anti-inflammatory drugs (NSAIDs) were able to block prostaglandin (PG) synthesis was instrumental in promoting extensive research into PGs and their role in inflammation during the past decade (JOHNSTON et al 1979; VAN DE STADT 1982).

Studies in this field are hampered by the fact that these arachidonic acid metabolites are not stored in tissue, but are newly synthesized in small quantities in the cells as a result of membrane perturbations (HARLAN & HARKER 1981; VAN DE STADT 1982). (Some suggest a continuous low level synthesis modulated by formation and enzymatic breakdown, ARTURSON 1979; NASJLETTI & MALIK 1981). These membrane

disturbances may originate from hormones, chemotactically active substances, phagocytosed particles (including immune complexes), calcium ionophores, ultraviolet light, or even mechanical stimulation (VAN DE STADT 1982).

Almost all mammalian cells have the ability to synthesise prostaglandins and related acidic lipids (JOHNSTON et al 1979; HORROBIN 1980). Current research suggests that they may have major roles/effects in many tissues and in inflammation (HORROBIN 1980); some of these effects will be demonstrated in the course of this discussion.

These substances were first identified in seminal vesicles by the independent investigations of Goldblatt and von Euler nearly 50 years ago, being named "prostaglandins" by von Euler (NALBANDIAN & HENRY 1978). These and related substances are derived from 20 carbon unsaturated fatty acids situated within the cell membrane. Their synthesis occurs following esterification of the fatty acids to phospholipids. This esterification is controlled by phospholipidases; it probably represents a major control mechanism of these metabolic pathways (HARLAN & HARKER 1981).

Arachidonic acid (or eicosatetraenoic acid), containing four double bonds, is the most abundant precursor in animal tissues. It is almost entirely bound to phospholipids and triglycerides particularly in the plasmalemmal membranes of platelets, leucocytes and endothelial cells. Arachidonic acid (AA) is the precursor of the "2-series" acidic lipids (PGE₂, PGD₂, PGI₂, Thromboxane A₂, and so on) (HARLAN & HARKER 1981).

Two other series are described, also relating to the number of double bonds in the chain; dihomogammalineolic acid (DGLA) having three

double bonds (C20:3) is the precursor of the "1-series" (PGE₁, PGI₁, Thromboxane A₁ ...). Eicosapentaenoic acid (EPA) is the five double-bonded precursor (C20:5) of the "3-series". The physiologic importance of the C20:3 and C20:5 fatty acid precursors and their derivatives is unclear (HARKAN & HARKER 1981). The series 1 group (particularly PGE₁) has been implicated as part of a major feedback mechanism in the prostaglandin system (HORROBIN 1980).

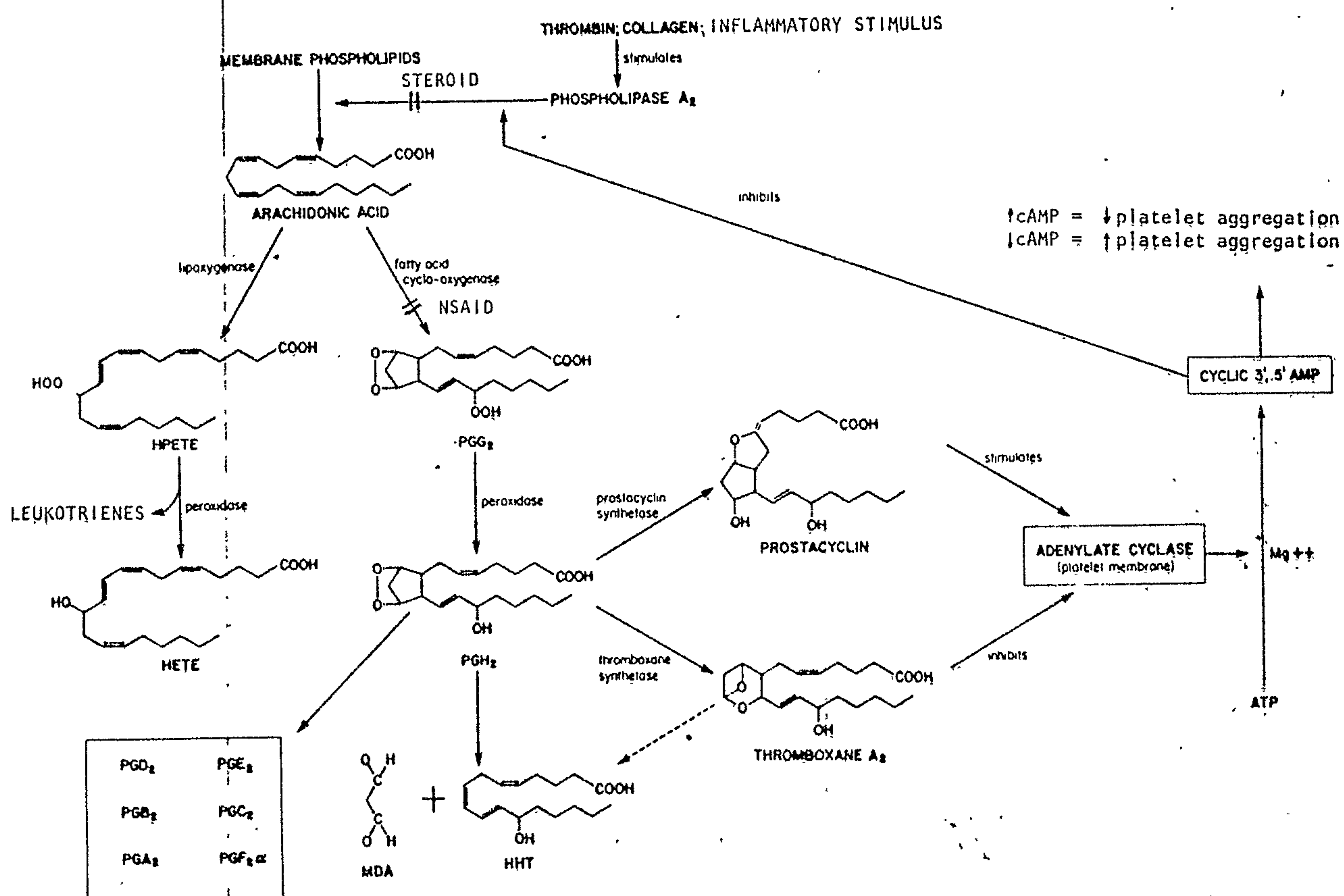
Discussion here will concentrate on the 2 series since it has been most clearly identified, though even this series contains many unexplained phenomena. Consideration of the postulated systemic effects of the PGs in normal physiology does not form a part of this thesis.

