

CHAPTER 4 MICROCIRCULATION AND OEDEMA FORMATION.

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4.1 INTRODUCTION.

The development of oedema is an obvious result of many oral surgical procedures. As has been seen, this oedema follows changes in the blood vessel walls in response to injury. These changes result in increased movement of fluid, macromolecules and cells across the wall. Other factors must however be involved since exercise may also produce similar endothelial alterations with increased fluid filtration and abnormal cell migration (SMITH et al 1970), but without the formation of oedema.

Understanding of the clinical picture of post-surgical oedema is further confused by the considerable variability of the magnitude of the reaction, even to apparently identical surgical procedures (BARCLAY 1970, OSBORNE 1973). Clinicians and researchers have implicated many factors in the modulation of oedema formation, thereby hoping to explain this variability. These factors will be considered in Chapters 5 and 6. However, as will be seen, there is little agreement even as to what aspects of surgery exacerbate the oedematous response, let alone to provide an explanation for the observed individual variability.

That the basic physiological and physico-chemical tissue responses to injury and inflammation remain hypothetical probably explains this clinical dilemma. A detailed review of these responses and the associated physiology is therefore critical to the drawing of conclusions concerning post-surgical oedema.

4.2 THE STARLING HYPOTHESIS.

4.2.1 Capillary Filtration Forces.

The net exchange of fluid between the blood and extravascular tissue mainly involves convective or bulk flow as determined by hydrostatic and osmotic pressure gradients across the wall of the blood microvessels (ZWEIFACH 1972).

This filtration is influenced, under both physiological and pathological conditions, by three factors - adjustments in blood fluid distribution, changes in capillary pressure, and changes in capillary colloid osmotic pressure (COP). The rate of fluid transfer relating to these changes in capillary and colloid osmotic pressures is designated as the capillary filtration co-efficient (CFC). The CFC is primarily determined by the magnitude of the capillary surface area (that is, the number of functionally open capillaries) and by capillary permeability (which is relatively constant under normal circumstances) (ZWEIFACH 1972).

Under physiological strain (that is, exercise), a progressive rise in CFC, parallel to increasing flow, is described; a three-fold increase in flow is thus followed by a two-to-three-fold increase in CFC.

In inflammation, as a result of various chemical mediators, increases in blood flow of up to ten fold are demonstrable (ZWEIFACH 1972; WELLS 1973; HURLEY 1981). Capillary filtration is unable to maintain parallel increases in such extreme circumstances and its corresponding rise is much lower (ZWEIFACH 1972). This is possibly a result of tissue resistance preventing such rapid fluid outflow. See 4.2.3 for further discussion on this point.

4.2.1.1 Capillary hydrostatic pressure.

As blood moves from the heart and through the vascular system, the resistance offered by the repeatedly branching, narrowing ramifications of the arterial system gradually erodes the pressure which results from cardiac contraction (STROMBERG & WIEDERHIELM 1977). While little pressure reduction occurs in the large arteries, pressure loss becomes progressively greater in the smallest "branches" of the arterial tree.

Landis in 1926 provided the first pressure profile of a microvascular bed. He demonstrated that the greatest pressure reduction occurred between the terminal arteries and the arterial capillaries, and thus that changes in arteriolar calibre exerted the greatest influence on blood flow and hydrostatic pressure in capillaries (cited by STROMBERG & WIEDERHIELM 1977). The effect of this reduction is discussed in 4.2.3.

Mean hydrostatic pressure is determined by the volume of blood in the microvessel. Consequently it depends on the relationship between inflow (as a function of arterial pressure and the resistance to blood flow through the arteries) and outflow (determined by resistance to blood flow through the veins and the level of outflow or venous pressure). This is assuming constant vessel wall compliance in the region (HADDY et al 1976).

Capillary hydrostatic pressure represents the driving force for longitudinal flow of blood in the capillary lumen. It is also important in radially-oriented fluid exchange through its interactions with osmotic gradients (HADDY et al 1976). Under physiological conditions, this pressure may vary both spatially along the length of a capillary

and as a random function of time in a given capillary, and among different capillaries in the same microvascular bed (DIANA & FLEMING 1979).

This variation in flow occurs in response to external (neuroendocrine) and local metabolic influences. Such changes are mediated by the level of contraction of the vascular smooth muscle and may dramatically affect the net transcapillary exchange (DIANA & FLEMING 1979).

Capillary Hydrostatic Pressure in Inflammation

Little consideration has been specifically directed to the changes which occur in capillary hydrostatic pressure in areas of inflammation. It may be reasonably assumed that the above mentioned increases in blood flow (up to ten fold) which characterise the initial phase of inflammation would be associated with markedly increased hydrostatic pressure.

However, as the inflammatory reaction progresses, the vessel wall compliance alters in response to injury and mediators. The resulting increased permeability allows marked fluid loss from the vasculature. Flow rate therefore declines because the microvessels (particularly venules) are perfused with blood of increasingly higher viscosity (in other words, post-capillary resistance increases). At stasis in these vessels, the full arterial hydrostatic pressure is brought to bear on the capillaries (ZWEIFACH 1972). Fluid transudation is therefore maintained.

Progressing further, the arteriolar dilatation subsides, stasis generally resolves and normal flow resumes where vessel integrity is intact. Capillary hydrostatic pressure is likely to follow accordingly.

Where vessels have been severed, haemostatic measures become fully operant and platelet and fibrin thrombi completely arrest flow. Hence, hydrostatic pressure here is minimal until flow resumes with recanalisation of the obstructed vessels.

The effect of these changes will be discussed shortly.

4.2.1.2 Plasma oncotic pressures

The osmotic activity of the plasma is, for convenience, separated into two components: the activity attributable to macromolecules (the plasma proteins) - that is, the colloid osmotic pressure, and that attributable to low molecular weight substances (including salt, glucose) - the crystalloid osmotic pressure. (Note: The terminology used here describing the osmotic activity of these substances as "pressures" is in keeping with that of the physiological literature where current investigation is generally based on hypothetical experimental models. Since these models provide simplicity and correlate reasonably well with observed in vivo functions, the terminology persists although no such osmotic "pressure" exists under thermodynamic and mechanical laws. (See SILBERBERG 1979)).

In his description of the microcirculation, STARLING (1896) postulated that there was a "balance of forces" acting across the capillary wall. This balance explained how intravascular volume was maintained when it would otherwise be expected to be gradually transudated into the interstitium as a result of intravascular hydrostatic pressure (STROMBERG & WIEDERHIELM 1977).

The plasma proteins are most significant in this balance because they are, for the most part, retained within the internal compartment

under physiological conditions. The plasma crystalloids, being able to freely diffuse to and fro across the vessel wall, are of limited significance, though not without some effect, as will be seen.

The plasma colloid osmotic pressure.

That force which is required to balance the osmotic attraction which the plasma proteins exert across the semipermeable membrane (vascular wall) is "operationally defined" ... as the "protein oncotic pressure" or "plasma colloid osmotic pressure" (STROMBERG & WIEDERHIELM 1977). This pressure is chiefly responsible for resisting the loss of fluid from the vessels, thus maintaining the fluid balance. In man, this pressure is usually recorded at between 24 and 27 mm Hg (LANDIS & PAPPENHEIMER 1965; HADDY et al 1976; STROMBERG & WIEDERHIELM 1977).

The plasma colloid osmotic pressure (COP) is dependent on the concentration of protein in the plasma, and thus upon the relative amounts of circulating protein and water. The amount of circulating protein is chiefly influenced by protein production and losses; the amount of circulating water by intake, output and the level of tissue hydration (governed by Starling's forces) (HADDY et al 1976).

The various plasma proteins contribute different proportions to the total COP. Albumin is normally considered the most significant. This is because the albumin molecule is approximately half the size of other plasma proteins such as globulin, and because it is present at higher concentrations in most species (LANDIS & PAPPENHEIMER 1965, HADDY et al 1976).

The plasma COP shows considerable fluctuations under normal conditions suggesting that important control mechanisms are in operation, possibly involving alterations in lymph flow (STROMBERG &

WIEDERHIELM 1977). Other factors may also be involved. For example, it was commonly believed that the plasma COP would decrease following surgery, proportional to the amount of blood lost and the post-haemorrhagic hypovolaemia (resulting from dilution associated with the early phase of volume restitution). However, this was not found to be so, probably due to alterations in the level of tissue hydration (LADEGAARD-PEDERSEN 1974).

In almost all situations, the plasma COP must be regarded as exceeding the interstitial COP. Where frank extravasation or extensive tissue lysis has occurred, this balance between interstitial and plasma COP may be grossly altered. (See 4.2.3 for further discussion).

4.2.2 Interstitial Forces.

As reported in 3.4, the interstitium is thought to be a two-phase system; a gel-like phase consisting of a system of interconnected fibres and glycosaminoglycans (colloid-rich, water-poor phase - CATCHPOLE 1973, DIANA & FLEMING 1979), and an aqueous solution containing proteins (colloid-poor, water-rich phase - CATCHPOLE 1973, DIANA & FLEMING 1979), (HADDY et al 1976, MEYER & SILBERBERG 1977). These phases are suggested to be labile and in thermodynamic equilibrium with each other (DIANA & FLEMING 1979). The colloid-poor, water-rich phase appears to be generally organised into channels (CASLEY-SMITH & VINCENT 1978) which facilitate much higher fluxes of water and macromolecules through the tissues (see 4.3). Section 2.4 contains detailed discussion of the postulated interstitial structure.