Two pathways of arachidonic acid (AA) metabolism have been described - the cyclooxygenase or the lipoxygenase pathways (outlined in TEXT-FIGURE 3.4).

The major pathway in most cells involves conversion of AA to labile cyclic endoperoxides PGG₂ and PGH₂ by the membrane-bound enzyme, cyclo-oxygenase. Endoperoxide production is accompanied by a rapid burst of oxygen consumption and the release of oxygen radicals which are thought to limit the action of cyclo-oxygenase and thus limit further endoperoxide production (HARLAN & HARKER 1981).

That the characteristic arachidonic metabolites exhibit quantitative as well as qualitative differences in different classes of cells suggests that cell-specific oxygenation pathways exist (NOWAK 1979; KUEHL & EGAN 1980; GOETZL 1981). The direction of further metabolism of these labile endoperoxides is evidence of this, since it depends upon the enzymes which are present. Platelets, for example, contain thromboxane synthetase and thus thromboxane A₂ is the chief AA

metabolite in the platelet; endothelial cells, containing significant amounts of prostacyclin synthetase and minimal thromboxane synthetase, chiefly produce prostacyclin (PGI₂) (NALBANDIAN & HENRY 1978; HARLAN & HARKER 1981).



TEXT-FIGURE 3.4 Pathways of arachidonic acid metabolism. The formation of prostacyclin, thromboxane, the various PGs, and the chemotactic agents HETE and HHT are shown schematically. The counterpoised activity of prostacyclin and thromboxane on platelet adenylate cyclase (thereby adjusting the level of cyclic AMP formed) is also illustrated. So too is the negative feedback pathway by which cAMP controls the level of PG synthesis. The sites where NSAID drugs (NSAID) and steroids block this pathway are identified (see 7.3.2) (from NALBANDIAN & HENRY 1978, p104).

Stimulated leucocytes (particularly macrophages and PMNs) elaborate E-type prostaglandins along with some PGI₂, and traces of thromboxane A₂ and F-type prostaglandins (BRUNE et al 1979; FORD-HUICHINSON & DOIG 1979). These "stable" prostaglandins (D, E, F, and A) are also elaborated in varying quantities by other cells, including vascular endothelium (HARLAN & HARKER 1981).

The action of lipoxygenase produces hydroperoxyeicosatetraenoic acids (HPETE acids); these are then reduced to hydroxyeicosatetraenoic acids (HETE acids) both of which are proposed to have biological functions. An entirely new pathway starting from 5-HPETE has been described recently. This pathway leads to the formation of complexes of HETE molecules called leukotrienes.

As with cyclooxygenase metabolites, the quantity and type of the various HPETE and HETE acids and leukotrienes formed depends upon the cell type and its constituent enzymes (VAN DE STADT 1982). It is noted that the previously described slow reacting substance of anaphylaxis (SRS-A) is now believed to be a leukotriene (WEISSMANN et al 1980; SAMUELSSON 1981).

Leucocytes are the most active in the lipoxygenase pathway producing some products (for example, leukotrienes) which are unique to their metabolism (GOETZL 1981). Polymorphs are particularly active in the lipoxygenase pathway, but are less selective, producing a wide range of HETE acids and leukotrienes (LTs) of which 5-HETE and leukotriene B4 (LTB4) are probably the most significant (VAN DE STADT 1982).

The reported biological activities of the end products of these pathways demonstrate considerable variation and in some instances, highly developed positive and negative feedback mechanisms are suggested. Furthermore, apparently opposing actions are reported to be produced by the same AA derivative. It is clear that much of the biological action and interaction of the PG system remains hidden. It would seem however, that this system is significant in inflammation and other metabolic processes. Thus understanding of the PGs is critical in the consideration of appropriate pharmacological modification of inflammation.

Prostaglandins and inflammation.

As was mentioned, the discovery that aspirin and other acidic non-steroidal anti-inflammatory drugs (NSAIDs) inhibited the enzymatic transformation of AA to the primary prostaglandins (PGE₂, PGF₂α, and PGD₂), thereby diminishing the inflammatory response, instigated intense research into the role of these substances in inflammation (GRYGLEWSKI 1981). This research also followed the discovery of prostaglandins in the exudate of experimental inflammatory models (GREAVES et al 1971, cited by MONCADA et al 1973).

The role of prostaglandins in inflammation has undergone considerable evolution in the scientific literature. DI ROSA et al (1971) proposed a sequential release of mediators: the earliest permeability/ flow changes being attributed to histamine and serotonin, the intermediate phase to kinins, followed closely by the prolonged late phase which was attributed to the PGs (JOHNSTON et al 1979), coinciding with the maximal vessel response (WILLOUGHBY & DI ROSA 1971).

These concepts have been modified by WILLIAMS and colleagues (WILLIAMS & MORLEY 1973; WILLIAMS 1976; WILLIAMS 1977a; WILLIAMS & PECK 1977). The above outline is primarily based upon the concept that an inflammatory cell produced a chemical mediator which produced a vascular response. However, it became evident that the significant plasma exudation described to occur with PGs (see MONCADA et al 1973), related to their potentiation of other mediators (including histamine and bradykinin) rather than a direct effect of PGs on the permeability of the vascular endothelium itself (MONCADA et al 1973; WILLIAMS & MORLEY 1973).

Indeed, WILLIAMS & PECK (1977) demonstrated that arachidonic acid-derived products themselves promoted little exudation at all. They suggested that the capacity of these products to provoke modest oedema and to significantly potentiate the oedema produced by other mediators represented a reflection of the vasodilatation which they produce. (See also WILLIAMS 1977b; WEISSMANN et al 1980).

WILLIAMS (1977a) proposed the "two-mediator hypothesis" - that inflammation involved the production of two separate agents, one which mediated the increased vascular permeability (such as histamine, bradykinin), and a vasodilator (prostaglandins), which potentiated the plasma exudation of the permeability-increasing mediator.

This was confirmed by the observation that other vasodilators (for example, isoprenaline, ADP) also potentiated responses to histamine, and that vasoconstricting agents (such as noradrenaline) reduced the histamine response. This potentiation is suggested to be a consequence of increased vessel wall area (WILLIAMS 1977a).

These concepts are supported by the later studies of JOYNER et al (1979) which demonstrated that topical applications of PGE₁ produced marked increases in blood flow (red cell velocity) and arteriolar diameter. However, this increased flow rate was not seen to be accompanied by an increase in the number of intercellular leakage sites. (Note that this study did not specifically investigate the PG potentiation of histamine-induced permeability changes, but concurred with this possibility).

Note that while other authors support the concept of prostaglandin potentiation of kinins, some (GREGA 1983) dispute that vasodilatation is the mechanism of this potentiation, though no other is yet apparent.

The range of biological effects ascribed to the prostaglandin series is outlined in TABLE 3.3. As will be noted, the literature contains a number of discrepancies in discussion of this subject. Confusion also arises from the fact that, whilst many of these compounds are clearly able to elicit (or potentiate) many of the phenomena which form the inflammatory response, their effects on the intracellular cyclic nucleotides appear contrary, with inhibitory effects seeming to result (GOLDSTEIN 1977). This subject will be considered further in 3.3.2.6.

It is also apropos here to mention the recent work of HORROBIN (1980) concerning negative feedback mechanisms within PG synthesis. These are chiefly attributed to interactions between the 1 and 2 series. Thus, for example, PGE₁ is suggested to inhibit AA mobilisation while TXA₂ and PGE₂ stimulated DGLA and its conversion to PGE₁. In addition, the AA pathway itself is described to be autoregulated since the formation of PGI₂ and TXA₂ probably also interferes with the mobilisation of AA or with the initial steps of its conversion to the endoperoxides.

Although these concepts are tentative at this stage, HORROBIN (1980) presents multiple clinical and experimental evidence of the effect of blockage of these feedback pathways. In view of the other interactions in this system, particularly as described in relation to the cyclic nucleotides, these concepts certainly deserve investigation.

TABLE 3.3 (see following page).
REFERENCE CODE:

- | | | |
|------------------------------|----------------------------|--------------------------------|
| 1. MONCADA et al (1973) | 9. PESKAR & BRUNE (1979) | 17. WEKSLER & GOLDSTEIN (1980) |
| 2. WILLIAMS & MORLEY (1973) | 10. SAMUELSSON (1979) | 18. BONTA et al (1981) |
| 3. WILLIAMS (1977a) | 11. SMITH (1979) | 19. GOETZL (1981) |
| 4. WILLOUGHBY (1977) | 12. WEISSMANN et al (1980) | 20. GRYGLEWSKI (1981) |
| 5. NALBANDIAN & HENRY (1978) | 13. WESTWICK (1979) | 21. HARIAN & HARKER (1981) |
| 6. JOHNSTON et al (1979) | 14. WILLIAMS (1979) | 22. VAN DE STADT (1982) |
| 7. JOYNER et al (1979) | 15. HORROBIN (1980) | |
| 8. LEWIS (1979) | 16. KUEHL & EGAN (1980) | |

TABLE 3.3 The Biological Effects ascribed to Acidic Lipids in Inflammation.