These features are particularly important determinants of interstitial fluid and macromolecule movement. The interstitium thus forms the second limb in the balance of forces which directs the

transfer of fluid and molecules across the capillary wall (STROMBERG & WIEDERHIELM 1977). Both hydrostatic and osmotic pressures within the tissues are again involved.

The formation of oedema is seen to progressively disrupt the dynamic balance of these forces (see 4.2.3).

4.2.2.1 Interstitial fluid hydrostatic pressure.

This pressure depends upon the interstitial fluid volume and tissue compliance.

Interstitial fluid volume

All fluids in and around a particular tissue can contribute to its "interstitial fluid pressure". This includes fluid in the interstitial compartment itself, and fluid in the cell compartment, and in the lymphatic and blood vascular beds.

In the hypothetical situation where the volume of fluid in the cell compartment is constant, the volume of fluid in the interstitial compartment of simple tissues (of which skin and muscle are examples), depends on the net fluid influx into the interstitium from the microvessels, and the efflux from the interstitium via the lymphatics. Increased influx or decreased efflux will raise the volume and hence the pressure, especially in tissues with low compliance (see later). The resulting raised interstitial hydrostatic pressure will tend to automatically limit further increases in tissue volume, in other words, to defend against the formation of oedema. Thus influx from the vessels will tend to be suppressed and lymphatic efflux will tend to be increased (HADDY et al 1976).

Obviously this is a somewhat simplified picture, and in fact, the volume of the cell compartment is not constant, which thus adds an additional variable. It does however illustrate the considerable capacity of the tissues to modify their fluid volumes and pressures so as to limit the development of oedema.

The measurement of tissue hydrostatic pressure (THP) has proved exceedingly difficult. Not least of the difficulties is the very small dimensions which are involved (STROMBERG & WIEDERHIELM 1977). Various techniques have been used in an effort to quantify the magnitude of tissue hydrostatic pressure. The excellent studies of McMASTER (1946), using the smallest available hypodermic needles, along with more recent studies using similar techniques with micropipettes (WIEDERHIELM 1969, cited by STROMBERG & WIEDERHIELM 1977), demonstrate slightly positive pressures (with respect to atmospheric pressure), around 2.5 mm Hg in subcutaneous tissues (STROMBERG & WIEDERHIELM 1977) - needle/pipette technique.

The "capsule technique" developed by Guyton (1963, cited by GUYTON et al 1971) involved measurement of the pressure found in the fluid space within an implanted hollow perforated plastic sphere or cylinder. This "capsule" was allowed to heal in situ for four or more weeks (on the assumption that this "healing-in period" is not representative of normal connective tissue). Using this technique, Guyton demonstrated pressures of -6 to -7 mm Hg.

Scholander et al (1968, cited by STROMBERG & WIEDERHIELM 1977) developed a "wick technique" in which a catheter is inserted into tissue through a relatively large trocar. The tip of the catheter is filled with a porous material and connected at the other end to a pressure transducer. This technique also demonstrates negative tissue

pressures but of lower magnitude than those found with the capsule (-3 to -4 mm Hg).

It is difficult to reconcile the differences between the needle/pipette techniques and those results obtained with capsule or wick. The former are suggested to be limited because they are thought to necessarily involve significant tissue distortions to "create" spaces in which the pressures are measured. On the other hand, the measurements obtained using the capsule and wick techniques may be measuring osmotic factors in addition to hydrostatic pressure (STROMBERG & WIEDERHIELM 1977). These techniques are well reviewed by RENEMAN and ARTS (1977), STROMBERG and WIEDERHIELM (1977), and WIEDERHIELM (1977b).

Although an exact value for THP is not yet available, it is an important factor in the regulation of the rate and direction of transcapillary pressure gradients. It is also important in discussions of the entry of fluid to lymphatics. As was illustrated in introducing this section, the relationship between THP and the volume of the interstitial fluid demonstrates significant capacity for the moderation of fluid movement and it may thus represent part of a complex feedback control system for body fluid balance (DIANA & FLEMING 1979).

Tissue compliance

The compliance of a tissue is a function of its ability to undergo dimensional, particularly volumetric, change. It is chiefly governed by the nature of restraints by which such a tissue is surrounded, though the nature of its connective tissue matrix (loose, areolar/dense, fibrous, and so on) is also significant (see later).

Thus, bone marrow and brain, being enclosed in a rigid "box" have low compliance. Likewise the kidney, (which has a rigid stroma and is enclosed in a fibrous capsule), skeletal muscle (which is covered by fascia), and the abdominal viscera (which is retained by the abdominal wall). All such tissues have low effective tissue compliance and consequently, for a given interstitial fluid volume, interstitial pressure is high (HADDY et al 1976).

It has been proposed that subcutaneous tissue has a non-linear compliance, being at first very low (strongly resisting the initial development of oedema) and then very high as the tissue becomes hydrated (HADDY et al 1976).

Tissue compliance is also governed on biochemical grounds, by the two phase structure of which its connective tissue is arranged. Thus, the presence of large polyanionic mucopolysaccharides in the gel phase implies the existence of a non-uniform distribution of both small and large charged molecules (sodium, potassium, and albumin, respectively). As will be seen, the proteoglycans of the gel phase tend to exclude such molecules both sterically and ionically. This exclusion is graded, increasing with increasing molecular weight. Its effect is that the gel-phase provides high resistance to flow and diffusion, whereas the fluid-phase facilitates such flow (DIANA & FLEMING 1979). Thus the compliance of a tissue varies depending on the concentrations of the various connective tissue components, as well as on their spatial arrangement. See also 4.4.

It might be postulated on the basis of this information that palatal tissues, having a dense fibrous stroma, might have a relatively low compliance. Buccal tissues (mucosa through to the skin) on the other hand, contain fairly loose areolar connective tissue and might be

considered to have high compliance.

Inter-patient differences in response to injury are likely to result, at least in part, from differing genetically-determined connective tissue compositions and arrangements. These may be altered in various disease states, and in response to ageing (see Chapter 5).

4.2.2.2 Interstitial colloidal osmotic pressure (COP).

As in the plasma, the interstitial colloidal osmotic pressure depends upon the concentration of macromolecules in the tissues. Protein leakage from the vasculature (see 2.3.5) accounts for most of this. It is balanced by the lymphatic efflux. On average it is calculated that the interstitial concentration of such plasma proteins is between 1.7 and 2.0 per cent, yielding an osmotic force of 4.5 mm Hg (STROMBERG & WIEDERHIELM 1977). The amount of water in the tissues is again a function of the dynamic balance of Starling's forces (HADDY et al 1976). This is not, however, the whole story.

Other factors also contribute to this COP, particularly the presence of other osmotically active particles (such as the proteoglycans) within the interstitium. The relative amounts and compositions of these macromolecules vary widely among the tissues (HADDY et al 1976). As has been described (2.4), these molecules are not randomly dispersed throughout the interstitium but are concentrated in association with the entangled "structural" fibre network of collagen and elastin (the "colloid-rich phase") (MEYER & SILBERBERG 1977; DIANA & FLEMING 1979). The interstitial plasma proteins tend to be excluded from this phase by the proteoglycans and are therefore usually limited to the colloid-poor phase (DIANA & FLEMING 1979).

While both protein and proteoglycan exert osmotic pressures of their own, measurements of tissue osmotic pressure suggest that the "oncotic pressure of the protein-hyaluronate mixture is greater than the algebraic sum of the two oncotic pressures measured individually" (DIANA & FLEMING 1979). The effective exclusion of protein from the colloid-rich phase (mentioned above) with the subsequent reduction of the interstitial fluid volume available to the plasma proteins may explain this. Known as the "volume exclusion effect", this phenomenon results from the obvious fact that two molecules cannot occupy the same space at the same time, and thus their centres do not come closer than the sum of their radii. Excluded volume is therefore larger for larger molecules (WIEDERHIELM 1977a;b; DIANA & FLEMING 1979). This increases the effective protein concentration, which in turn increases the tissue oncotic pressure (WIEDERHIELM & BLACK 1976; STROMBERG & WIEDERHIELM 1977; DIANA & FLEMING 1979).

Interstitial collagen also appears to contribute to the tissue COP. Because of its insolubility, this molecule was thought to lack osmotic activity and therefore to be inert in terms of its effect on the capillary fluid balance. Recently however, collagen (and probably also elastin) was found to profoundly influence interstitial fluid osmotic pressures. This is probably through its contribution to the volume exclusion effect (WIEDERHIELM & BLACK 1976; STROMBERG & WIEDERHIELM 1977).

4.2.3 Gradients of fluid movement/plasma escape.

The flow of materials occurs along gradients of chemical potential, with each molecular species having its own potential. Any realistic model of transport between blood and tissue would require a very complex statement of all such gradients. Such a model would be

further complicated by the variety of constants which are necessary to describe such movement, and by the "mutual influence of the movement of one species upon another" (SILBERBERG 1979).

This situation can therefore only be roughly approximated. Hence, most discussion of the fluid and macromolecular fluxes between blood vessels, and between blood and lymphatic vessels, is primarily based on simplified hypothetical models. In such models, these fluxes occur chiefly because of gradients which develop following dynamic interactions between the previously mentioned forces. Such gradients operate under a variety of physiological conditions. Though disruption of the balance occurs in inflammation, these gradients are also significant in fluid/macromolecule fluxes here.

The colloid osmotic pressure gradient is defined by the difference between the interstitial and plasma COP's. This difference is primarily governed by the permeability of the vessel wall to the plasma proteins (HADDY et al 1976). With the plasma protein concentration being almost always higher within the microvessel, the COP gradient tends to prevent "reabsorption" of protein from the tissues (SILBERBERG 1979).

Similarly, the hydrostatic pressure gradient is the difference between the interstitial and vascular hydrostatic pressures. It is this gradient which is the driving force of fluid movement out of the vascular compartment. It is reiterated that the magnitude of this gradient continues to be disputed because of the polarisation of investigators concerning the size of the interstitial hydrostatic pressure (4.2.2.1). Resolution of this impasse would appear to require technological advances (HADDY et al 1976; STROMBERG & WIEDERHIELM 1977).

It was Landis who demonstrated that the hydrostatic pressure in arterial capillaries (average 32mmHg) exceeded the osmotic pressure exerted by the plasma proteins (24-27mmHg) (see 4.2.1.2) whilst the situation was reversed at the venous end of the microcirculation (average HP 12mmHg). This reversal results from the gradually decreasing capillary hydrostatic pressure along the length of the vessel as the internal pressure is dissipated. The relationship between these two gradients in the various segments of the vasculature therefore governs fluid transudation and reabsorption across the endothelial wall, as Starling had hypothesised (HADDY et al 1976; STROMBERG & WIEDERHIELM 1977; SILBERBERG 1979).

This concept of arterial outward filtration and venous reabsorption is chiefly a statistical concept since the arterial and venous limbs are in fact not separate but form a continuous network. Further, whole regions may at times be devoted to filtration or reabsorption. The concept does however provide a reasonable approximation and it has been valuable in testing these hypotheses against physiological and thermodynamic principles (CASLEY-SMITH 1976a). These aspects are thoroughly reviewed by HADDY et al 1976.

The dynamic nature of the interactions between these gradients permits great physiological variability and many aspects tend to limit excessive fluid shifts into the interstitium. The balance is highly dependent upon water and protein movement into the tissues and its clearance from them by capillary reabsorption and lymphatic drainage. This "coupling between lymphatic drainage of fluid, capillary reabsorption of fluid, interstitial hydrostatic and osmotic forces and their relation to interstitial fluid volume and colloid concentration remains to be explicitly clarified" (DIANA & FLEMING 1979). Whatever

the mechanisms, it is obvious that rapid accommodations may occur should one component (for example, capillary hydrostatic pressure) undergo alteration. Again, the physiological balance tends to limit fluid accumulation (DIANA & FLEMING 1979).

In addition to these plasma protein gradients, osmotic gradients may also occur following alterations in the concentration of the small molecules to which the microvascular membrane is normally permeable. Such a gradient can induce a consequent transcapillary flux depending upon the direction of the gradient. This flux is however transient because the small molecules quickly equilibrate, thus abolishing the gradient (HADDY et al 1976). An influx of such molecules into the interstitium would be expected with initial haemorrhage.

These gradients are illustrated in FIGURE 4.1.

Fluid gradients in inflammation.

Turning now to the changes which occur in this dynamic balance in response to inflammation, it is again apparent that the subject is only sparsely covered in the literature. The most obvious feature is that the increased vascular permeability and hyperaemia produce exudation of protein-rich fluid in such quantities that the normal wide range of physiological adaptability is increasingly unable to restore the fluid/protein balance (FLOREY 1970b).

This progressive collection of fluid within the tissues leads to a number of changes in the interstitium (as described in Chapter 9). However, in many respects it is probably the increased tissue protein concentration which has the greatest significance. This concentration increases following frank extravasation and vessel permeability alterations, and later, from the breakdown of cellular debris. All

such changes dramatically increase the concentration of osmotically active molecules within the tissues, further reducing the COP gradient responsible for drawing fluid back towards the blood vessels (FLOREY 1970b). This fall in the COP gradient is concurrent with the markedly increased vessel hydrostatic pressure (5.2.1.1), causing intense fluid accumulation.

As will be seen later, the lymphatics and the tissue protein concentration must also be considered in discussions of the development of oedema (see 4.7 and 4.8). These gradients in inflammation are illustrated in FIGURE 4.2.

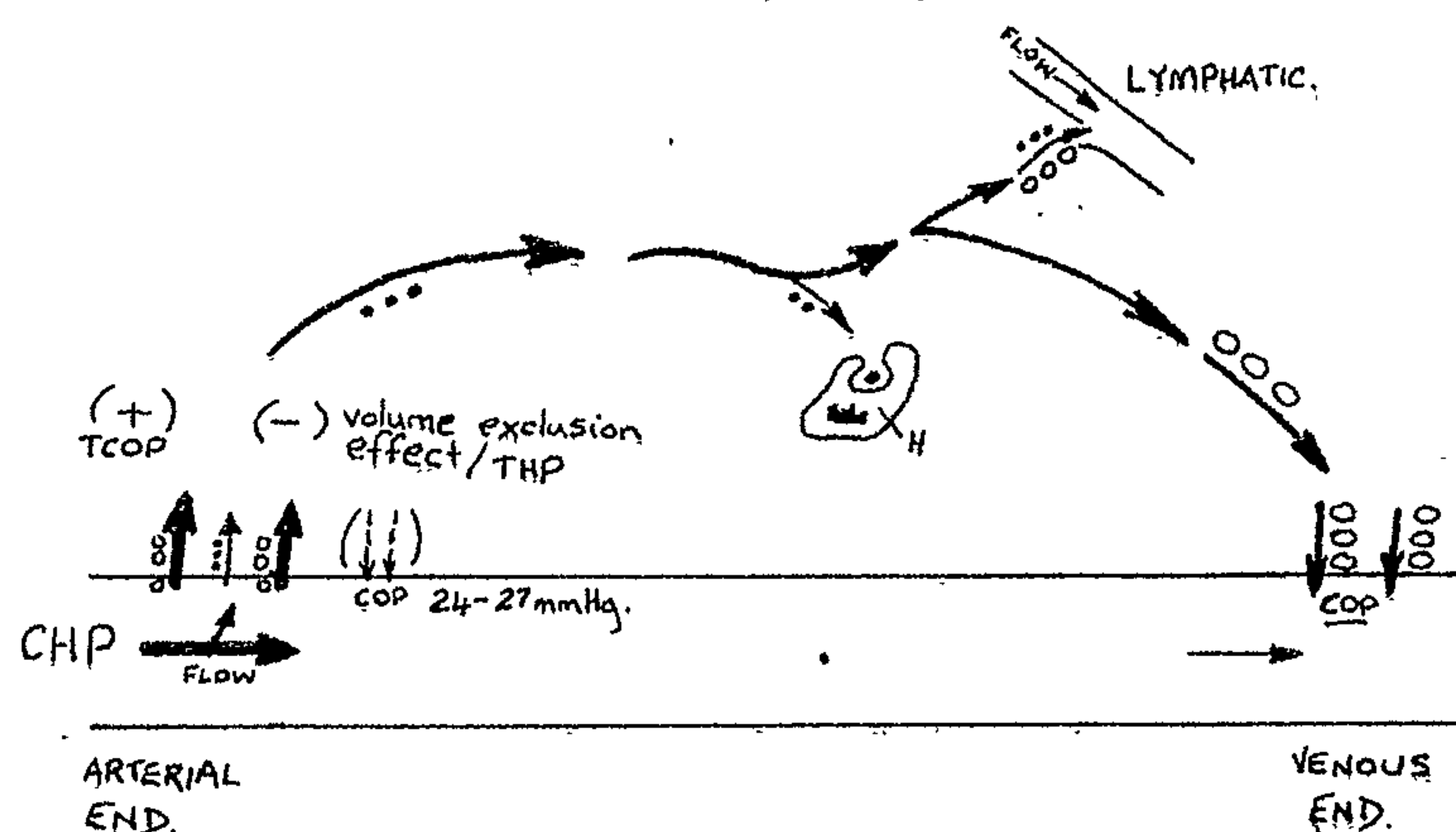


FIGURE 4.1 Forces governing movement of fluid and proteins across the un-inflamed capillary wall.

- | | | |
|----------------------------------|---------------------------------------|----------------------------------|
| → gradient causing movement. | ○ fluid. | H tissue histiocyte (phagocyte). |
| --- gradient resisting movement. | ● protein/macromolecules. | |
| + attracting force. | THP tissue hydrostatic pressure (HP). | |
| - resisting force. | CHP capillary HP. | |
| | COP colloid osmotic pressure (COP). | |
| | TCOP tissue COP. | |

NOTE. The size of the symbol quantifies the volume of the component concerned.