	CYCLIC NUCLEOTIDES	PAIN	LEUCOCYTE CHEMOKINESIS/ CHEMOTAXIS	MICROVASCULAR PERMEABILITY	MICROVASCULAR TONE	LYSOSOMAL RELEASE	PLATELET AGGREGATION	OTHER ACTIONS
ARACHIDONATE	-	overt pain (12)	chemokinetic (12)	-	vasodilation + (14)	-	-	-
PGG2 & PGH2	-	-	-	mild ↑, ? related to release of oxygen radicals at conversion PGG2 to PGH2 (20).	vasoconstriction then vasodilation (13,17), but only if later pathway to PGI2 not inhibited (12).	-	enhance but ? only via TxA2 (12).	-
PGD2	-	transient pain (18)	chemokinetic* (18)	little effect alone (3,7,8,14).	vasodilation (9,19) vasoconstriction; 13	suppresses (19)	inh. + (17,19)	-
PGI1	↑ cAMP (6,15)	.sensitises to other stimuli (1) .high dose: overt pain (6)	little effect (11)	enhances vasc. permeabil. of other mediators (8,13)	.d-d vasodilation; .inhibits vasc. response to symp. N.S (6)	suppresses* (22)	-	↑ macrophage stim. of C.T synthesis* (18)
PGI2	↑ cAMP (6)	.sensitises to other stimuli (1) .high dose: overt pain (6)	chemokinetic* (6,19,22) ?chemokinesis (12)	.little effect alone (3,7,8,14) .enhances vasc. permeabil. of other mediators (1,3,7)	.d-d vasodilation (6,8,12,14,16,17,19) .prolonged effect (2-10hrs) (6)	suppresses* (22)	potentiates (low dose) inh. (high dose) (17)	+ PGs generally reported to ↓ collagen deposition & formation of proteoglycan (12)
PGF2α	↑ cGMP (15)	.sensitises to other stimuli (1) .inh. algnesia of Bk (6)	chemokinetic* (6,12,22)	little effect alone (2,3,7,8,14)	.little effect unless supraphysiol (6,13,14,17) .inh. response to other mediators; 4,20	-	-	-
PGI2	↑ cAMP (15)	sensitises to other stimuli (1)	inh. chemotaxis (17) stim. chemokinesis (19)	.little effect alone (11,14,16,22) .enhances vasc permeabil. of other mediators (3,7,8,14)	d-d vasodilation (6,8,12,14,16,17,19)	suppresses* (19,22)	potent inh. (12,20,21)	↑ adherence of PMNs to endothelium (22)
THROMBOXANES	↓ cAMP	-	TXB2: little effect (11) TxA2: chemotactic + (12)	-	vasoconstriction (12,17,19,20)	-	potent stim. (6,12,17,21)	-
HHT	-	-	chemotactic ++ (5,12,17,22)	-	-	-	-	-
HPETE acids	-	-	chemotactic + (22)	-	vasoconstriction (12)	-	-	-
HETE acids	↑ cGMP (19)	-	chemotactic +++ (17,22) 5-HETE > 8&9-HETE > 11&12-HETE > 15-HETE (19)	-	-	-	-	-
LEUKOTRIENES	-	-	chemotactic +++ (LTB4) LTB4 > 5-HETE (10,16,19)	-	vasodilation-LTB4, LTC4; vasoconstriction-LTC4 (19)	-	-	-

* = action related to changes in cAMP; inh.= inhibits; stim.= stimulates; d-d = dose-dependent; vasc. permeabil.=vascular permeability; Bk=bradykinin
 Lipid-Oxygenase Pathway
 Symp. & S. = sympathetic nervous system

3.3.2.3 Lysosomal hydrolases and inflammation.

As has been mentioned, the role of polymorphs in tissue lysis is perhaps their most significant contribution to inflammation. Normally, lysis causes the breakdown of necrotic or effete tissue in preparation for macrophage phagocytosis. However, an exaggerated lytic response may lead to marked destruction of adjacent tissue. Some appreciation of these processes is therefore essential to understanding tissue destruction. Central to PMN lysis is the large number of inflammatory substances contained within these cells, particularly those sequestered within their cytoplasmic granules (lysosomes) (GOLDSTEIN 1977) (See 3.2.2.5).

Whereas the relevance of many of these substances is unclear, lysosomal neutral proteases and other trypsin-like enzymes are clearly capable of tissue lysis. These enzymes have been shown to be capable of many functions, including the degradation of collagen (collagenase), elastin (elastase), vascular basement membrane and connective tissue proteoglycans. They may also provoke blood coagulation and fibrinolysis, or generate biologically active products, (such as complement fragments or kinins) by their action on several plasma components (GOLDSTEIN 1977).

The postulated inflammatory effects of these substances are summarised in TABLE 3.4. The mechanisms of their release are described in 3.2.2.7. It is noted that the in vivo significance of the lysosomal contents is unclear since inflammation proceeds normally in agranulocytic gnotobiotic rats (other than the obvious absence of neutrophils) (WILLOUGHBY & DI ROSA 1971).

Later studies (DI ROSA & WILLOUGHBY 1972) did however, suggest that depletion of polymorphonuclear cells did lead to a slight reduction in oedema formation during the later phase of inflammation. Inhibition of mononuclear infiltration was seen to produce even greater diminution of this phase of oedema (WILLOUGHBY 1973).

TABLE 3.4 Inflammatory Effects of Lysosomal Products.

Cationic proteins

Increased vascular permeability:

Factor dependent upon mast cell degranulation

Factors independent of histamine release

Chemotaxis of mononuclear phagocytes

Neutrophil immobilizing factor

Acid proteases

Degradation of basement membranes, etc (if pH acid)

Release of leukotoxin from plasma "leukokinogen"

Neutral proteases

Degradation of:

Collagen

Elastin

Cartilage

Fibrin

Generation of chemotactic fragments from C3 and C5

Release of kinin from plasma kininogen

(adapted from RYAN & MAJNO 1977, p240).

The concomitant release of oxygen-derived free radicals from within lysosomes probably also contributes to the tissue damage associated with lysosome discharge (JOHNSTON & LEHMEYER 1976; GOLDSTEIN 1977; KUEHL & EGAN 1980; ZABBUCCHI et al 1980).

It may be that in an "ideal inflammatory process", neutrophils are relatively quiescent, with only limited lysosomal discharge. However, where for example, injury is severe, or bacterial contamination is high, neutrophil emigration and activation is high, as is lysosomal release. Thus, there is significant neutrophil-derived tissue lysis and increased oedema (ARFORS 1983).

3.3.2.4 Other tissue derived mediators.

As a result of injury, and inflammation itself, the tissue in which the inflammatory reaction occurs may contribute factors which affect the inflammatory process. Tissue necrosis may result from infarction (secondary to thrombosis), from destruction by lysosomal enzymes, or complement- or lymphocyte-mediated cytolysis. Such necrosis may liberate additional antigen (thereby boosting the immune response) or active enzymes capable of activating prekallikrein, plasminogen and prothrombin (GILMORE 1976). Other products of tissue breakdown may be chemotactic for leucocytes (RYAN & MAJNO 1977).

Tissue injury is also suggested to lead to the modification of tissue collagen and other protein as a result of the activation of dermal proteases. Such modified tissue proteins, also formed by the action of lysosomal enzymes, are suggested to represent the "true trigger mechanism" of the inflammatory response, the "triggering" being the contact between plasma and these modified tissue proteins (WILLOUGHBY & DI ROSA 1971). While ignored for some years, this concept has now been revived (WILLIAMS 1983). It certainly offers an attractive mechanism for the initiation of the body's response to injury but further investigation is required.

Dermal proteases themselves are also reported to cause a significant increase in vascular permeability and to be chemotactic for leucocytes (particularly PMNs) (FRAKI 1977).

3.3.2.5 Modulation of cell function in inflammation: cyclic nucleotides.

The work of SUTHERLAND (1972) (for which he was awarded the 1971 Nobel Prize for Medicine), was pre-eminent in laying the foundation for

our present understanding of the role of cyclic nucleotides in cell function. Much of this material is as yet hypothetical, or at least chiefly based on in vitro evidence. However, some correlation to the in vivo situation seems to have experimental support.

SUTHERLAND (1972) and others proposed the concept that adenosine 3',5'-monophosphate (cyclic AMP, cAMP) acts as an intracellular "second messenger", mediating the action of various hormones on cell function. (See also NALBANDIAN & HENRY 1978; JOHNSTON et al 1979). Initial studies suggested that cAMP acted as a "turn on" switch whereby hormones could stimulate various organs (for example, heart, liver) or various target endocrine glands (such as the thyroid, adrenals). In the course of investigations it became evident that cAMP also acted to "turn off" or inhibit other cell functions (BOURNE et al 1974). This later research suggested that many inflammatory and immune responses were similarly modulated by the action of cAMP, this cyclic nucleotide again acting as the "second messenger" from the various hormones and mediators of these processes (BOURNE et al 1974).

The functioning of cyclic AMP is now suggested to fall within a complex system of chemical modulation of cell function. Such modulation is suggested to occur in two ways with two major types of compounds involved. Firstly, there are those compounds which directly affect the state or function of cytoplasmic microtubules, and secondly, those compounds which influence the accumulation within the cells of the cyclic nucleotides - cyclic AMP (as described above) and cyclic 3',5'-guanosine monophosphate (cyclic GMP, cGMP) (GOLDSTEIN 1977).

Cytoplasmic microtubules, found in many plant and animal cells, are thought to be significant in the stabilization of cellular form, in

the control of cellular motility and chemotactic responsiveness, and in the regulation of intracellular granule flow (GOLDSTEIN 1977). Such tubules are considered to be in a dynamic state of assembly and disassembly. It is however thought that the assembled form is the most significant to cell mechanics, this being the most conducive of cell activity (GOLDSTEIN 1977).