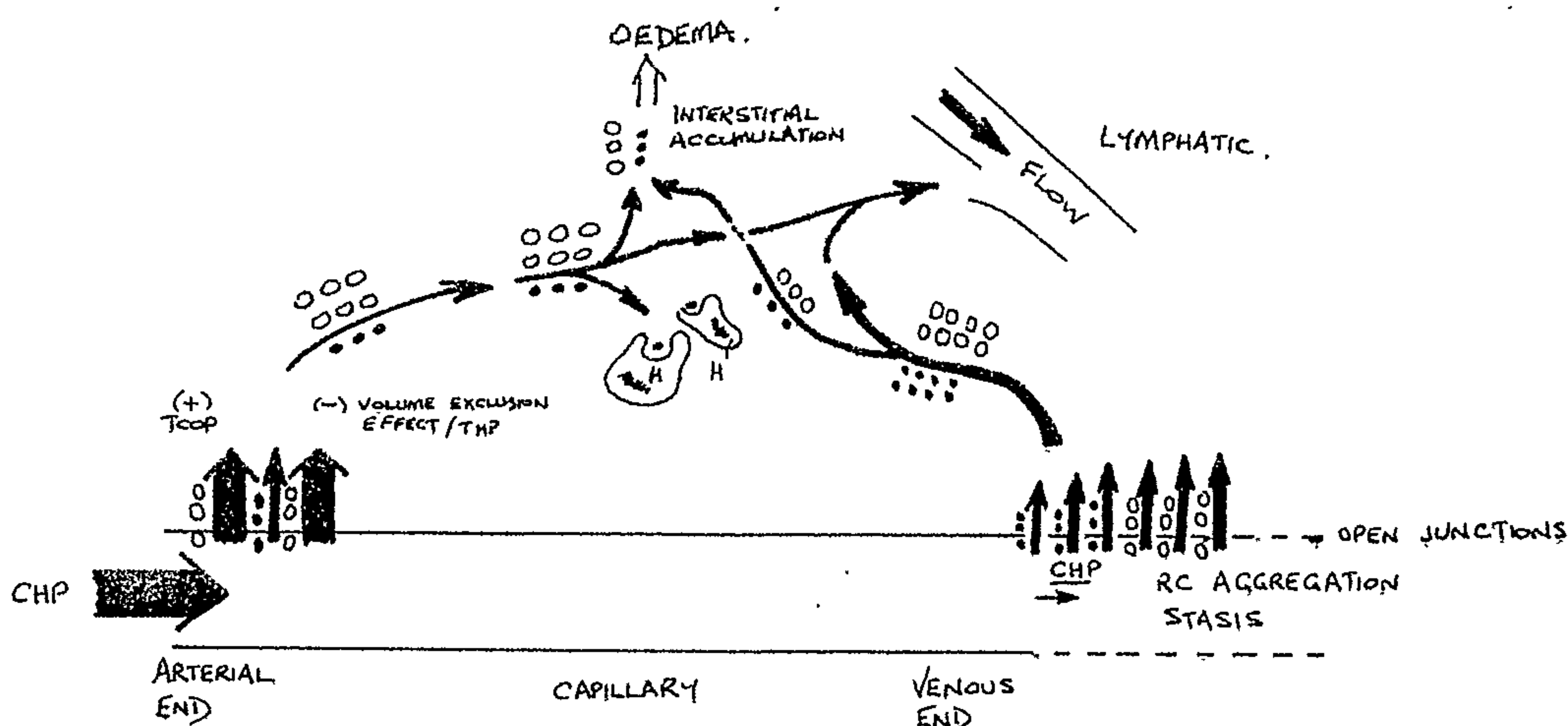


FIGURE 4.2 Forces governing movement of fluid and proteins across the microvessels in inflammation, RC= erythrocyte; other symbols as in FIGURE 4.1.

With the progression of time, these initial changes are modified. In particular, some of the vessels which initially permitted significant macromolecular flux develop vessel stasis and microthrombi. However, closure of transendothelial avenues (the intercellular junctions) in these circumstances is not seen to be accompanied by the resumption of normal transendothelial exchange. This is primarily because vessel flow is occluded, thus aborting any possible reabsorptive functioning. Eventually, as recovery proceeds, stasis may resolve, or thrombosed vessels may be recanalized or replaced (SMITH et al 1970).

4.3 TRANSPORT FUNCTION OF CONNECTIVE TISSUE.

It is suggested that several types of flow may occur through the interstitium. Principally these are i) the diffusional flow of water, ii) the bulk flow of solvents in general, and iii) the translational transport of macromolecules (HRUZA 1977). Such flow is influenced to a greater or lesser extent by the two-phase constitution of connective tissue, being chiefly a function of the degree of exclusion of molecules from the colloid-rich, water-poor phase (DIANA & FLEMING 1979).

While such exclusion is related to molecule size, the immensity of the proteoglycan network is so great that the movement of small molecules may also be impeded (HADDY et al 1976; DIANA & FLEMING 1979). The channels into which the colloid-poor phase is arranged thus facilitate fluid and molecular movement (SILBERBERG 1979). The size and number of these channels greatly increase in situations of oedema (CASLEY-SMITH 1979a). That fluid movement occurs at all is obviously dependent on the transcapillary filtration, and on reabsorption (venular and lymphatic) as a function of the Starling balance.

Whilst the arrangement of the proteoglycans is important in controlling the filtration rate and the porosity of the interstitial space, under normal circumstances it does not selectively retain serum proteins during such filtration. Thus, at rest, no concentration gradients are thought to occur; lymph and interstitial fluid will possess the same composition (MEYER & SILBERBERG 1977). This position is altered in inflammation when lymphatic function is disrupted.

The permeability of a tissue to macromolecules is said to be a function of the percentage composition of proteoglycans and their level of aggregation (HRUZA 1977). Increased aggregation may result from ageing or from the application of cortisone; aggregation decreases following hyaluronidase treatment or in scurvy. Tissue permeability would be seen to decrease should aggregation increase and vice versa (HRUZA 1977). The biologically active amines released in inflammation promote similar disaggregation of the connective tissue matrix, thus reducing the natural tissue resistance to oedema formation (ZWEIFACH 1972; HRUZA 1977).

4.4 TISSUE RESISTANCE TO FLUID ACCUMULATION.

The ultrastructure of the interstitium is suggested to maintain the fluid balance in various ways. The structural configuration of the colloid-rich or "fixed" phase is thought to provide tissue shape via the fibre network. This network also provides elastic recoil which tends to resist extension from the resting state (MEYER & SILBERBERG 1977). It probably also contributes to definition of the compliance of a tissue. Further, the fluid partition between the colloid-poor phase and the colloid-rich phase is suggested to produce an osmotic buffering effect which will limit excessive fluid movement between the two phases (DIANA & FLEMING 1979).

It is noted however that tissue which has been digested by hyaluronidase does not swell in an excess of Ringer's medium, unlike normal tissue which approximately doubles its initial volume. This demonstrates that swelling results from the molecularly dispersed tissue proteoglycans tending to dilute themselves (MEYER & SILBERBERG 1977). In the undigested state, the accumulated fluid is maintained within the tissue section, whereas tissue which has been digested by hyaluronidase allows rapid dispersion of fluid and molecules.

These features are clinically and experimentally evident. LANDIS and PAPPENHEIMER (1965) demonstrated as an incidental finding, that considerable resistance had to be overcome if rapid increases in interstitial fluid volume were to be achieved. Resistance was apparent even to more gradual increases. This resistance is similarly apparent in the infiltration of local anaesthetic solution, as is the effect of differing connective tissue constitutions on fluid movement; palatal infiltration requiring greater force than buccal.

These observations suggest that, under increased tissue pressure, water flow to surrounding tissue spaces is slow, as is its return after the pressure is released. These illustrations thus demonstrate variations in tissue resistance to fluid movement and accumulation (HRUZA 1977). This resistance is probably a result of the above described interactions involving the proteoglycans, together with the effects of the support structure provided by the connective tissue fibre network (MEYER & SILBERBERG 1977) and the "compliance" of the tissue.

These protective effects are obviously overwhelmed by surgical injury and the inflammation which it initiates.

4.5 CLASSES OF OEDEMA.

Casley-Smith has been instrumental in the development of knowledge of the lymphatic system in normal function and in oedema (See CASLEY-SMITH 1977c; 1983b). In conjunction with Foldi, he has broadly classified oedema into three groups according to the protein concentration of the tissue fluid (and lymph) and the rates of flow of this fluid.

These are: i) the low-protein concentration (less than approximately 1 g/dl), high-flow,
ii) the high-protein concentration (greater than approximately 2 g/dl), high-flow, and
iii) the high-protein concentration, low-flow oedemas (FOLDI & CASLEY-SMITH 1978).

4.5.1 Low Protein, High-Flow Oedemas.

These are reported to be caused by disturbances of the Starling equilibrium. Increased vascular hydrostatic pressure or reduced plasma colloid osmotic pressure thus produce excessive net water outflow, more than can be removed by the lymphatic system. In these situations, the permeability of the vessel wall is essentially unaltered and therefore, the transudate has a low protein content (CASLEY-SMITH 1977c).

When the limit of lymphatic capacity to remove fluid is reached and tissue hydrostatic pressure continues to increase, fluid accumulates and oedema forms. In this situation, lymphatics are acting as "safety valves" (FOLDI 1969, cited by FOLDI & CASLEY-SMITH 1978), tending to filter away the excess fluid until they can no longer match vessel outflow (CASLEY-SMITH 1977c).

This type of lymphatic failure may be seen, for example, in chronic hypertension in which both intravascular fluid retention (diluting the plasma proteins) and raised intravascular hydrostatic pressure are operative.

4.5.2 High-Protein, High-Flow Oedemas.

These result from trauma (including surgical) or diseases which produce actual breaks in the capillary wall. The situation here is more complicated than in the low protein oedemas. Again the lymphatics tend to provide increased fluid removal to correct the imbalance between inflow and outflow, and again, small molecules may continue to re-enter the blood vessels in the venous limb with only a relatively small amount passing to the lymphatics. However, macromolecules are in a quite different position, tending to accumulate within the tissues (CASLEY-SMITH 1977c).

Normally these molecules chiefly depend on the lymphatics and to some extent on tissue protein catabolism (see 4.7) for their removal. While these mechanisms do increase here, they may be only partially successful and the accumulated protein will then retain fluid by virtue of its osmotic action. Thus oedema will remain as long as the excess protein does (CASLEY-SMITH 1977c).