Various agents which interfere with microtubular function by enhancing disassembly have been identified, including colchicine and vinblastine. Such agents have been shown to inhibit phagocytosis and the redistribution of granule-associated hydrolases (See GOLDSTEIN 1977).

It is tentatively suggested that the cyclic nucleotides exert their modulatory effects partly through alteration of this dynamic balance in microtubule assembly/disassembly. Thus, agents that raise intracellular levels of cAMP (cyclic nucleotides themselves, beta-adrenergic catecholamines, theophylline and E-type prostaglandins) are suggested to limit tubule assembly, thus leading to inhibition of various cell functions (GOLDSTEIN 1977). Conversely, agents which raise the intracellular levels of cyclic GMP promote tubule assembly, enhancing cell function (BOURNE et al 1974; GOLDSTEIN 1977).

This is termed the "Yin-Yang hypothesis" of mediator release, proposed by GOLDBERG et al (1976). This hypothesis is so named because it symbolises a dualism between opposing natural forces, which are postulated to be operative in a wide variety of organ and cell functions (including cardiac contraction, cell proliferation and hormone biosynthesis, storage and secretion (GOLDBERG et al 1976).

The full nature of the interactions of these nucleotides, microtubules and a variety of other cell functions may be merely a matter of convenient speculation. It is nevertheless apparent that the reciprocal effects of cAMP (inhibition) and cGMP (enhancement) on such cell functions correlate, on an ultrastructural basis, with the disassembly and assembly of cytoplasmic microtubules (respectively) (GOLDSTEIN 1977; WEISSMANN et al 1980).

With respect to inflammation and immunity, various actions of the cyclic nucleotides have been demonstrated (at least in vitro). A rise in cAMP inhibits lysosomal release from neutrophils, degranulation and aggregation by platelets, ^{and} lymphokine release from lymphocytes (JOHNSTON et al 1979). Increased cAMP is also suggested to decrease leucocyte adherence to endothelium, (VAN DE STADT 1982) and to inhibit their response to chemotactic stimuli (SPISANI et al 1982; VAN DE STADT 1982).

The opposite effects are reported with raised cGMP which is therefore seen to be stimulatory in many cell systems (BOURNE et al 1974; GOLDSTEIN 1977; JOHNSTON et al 1979; SPISANI et al 1982; VAN DE STADT 1982).

The mechanism of the postulated interaction between the extracellular hormone or mediator and intracellular cyclic AMP is described in FIGURE 3.7. Various clinical effects are attributed to such alterations, particularly in chronic inflammation. These are discussed in the excellent review of HORROBIN (1980).

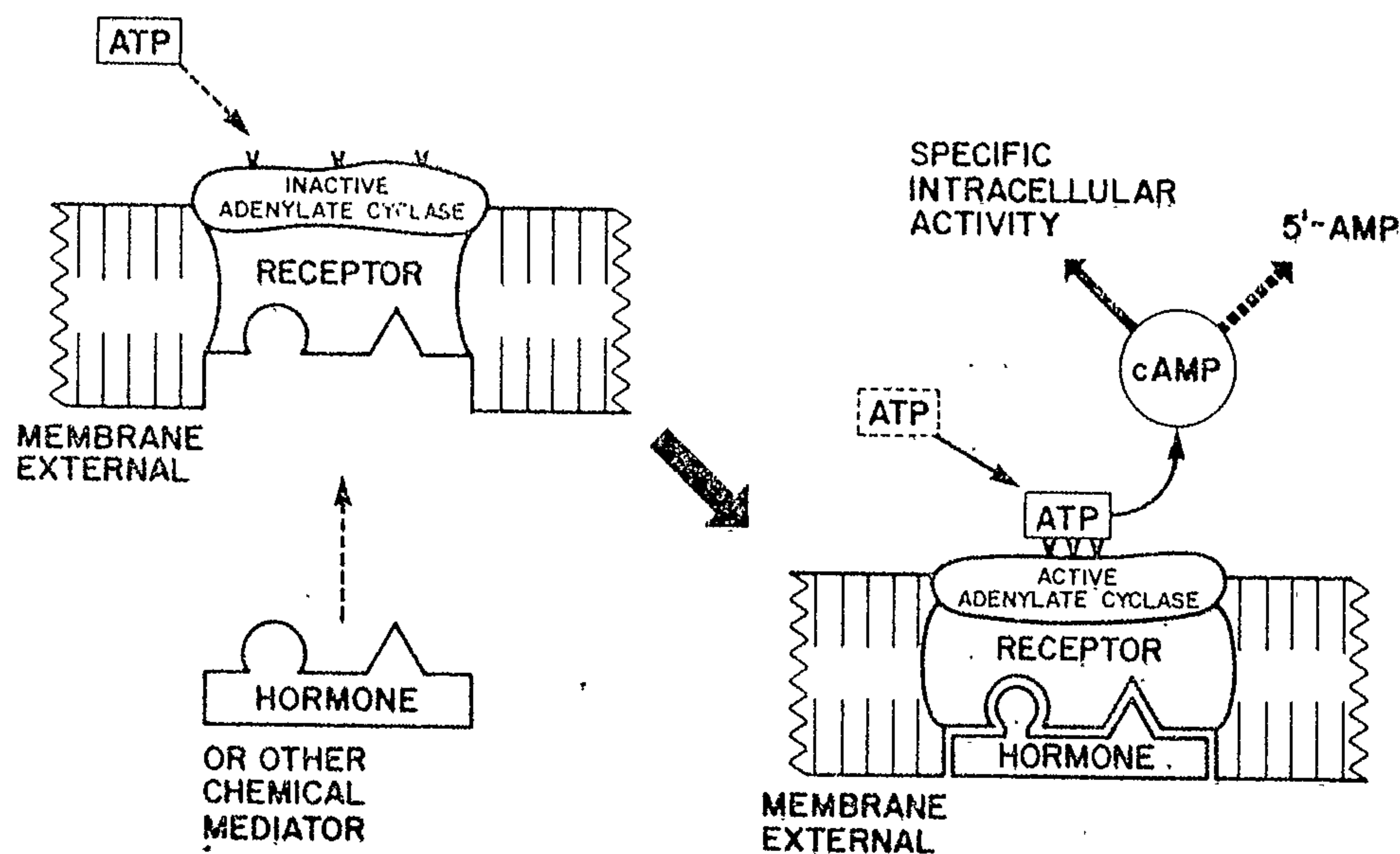


FIGURE 3.7 Mechanism of interaction between extracellular messenger and intracellular cyclic AMP (cAMP). This may be enhanced by specific stimuli which increases membrane fluidity and therefore the coupling between adenylate cyclase and receptor (adapted from NALBANDIAN & HENRY 1978, p101). See also TABLE 3.1.

As described in 3.2.2.6, the interaction of extracellular hormones and various inflammatory stimuli with receptors in the external cell plasmalemma is followed (or accompanied) by locally enhanced membrane fluidity which allows translocation of the outer plasmalemma with respect to the inner, thus permitting coupling of the receptor to adenylate cyclase (bound to the inner membrane). This coupling is enhanced by a second stimulus (for example, C5a) and it leads to the rapid generation of cyclic AMP. Again calcium metabolism is apparently the final event which triggers cell activation (WEISSMANN et al 1980) - compare with the platelet release reaction 1.3.2.2.

3.4 SYSTEMIC CONTROL OF INFLAMMATION.

As was seen in 2.3.8, the blood supply to any given area is controlled by both nervous impulses and humoral agents in addition to local vasomotion in response to tissue needs. Thus it may reasonably be assumed that these factors may be significant in the vascular response to inflammatory stimuli.

Since LEWIS (1927) defined the "triple reaction" (3.3), little attention has been given to this area. One study, however, has shed some light on these mechanisms. APPENZELLER & MCANDREWS (1966) demonstrated that the development of the "flare" in response to histamine was partially under central nervous system control. Significant alterations in flare size (both increased and decreased) were noted in patients with sensory CNS lesions, the definitive site of the CNS lesions determining whether the flare was increased or decreased. Thus, in the presence of sensorimotor cortex lesions on one side, enhanced flare was evident on the contralateral side. By contrast, decreased flare was seen with thalamic or spinothalamic lesions. No alteration was noted in purely motor lesions.

BROWN et al (1968) used a variety of experimental models to investigate these areas further. They demonstrated that in some inflammatory models (rat hind-paw oedema in response to serotonin injection), narcotic analgesics or spinal transection produced only slight diminution of the response. However, the inflammatory response to injections of formalin and carrageenin was considerably delayed by both of these measures. Local anaesthetics produced similar delayed responses. It was further noted that these alterations in response did not occur if narcotic administration or nerve sectioning was delayed until after inflammation had commenced.

On the basis of these observations, it was concluded that some components of the inflammatory response were purely peripheral and thus unaffected by systemic stimuli. Most inflammatory responses, however, were suggested to also involve augmentation of the peripheral reaction by systemic factors.

CNS involvement was suggested to be partly in response to pain impulses generated by algescic substances released in inflammation. Direct vascular effects were also postulated to occur by unknown mechanisms (BROWN et al 1968).

Three levels of activity are described in neural structures in relationship to inflammation - the axon reflex, specific receptor mechanisms, and combinations of effects reflecting the descending (corticospinal) tracts and peripheral efferent innervation (MORELL 1971).

Of these, inflammatory lesions related to the axon reflex are the most clearly defined. Bruce (1910), Krogh (1920), and Lewis and Grant (1924) were among the first to describe this response in which antidromic impulses pass along the sensory nerves to adjacent vessels, producing vasodilatation (cited by FLOREY 1970a) - illustrated earlier in FIGURE 3.2.

Other nervous system effects are poorly delineated. It is clear however, that whilst the manifestations of inflammation may continue in the absence of nervous connections, the nervous system does modulate the response (FLOREY 1970a; MORRELL 1971).