In this form of oedema there are thus two causes: excess fluid leakage, and excess protein accumulation (which attracts further fluid) (CASLEY-SMITH 1977c). The lymphatic system functions maximally to reduce both aspects but eventually fails in the attempt. This failure may partly be because the lymphatic vessels themselves are also affected by the tissue injury, either directly or indirectly (FOLDI & CASLEY-SMITH 1978). Also, the continued development of oedema will

itself ultimately result in lymphatic incompetence (See 4.8).

4.5.3 High-Protein, Low-Flow Oedemas.

These lymphoedemas occur as a result of absolute rather than relative lymphatic deficiency, so that oedema occurs because the lymphatics are unable to remove normal protein and fluid loads. Protein therefore accumulates, attracting excess fluid. A principal difference from other oedemas is that blood vascular leakage is not increased (CASLEY-SMITH 1977c; FOLDI & CASLEY-SMITH 1978).

The failure of the lymphatics to achieve normal function may be caused by their congenital lack, their blockage (for example, as a result of chronic fibrosis), or by malfunction of their pumping mechanisms (CASLEY-SMITH 1977c).

Whilst this form of lymphatic failure is not the primary cause of the formation of inflammatory oedema following surgery, it certainly may compound the problem. For example, mechanical obstruction may occur as a result of intraluminal thrombi or reflex spasm of the muscular lymphatics. Surgical injury may also lead to incompetence of lymphatic cell junctions or valves (See 4.8). Under these situations, the effects of lymphoedema are added to the failure which resulted from blood vessel permeability alterations.

4.6 INFLAMMATORY EXUDATE: CONTENTS.

Under normal circumstances, significant variation is noted in the composition of lymph. Its principal constituents are:

i) protein (50% to 100% of the circulating plasma proteins are suggested to be transudated from the intravascular compartment and into the lymph each day), and

ii) cells (particularly leucocytes, but also varying numbers of other cells, including erythrocytes).

These components are transported in varying amounts of fluid (depending upon vascular permeability and level of local tissue metabolism). The fluid is accompanied by small solutes in concentrations similar to plasma (SMITH et al 1970). Lymph is normally returned into the veins of the neck via the thoracic and right lymph ducts (CASLEY-SMITH 1977c).

The most significant feature of lymph is the protein concentration present in the initial lymphatics. This is reported to be approximately three times its concentration in the tissues, though some dilution may occur later as a result of fluid inflow in the collecting lymphatics (CASLEY-SMITH 1977c). Other than this feature, the composition of lymph and interstitial fluid are considered to be approximately identical under physiological conditions. For this reason, experimental investigations commonly use analysis of lymph to determine the interstitial fluid composition.

During inflammation, both the volume of lymph and its protein and cell content undergo drastic alteration as a result of membrane changes in the blood vessels. These changes again mirror those seen in the interstitium itself. Two areas may be identified. The first is that area which was the site of greatest damage. Here, vessels (blood and lymphatic) are ruptured producing gross extravasation; fibrin clots and cell aggregates form, and flow in both lymph and blood vessels is effectively halted. (See FOLDI & CASLEY-SMITH 1978).

Surrounding this is an area of progressive, rapid transudation of fluid and macromolecules. The fluid here has a high plasma protein concentration showing increases of all fractions (SMITH et al 1970). Of particular note is the significant presence of fibrinogen (FLOREY

1970b) which accumulates (along with the remainder of this serous exudate) within and around the cells and fibres. Hence, fibrin is deposited throughout (FLOREY 1970b, KORBEL 1970, SMITH et al 1970).

In addition to protein, inflammatory exudate contains significant amounts of lipids and lipoproteins, as well as microorganism and cellular debris, and various intracellular enzymes (SMITH et al 1970; GLASSER & BARTH 1982). It also contains various chemical mediators of inflammation (in active or inactive forms) including histamine and PGE₂ (SMITH et al 1970).

Whilst most of these changes are the direct result of altered vascular permeability, significant effects are also reported to follow changes in interstitial proteoglycans and collagen. These result from dilution due to fluid retention, (with consequent alterations of the exclusion effect) (ZWEIFACH 1972), and from enzymatic activity in digesting these components.

The tendency is thus towards the formation of a relatively stagnant, high-protein fluid. The effect of this fluid on the tissues is discussed in Chapter 9.

4.7 PROTEIN AND OEDEMA.

It has become increasingly obvious that tissue proteolysis plays a relatively small but nonetheless significant role in the interstitial protein balance (CASLEY-SMITH 1979a; CASLEY-SMITH & GAFFNEY 1981). Macrophages were suggested to be responsible for this proteolysis following the observation of BRUNS and PALADE (1968a;b) that ferritin was taken up by these cells (as histiocytes) only five minutes after its injection into non-inflamed tissue (note: ferritin is a protein of

similar nature and dimensions to plasma proteins, FOLDI & CASLEY-SMITH 1978). The large numbers of these cells present in sites of inflammation suggests a similar role in inflammation (FOLDI & CASLEY-SMITH 1978).

This theoretical role of macrophages in managing extravasated plasma proteins by proteolysis has been recently confirmed by PILLER and CASLEY-SMITH (1975, cited by FOLDI & CASLEY-SMITH 1978). Using radiolabelled albumin and polyvinylpyrrolidone (PVP - a similarly sized but non-metabolisable molecule), these authors demonstrated that if macrophage activity was stimulated by the administration of benzo-pyrones (discussed in Chapter 7), the clearance of radio-albumin from lymphoedematous and thermally-injured areas was enhanced. This enhancement was abolished if the macrophages were destroyed by silica. The clearance of radio - PVP, however, was unaffected in either situation (CASLEY-SMITH 1977c; FOLDI & CASLEY-SMITH 1978).

The quantitative function of these cells relative to that of lymphatics in protein removal is uncertain, "but obviously appreciable" since the destruction of macrophages considerably worsened the oedema (CASLEY-SMITH 1979a). Leucocyte proteases probably also contribute to protein degradation (CASLEY-SMITH 1979a; FOLDI & CASLEY-SMITH 1978). Thus a high protein oedema is evidence not of lymphatic failure alone, but of combined failure of the lymphatic and tissue proteolytic systems (CASLEY-SMITH 1979a).

It should be mentioned that on physico-chemical grounds, the breakdown of plasma proteins into many smaller molecules would appear to markedly increase their osmotic attraction, thus leading to increased fluid retention. However, these protein fragments are generally small enough to re-enter blood vessels via their

intercellular junctions, and since they have marked concentration gradients towards reabsorption, they are rapidly removed from the tissues. Protein lysis therefore results in reduction of the quantity of osmotically active substances within the tissues and thus, oedema is decreased (CASLEY-SMITH 1977c).

4.8 LYMPHATICS AND OEDEMA.

4.8.1 Normal Function.

Lymphatics are recognised to be significant in normal function, providing a major route for protein and fluid movement from the interstitium (see 2.5.1.3). Were it not for such drainage of protein, the relative interstitial colloid concentration would approach that of the plasma, thereby eliminating the colloid osmotic pressure gradient across the capillary wall. The resorption of water and electrolytes into the venous limb would therefore cease, bringing about a widespread fluid shift which would stop only when tissue and vascular hydrostatic pressures roughly equilibrated. In this situation, significant loss of intravascular volume would occur with widespread tissue oedema (LEAK & BURKE 1973). Normal physiologic functioning of the lymphatics, however, maintains adequate return of proteinaceous fluid to the blood vasculature.

4.8.2 Lymphatic Function in Inflammation.

It was the pioneering work of PULLINGER & FLOREY (1935) which demonstrated some of the basic aspects of lymphatic functioning in inflammation. These authors demonstrated that lymphatics were dilated in oedematous tissue, rather than compressed by the increased tissue pressure, as might have been expected. They described the attachment

of fibres from the surrounding connective tissue to the lymphatic endothelium and suggested that raised tissue pressure would place tension on these fibres, causing the observed passive dilatation of the vessel. Pullinger and Florey also confirmed that lymph flow was greatly increased in inflammation (up to twenty fold, CASLEY-SMITH 1973) as shown earlier by Drinker and Field (cited by PULLINGER & FLOREY 1935).

The dilatation of the initial lymphatics in inflammation causes the opening of many more of the endothelial junctions than normal. This readily allows the protein-laden interstitial fluid to enter the vessels. (See FIGURE 4.3). Possibly functioning according to the hypothesis of FOLDI and CASLEY-SMITH (1978) (described in 2.5.1.3), these initial lymphatics pump away greatly increased amounts of protein and fluid (CASLEY-SMITH 1979a).