In view of the morphologic continuity between neural and endocrine systems, personality and emotional disturbances are also reported to alter inflammatory/immunological responses (MORRELL 1971). Furthermore, CNS impulses and peripheral sensory information may modulate the various endocrine cells, perhaps explaining the sex predilection observed in some chronic inflammatory diseases, for example, systemic lupus erythematosus and rheumatoid arthritis (MORRELL 1971). The effect of these factors in acute inflammation is a matter

for speculation.

The role of adrenocortical secretions is, however, well known in the maintenance of "vascular tone" through the partial contraction of arteriolar and venular muscle cells (ZWEIFACH et al 1953). It is reported that the precapillary sphincters associated with vessels in inflamed tissues become refractory to these vasoconstricting stimuli both humoral and alpha-adrenergic (ZWEIFACH 1973a). This alteration in receptiveness is suggested to be in response to prostaglandins E1, E2 and A2 (BAEZ 1977a).

Further work is required to clarify these factors. Some further discussion of various humoral effects (both physiologic and pathologic) is presented in Chapter 5.

3.5 CONCLUSION.

The preceding discussion may, to the clinician, appear to contain a mass of facts and hypotheses which frequently seem to be in contradiction with each other. The position is further complicated by the fact that there are a variety of experimental models of inflammation and different animals under investigation. These models are generally designed to examine specific aspects of the inflammatory process. However, this isolation of components of inflammation is not seen in vivo, and the clinician is left with the unenviable task of having to determine which portions of experimental data are relevant to the particular human condition with which he or she is concerned. He or she must then assimilate this information and develop diagnostic and treatment protocols which are thus in harmony with physiological considerations. An attempt has been made at this synthesis in Chapter 10.

In summary, inflammation in response to tissue injury is clinically characterised by redness and swelling, with heat and pain (CELSUS). In addition, surgical injury also includes frank extravasation and the haemostatic fibrin clot.

Physiologically, the injury is seen to produce tissue and vessel injury in varying degrees, from minor reversible changes, to irreversible damage and cell death. Macroscopically, gross disruption of the tissues and vasculature is evident, obviously concomitant to surgical incision. Such disruption and tissue damage causes significantly increased vascular permeability with resultant protein and fluid exudation (clinically evident as oedema) and hyperaemia (manifested as erythema). The significance of these alterations is that they facilitate contact of circulating leucocytes and components of the plasma with the damaged tissues.

Multiple pharmacologically active substances are evolved as a result of this contact. These substances are significant in various ways. It is important to note that experimental evidence suggests that the microscopically observed events which are seen to follow injury may be mediated by several of these substances together producing additive, synergistic or (sometimes) inhibitory effects.

Chemical mediators of this nature are reported to act in the following ways, producing the processes which are together known as "inflammation". Mediators may:

1. be chemotactic for thrombocytes (TXA₂) and leucocytes (C5a, leukotrienes and others) leading to aggregation of these cells in the vicinity and the migration of leucocytes into the tissues;
2. produce haemostasis in combination with the aggregated thrombocytes.
3. maintain and extend the vascular response (outlined above) -

histamine, bradykinin, PGs;

4. cause lysis of damaged cells and tissue components, and particularly extravascular fibrin. This lysis is enhanced by the function of PMN leucocytes as a result of the release of intracellular lysosomal contents, and later (24-48 hours) by their degeneration. (Monocytic macrophages are also active here);
5. sensitise local nociceptors;
6. be pyrogenic, producing systemic fever.

These responses may be significantly compounded by the presence of bacterial contamination, or large amounts of extravascular clot and necrotic tissue (see 1.4).

It is important to re-emphasise that the direct surgical injury itself is primary to the altered vascular response as has been proposed by HURLEY (1972; 1981; 1983). Thus significant leakage will be maintained until this damage can be repaired - up to 48 hours later (RYAN & MAJNO 1977). This is now believed to commonly involve regrowth of endothelium to replace terminally injured cells (U. RYAN 1983; HURLEY 1983).

Obviously, the release of chemical mediators which advance the inflammatory "lesion" will occur automatically upon tissue/plasma contact, so enhancing the effect of tissue damage. The proportional relationship between mediated and direct effects has not been determined. Mediated exudation must however, have some significance since its pharmacological suppression does appear to limit the amount of oedema which is formed. This important question is discussed further in association with anti-inflammatory agents and oedema control (Chapter 8).

An interesting postulation followed the work of GRYGLEWSKI

(1981) with chlorpromazine. The action of this anti-inflammatory drug was found to depend upon the relative concentrations of essential co-factors, with opposite effects being seen with different combinations. Thus the in vivo milieu at the site of inflammation may be critical to the expression of action of many such drugs and possibly also of the proposed mediators of inflammation. This may be one reason why experimental results conflict.

The other critical question for the clinician must be related to the role of inflammation in wound repair. This matter, which remains widely debated, is obviously an essential part of the clinician's assessment of the need for pharmacological modulation of these factors. Chapter 9 is devoted to assessment of these considerations.

Prerequisite to these discussions is an examination of the physiological mechanisms by which oedema accumulates, and of situations in which oedema formation may be altered (both endogenous and exogenous). These questions are examined in Chapters 4, 5 and 6 respectively. Chapter 4 also describes the physiological nature of oedema fluid.