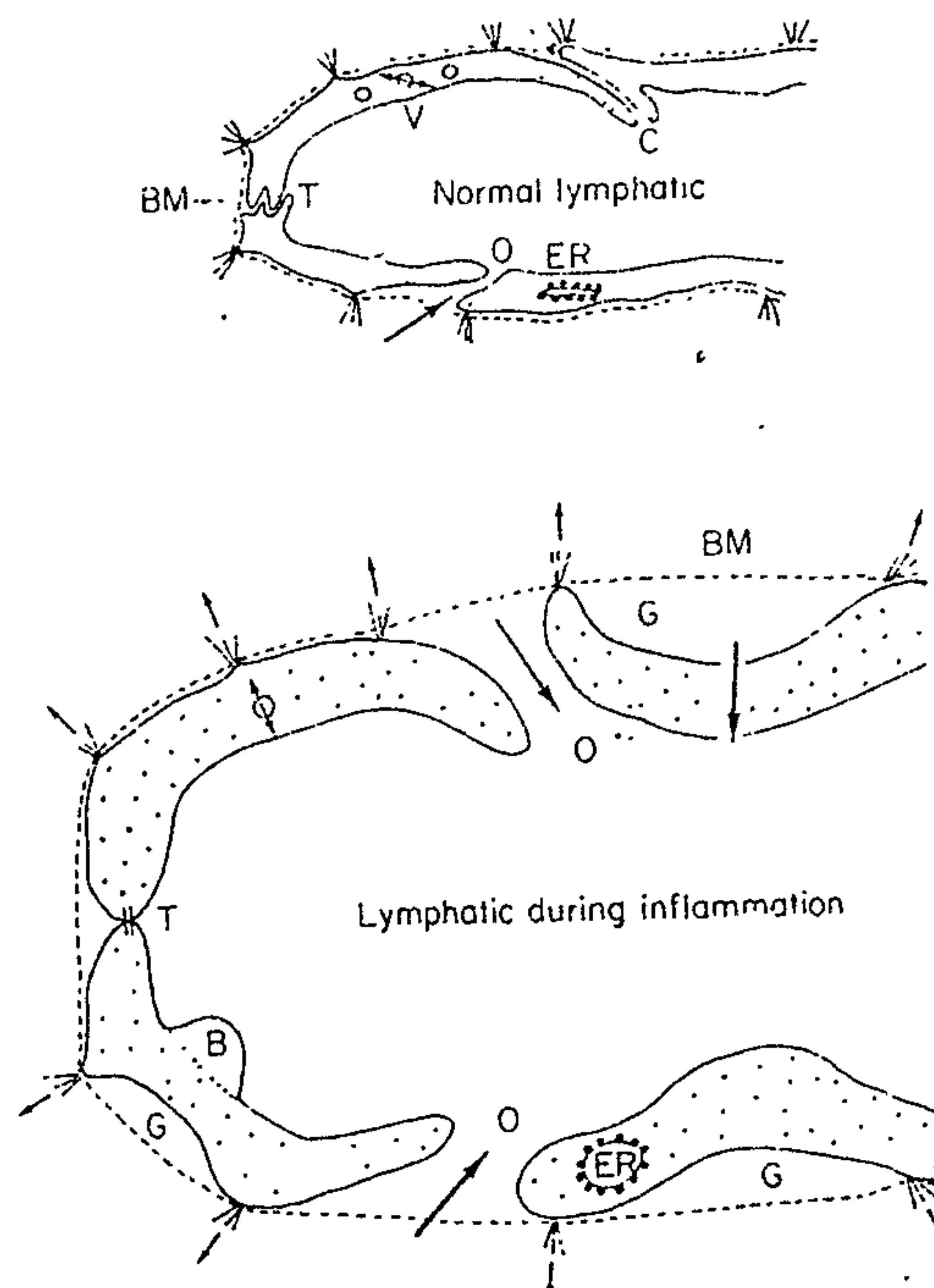


FIGURE 4.3 The effects of inflammation on the initial lymphatics. The vessel is dilated and the cells are pulled apart by the filaments attached to the abluminal surfaces; intervening portions are forced inward, leaving gaps (G) between them and the basement membrane (BM). The close junctions (C) become open; the tight ones (T) probably remain closed. However, the convolutions and projections tend to disappear as the very swollen cells stretch and break their plasma membranes. Probably for the same reason, the vesicles (V) decrease in numbers becoming incorporated in the plasma membranes. Blebs (B) are seen on the cells, and the endoplasmic reticulum (ER) dilates. Fluid (thin arrows) and fluid and protein (thick arrows) pass via the junctions, vesicles, and disrupted plasma membranes in directions and amount which depend on the conditions, the phase of the cycle, and their relative dimensions (from CASLEY-SMITH 1977c, p434).

That the lymphatics are of considerable importance in limiting the extent of oedema was clearly shown by CASLEY-SMITH and PILLER (1973, cited by CASLEY-SMITH 1977c) who found that oedema was significantly increased if the draining lymphatics of thermally-injured tissue were occluded. It is noted that the controversy surrounding vessel filling in normal tissue does not apply here since tissue hydrostatic pressure is clearly positive so that a distinct hydrostatic gradient into the lymphatics is present (CASLEY-SMITH 1979a).

4.8.3 Impairment of Lymphatic Function in Inflammation.

As increased exudation continues, too much fluid and protein enters the tissues and the lymphatics are overwhelmed. The tissue hydrostatic pressure then progressively increases and oedema occurs - described as a "high-protein, high-flow" type in post-surgical inflammation (FOLDI & CASLEY-SMITH 1978; CASLEY-SMITH 1979a). (See 4.5).

These changes are followed by alterations in lymph flow rates. Flow is usually greatest in the early stages, decreasing with the commencement of tissue oedema. (CASLEY-SMITH 1973; 1976a). These changes appear to follow decreases in the rate of fluid loss from the vessels, due to vessel stasis and the interstitial disruption which accompanies oedema. Lymph flow is also decreased by the application of pressure to the area or by cooling (CASLEY-SMITH 1973).

In addition to the overwhelming of maximally functioning lymphatics, it must be remembered that the surgical injury damages lymphatic and blood vessels alike. Lymphatic damage is of similar appearance to that in blood vessels, except that lymphatic endothelial cells are apparently more susceptible to rupture than blood

endothelium. Where damage leads to the loss of lymphatic endothelium, or where the supporting fibres have been sectioned or enzymatically degraded (for example, by lysosomal hydrolases), the efficient functioning of that vessel is greatly restricted or stopped (CASLEY-SMITH 1979a).

Impairment of lymphatic function may also result from secondary mechanical obstruction due to intralymphatic fibrin thrombi (SMITH et al 1970), or reflex spasm of collecting lymphatic smooth muscle (CASLEY-SMITH 1979a). Such obstruction may be partial or complete. In any case, acute lymphoedema is added to the initial inflammatory response and lymph flow falls or stops (CASLEY-SMITH 1979a).

Under these circumstances, and in the continued transudation of serous fluid, two alternative descriptions of the lymphatics are given. In most tissues, the vessels dilate and intralymphatic pressures rise as a result of obstruction. With time, dilatation occurs to such an extent that the valves of involved lymphatics become incompetent. The raised intralymphatic pressure is thus transmitted back to the initial lymphatics, forcing the cell junctions open, so preventing sealing of these vessels. This situation will occur in most tissues where the lymphatics are surrounded by a relatively loose stroma (FOLDI & CASLEY-SMITH 1978).

In regions such as muscles and glands (especially of older animals), the lymphatics pass through dense connective tissue. They are thus prevented from achieving full dilatation, and the raised intraluminal pressures (being greater than interstitial hydrostatic pressures) force the intercellular "flap junctions" closed (FOLDI & CASLEY-SMITH 1978). (See FIGURE 4.4).

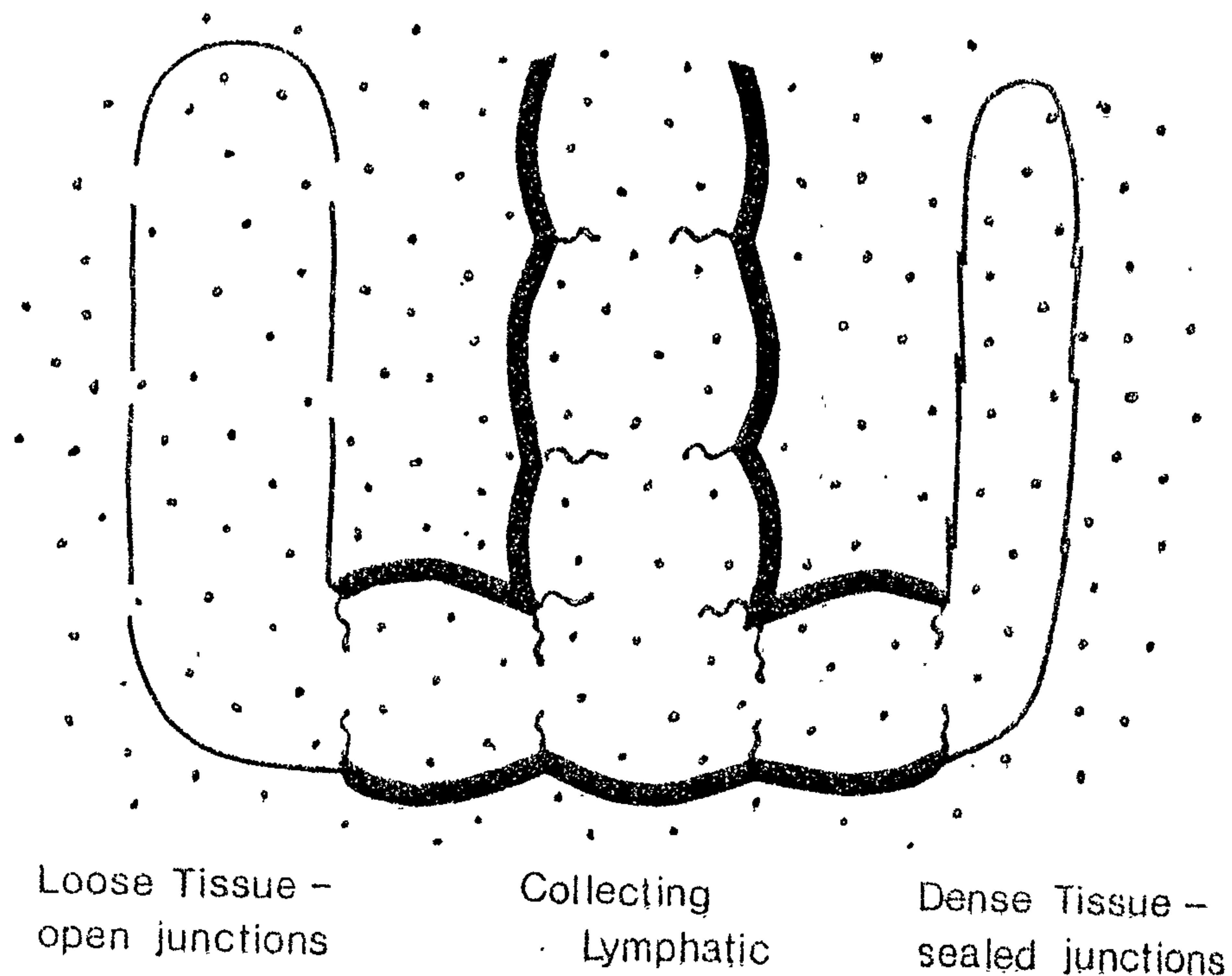


FIGURE 4.4 Illustration of the two effects which may occur during lymphoedema. The left initial lymphatic is in loose connective tissue and has dilated so greatly that its junctions have become incompetent; the one on the right is in dense tissue and therefore cannot dilate so much. Its junctions are closed by the raised internal pressure. In neither case can the vessels function (from FOLDI & CASLEY-SMITH 1978, p116).

The end result of both patterns is the same, with gradual equilibration between intra- and extravascular fluid. The final progression of these changes is that the raised lymphatic pressures spill over into the tissues themselves, enhancing distortion of the two-phase system, with marked increases in the size and numbers of the interstitial channels of the fluid-phase (FOLDI & CASLEY-SMITH 1978; CASLEY-SMITH 1979a).

4.9 CONCLUSION.

Understanding of connective tissue structure and the balance of forces which govern fluid movement through it suggests that tissues are relatively resistant to fluid accumulation in normal function. Notwithstanding, the circumstances which develop as part of the post-

surgical inflammatory response, in addition to the surgical injury itself, produce plasma exudation of such dimensions that the dynamic equilibrium provided by tissue function is grossly overwhelmed. There is therefore a considerable shift of fluid and, more importantly, plasma protein into the interstitium.

Even under these conditions, the lymphatic system attempts to maintain the balance, with up to twenty fold increases in flow. Tissue proteolysis also increases. Nonetheless, these mechanisms are soon inundated and clinically-evident oedema forms.

A significant contribution to this fluid and protein pooling is the direct damage which blood and lymphatic vessels sustain at surgery. Lymphatic function is further compromised by the development of fibrin thrombi within the inflammatory exudate and within the lymphatics. Such thrombi cause secondary obstruction, and together with the occurrence of reflex spasm of the collecting lymphatics, they superimpose acute lymphoedema upon the oedema developing from inflammatory processes.

In the following chapters, discussion is directed to the various endogenous (both physiological and pathological) and exogenous (that is, environmental and intra-operative) factors which are supposed to influence oedema formation.

A significant omission from this chapter is the effect which oedema produces within the tissues. It is felt that this subject is more relevant to discussion of the role of inflammation and oedema in the healing of oral surgical wounds (Chapter 9).

CHAPTER 5 THE EFFECT OF ENDOGENOUS FACTORS ON OEDEMA.

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5.1 INTRODUCTION.

Whilst it can be said, in principle, that the sequence of events in all wounds is similar, irrespective of the noxious agent by which they were induced, some variation is evident. This variation may particularly involve the time course, as well as the magnitude of the response; the complex interactions however are the same. Thus physical factors (such as tension, pressure, cuts, heat and radiation), chemical agents (acids, alkali and toxins), and biological factors (infection) all produce similar tissue lesions (CHVAPIL & KOOPMANN 1982).

The tissue injury may result from direct rupture of cells or from indirect cell damage as a result of peroxidative changes in the tissues, causing labilization of biomembranes (CHVAPIL & KOOPMANN 1982). This response to injury will commonly lead to the subsequent formation of interstitial oedema.

The question of post-surgical oedema has particularly puzzled the clinician because of the wide range of its expression. For example, removal of a mesioangularly impacted lower third molar tooth from one patient may result in quite a different degree of oedema from the removal of a tooth of similar impaction in another patient. Even more difficult to comprehend is the observation that removal of deeply impacted teeth may lead to less oedema postoperatively than more simple surgical exodontia.

These unexplained variations have led to the implication of many factors as possible modifying agents. As is evident from a brief perusal of the chapter outline, the spectrum of such proposed factors is kaleidoscopic. It will be readily apparent that the ensuing discussion pays more than passing attention to their effect on wound

healing as a whole rather than just to oedema per sé. Inclusion of these aspects is not intended to provide a comprehensive review of the factors which may modify wound healing. Oedema however cannot be reviewed outside the context of healing as a whole. In any case, the reparative processes are so interdigitated that many agents which affect oedema appear to also affect other aspects of repair, and vice versa.

It is emphasised here by way of general introduction that much of the following material is conjecture, particularly with regard to oedema. This has been necessary since experimental and clinical understanding remains inadequate despite intensive research endeavours in the past decade and more.

It will be seen that the separation of various points, including the demarcation between Chapters 5 and 6, is somewhat arbitrary. Such demarcation is for convenience, and is explained where appropriate. In the discussion of some subjects, background pathophysiology has been included so as to provide as complete a framework as possible for any speculation. It is not intended that this background discussion should completely elucidate these various disease states.

The achievement of understanding in these areas is further complicated by the difficulty of obtaining much experimental data on man. The differences between man and experimental animals are largely unknown, and as was seen earlier, there is a wide variance even between experimental animals. This further complicates the necessary correlation to the human situation.

This dilemma is illustrated by the observation that the healing of simple incisions is much slower in man than in animals commonly used

in experimental investigation. In particular, the rate of collagen deposition is at significantly lower rates, occurring at about the fifth day in man compared with about the third day in most experimental animals (LEVENSON 1969). It is therefore likely that much experimental information is not directly applicable to man.

Concerning the pattern of oedema formation, there are few studies which undertake to "continuously" measure the oedema and there is little consensus amongst these. Carrageenin, for example, is reported by some authors to cause a bi-phasic response (initial increase to the third hour, then a plateau, and then renewed increase after the fifth hour) when injected into rat paws. On the other hand, oedemas produced by other substances, (that is, yeast and formalin), appear to induce only a single phase response in the same experimental model. (See VINEGAR et al 1979; BEKEMEIER et al 1982).

The response to thermal oedema has been thoroughly investigated by ARTURSON (1979). This author demonstrated that the rate of fluid accumulation was highest in the early phase. It began to subside after 15 to 20 minutes, though by this time the tissue had already doubled its original weight as a result of fluid transfer. Maximum oedema was reported to occur eight hours after moderate thermal injury (ZWEIFACH 1973a).

By comparison, POLLMANN and HILDEBRANDT (1982) daily recorded the swelling following removal of four third molar teeth and suggested that oedema peaked after the first day. Daily measurements however are too infrequent to provide adequate description of this pattern. In the author's subjective opinion, this peak would seem to come a little later (perhaps 36 hours). However, close measurement would be necessary to establish this.

What is apparent from this survey is that the post-surgical response is considerably prolonged when compared with the experimental models (in the order of 18 hours). This is probably a function of the severity of injury and particularly of the degree of direct vessel damage causing both vascular leakage and impairing lymphatic drainage. This issue will be discussed further in subsequent sections.

5.2 PHYSIOLOGICAL FACTORS.

A study of physiology clearly illustrates the wide ranging capabilities which the human body may achieve in health. These capabilities are based upon accommodation of function to changing environmental conditions (both internal and external), for example, the classic flight/fight response to stress which rapidly generates greatly altered function in the whole organism. Such accommodation is also evident in many of the processes of inflammation, as in the control of haemorrhage from severed vessels. Close examination of these capabilities reveals intimate interrelationships between tissue systems. This feature of human structure is the basis for the extensive gamut of factors suggested to modify the oedema response.

5.2.1 Age

It is generally believed that wounds heal more slowly in the old. While there is some experimental evidence for this, the specific reasons for the impaired function are unknown. The reason for this lack of understanding is probably that both wound healing and ageing are multifaceted and exceedingly complex (SCHILLING 1975).

Why ageing occurs is itself a mystery. That there are many conflicting views on this subject is reflected in the numerous

hypotheses on the affect ageing has on wound repair (CHVAPIL & KOOPMAN 1982). A further difficulty is that it is often difficult to distinguish between the effect of ageing itself and the effects of other factors with which increasing age may be associated. The social circumstances of the elderly may, for example, lead to inadequate diet. Limited body reserves, diminished function of essential organs and the presence of concomitant disease are identified as other complicating factors (SCHILLING 1975).

In general it would seem fairly correct to state that during senescence there is a progressive deterioration, both quantitative and qualitative, of metabolic functioning. Cell division and differentiation are particularly affected, and thus synthesis is commonly deficient (SCHUMANN 1979).

Of particular note in wound healing of the aged is decreased multiplication of repair cells and diminished or altered synthesis of connective tissue components (collagen, elastin and glycosaminoglycans) (SCHILLING 1975; CHVAPIL & KOOPMANN 1982). Nuclear and cytoplasmic components may also be similarly affected leading to defective DNA and RNA (SCHILLING 1975; GOODSON & HUNT 1979b). In fact, these intracellular features are probably responsible for many of the observed alterations in cell function, altered DNA leading to delayed and impaired mitosis, and altered RNA to modified synthesis of protein, and so on (SCHILLING 1975).

Though some report no differences following histological examination (KANTA et al 1981), several features are evident in the wounds of senescent animals when compared with wounds in young animals. The most obvious feature is that the whole process is slowed down (ROTH et al 1981). The rate of cellular multiplication of cultured

fibroblasts, for example, varies inversely with age. This is believed to result from both markedly reduced cell numbers (MANN & BEDNAR 1977), and diminished functional capacity (CHVAPIL & KOOPMAN 1982).

This is clearly evident in the synthesis of both collagen and elastin. Collagen appears later in the wound of the old (SCHILLING 1975). Also, the ratio of insoluble to soluble collagen which is formed is reversed in harmony with the general position throughout the body (that is, the insoluble form is increased, the soluble decreased). The equilibrium between collagen synthesis and decomposition is also altered such that synthesis dominates for much longer in older animals. This would account for the greater density of collagen which is found in the wounds of older individuals (CHVAPIL & KOOPMAN 1982).

This slowed tissue turnover rate also means that Type III collagen persists for longer and that the disoriented fibres which are deposited early are not remodelled and replaced. Together with apparently delayed fibre cross-linkage, these changes result in significantly reduced early wound strength in the elderly (GOODSON & HUNT 1979b). Although this position is much later reversed so that collagen fibres show increased density and insolubility (most likely the result of increased cross-linkage) (SCHILLING 1975), wound tensile strength remains low (MANN & BEDNAR 1977).

Other features of wound healing in the old are also relevant. Ageing leads to alterations in the epithelium and connective tissue which are generalised throughout the body, including the oral tissues. These changes include thinning of the epithelium, blunting of the rete pegs, decreased vascularisation, thinning of the tunica propria, decreased water, hyaluronic acid and elastin content with increased content of ground substance components which are less fluid-binding

(that is, dermatan and keratan sulphate). Together, the result of these changes is tissue which is more easily damaged, but less able to regenerate (CHVAPIL & KOOPMAN 1982, KOOPMANN & COULTHARD 1982).

Of particular note are the vascular changes which result from arteriosclerosis. Arteriosclerotic changes in peripheral arterioles (including intimal hyalinization, medial hypertrophy and endothelial proliferation) are generally seen to represent degenerative age-related change (BERNICK 1967). (Such changes have been observed in vessels of the oral mucosa (KEENE 1975) and of the dental pulp (BERNICK 1967; SELTZER & BENDER 1975)). Thus wound oxygen tension and nutrient supply is significantly impaired, and ischaemia may occur. In addition, delayed vascular regeneration (YAMAURA & MASUZAWA 1980) further compromises tissue vitality and repair.

As related specifically to inflammation and oedema, little work has been done. It is reported that exudation persists into the late phase of proliferation, perhaps as a result of diminished ability of wound collagen to maintain adequately fixed apposition between the wound edges (MANN & BEDNAR 1977).

The prolongation of this initial phase of healing suggests that leucocyte function (like that of connective tissue cells) is also impaired. The clinical observation of increased incidence of wound infection in the elderly would support this. SCHILLING (1975) suggests that the leucocyte defects are similar to those seen in the newborn, that is, decreased effectiveness of intracellular microbiocidal action and defective antibody formation.

How these changes quantitatively effect oedema is unknown. It has not been the clinical experience of the author that post-surgical

oedema is increased in the elderly, though visible signs of inflammation including oedema do persist longer with increased age. Perhaps the most obvious feature following surgery in elderly patients is the extensive ecchymosis which rapidly develops. This, being indicative of increased capillary fragility, would also support the persistence of increased vascular permeability and thus of continued oedema formation.

It must be emphasised that despite all these apparently marked alterations, healing in the elderly may progress uneventfully in the absence of other debilitating circumstances. Certainly, the healing response, though perhaps delayed, is essentially the same (SCHILLING 1975). MANN and BEDNAR (1977) state that perhaps the differences between healing of young and old individuals are exaggerated.

Of clinical significance to surgery on the mandible are the observations of BRADLEY (1972; 1981) that the inferior alveolar artery is affected by arteriosclerotic change approximately fifteen years earlier than the remainder of the carotid arterial tree. This is apparently because this vessel is "tethered" within the bone, thus altering its haemodynamic function and leading to increased stresses within the wall as a result of pulsatile flow. This artery is thus virtually non-existent in the elderly, mandibular blood supply being almost exclusively obtained via freely anastomosing extraosseous vessels.

5.2.2 Sex.

Differences in wound healing based upon a patient's sex are not readily obvious to the clinician because of the considerable inter-patient variation. Further, little experimental investigation of this

area has been performed to assist in clarifying this question.

Sexual differences manifest themselves not only in the composition of the connective tissue-rich structures (that is, skin, tendons, and arteries) but also in the reactivity of this tissue during repair. Animal studies indicate that females form connective tissue at a slower rate and with an ultimately lower level of deposition than males (CHVAPIL & KOOPMANN 1982). Otherwise little is known.

Interestingly, GOONATILAKE (1981) undertook to evaluate the relative significance of four patient parameters: age, sex, wound drainage and wound classification (according to level of contamination). In this model, the patient's age and sex were of little significance to healing in comparison to bacterial contamination and drainage.

As will be seen in the subsequent section however, female sex hormones do appear to play some role.

5.2.3 Hormones.

As has been stated, the repair of wounds is generally a non-specific process. In principle, hormones may alter this process in rate or in quality by influencing aspects of the phases of healing (outlined in Chapter 1) (AHONEN et al 1980). Various hormones have been suggested to have a potent effect in modification of healing as outlined in TABLE 5.1.

However, the results of experimental investigations into the endocrinology of repair have been contradictory, with many studies failing to demonstrate any influence. AHONEN et al (1980) state that

although the possible influence of these hormones is of great theoretical interest, it would seem fair to conclude that only the glucocorticoids and female sex hormones do in fact have any clinical consequences. This comment probably requires qualification in view of the work on insulin deficiency in diabetes (5.3.1), and on thyroid hormones (5.2.3.2).

TABLE 5.1 Hormones and Wound Repair.

Stimulatory Effect	Inhibitory Effect
TSH	Corticoids
Insulin	ACTH
Calcitonin	Thyroxin
Oestrogen	Hypophysectomy
Testosterone	Gestagens

The table indicates the supposed overall effect of specified hormones on the connective tissue component of repair. Adapted from CHVAPIL and KOOPMAN (1982, p264).

A significant point to this discussion is that many hormones are target-organ specific, therefore having limited whole-body action. For example, testosterone stimulates hyaluronic acid production in the cockscomb, but not in skin (CHVAPIL & KOOPMAN 1982), as supported by the studies of SHAMBERGER et al (1981).

This discussion gives chief consideration to the effect of female sex hormones on repair. Discussion of the glucocorticoids is included in relation to stress (5.2.4) and particularly in relation to the pharmacological modulation of inflammation (Chapter 8).

5.2.3.1 Female sex hormones.

Interest in the female sex hormones and their effect on wound healing originated from the observations of LINDHE and BJORN (1967) and HUGOSON (1970) that increased gingival inflammation was noted in

pregnant women, and in women using hormonal contraceptives.

Various other effects have been reported including decreased wound bursting strength after twenty days of healing (no difference at ten days) and increased collagen maturation (providing increased "rigidity" but not increased strength) in pregnant rats (ANDREASSEN et al 1977). Delayed collagen accumulation is also reported (HAGBERG et al 1980). Also, premenstrual exacerbation of the symptoms of the carpal tunnel syndrome is considered evidence of cyclic hormonal control of fluid balance in the female (WARD 1976). Similarly relating to the fluid balance, blood capillaries in pregnant women are reported to show increased permeability, particularly in the last trimester when peak hormone levels are reported (NOTELOVITZ 1972; CIMASONI 1974).

CIMASONI (1974) studying the gingival crevicular fluid and vessels of the attached gingiva, also found that vascular permeability and the flow of crevicular fluid showed fluctuations which paralleled the menstrual cycle, both being highest at ovulation. His work, together with that of Lindhe and Attstrom, and Loe and Holm-Pedersen (all in the late 1960's, cited by CIMASONI 1974) suggested that some of these changes were dependent upon the level of plaque deposits and gingival inflammation. Changes dependent upon the menstrual cycle and during pregnancy are prominent if oral hygiene is poorly maintained, whereas no change in crevicular flow was demonstrated in such women with minimal plaque. A similar situation is described in conjunction with the use of hormonal contraceptives. However, CIMASONI (1974) concludes that the evidence does seem to support the hypothesis that the female sex hormones do increase vascular permeability.

This effect on capillary permeability is supposedly related to a decreased colloidal osmotic pressure resulting from hydraemic dilution of the plasma proteins, or to specific hormone-induced alterations of the integrity of the microvascular endothelium (NOTELOVITZ 1972; MAHAJAN et al 1973).

The relationship of hormone levels to collagen metabolism in wounds is unclear but is partly explained by the physiological functioning of these hormones. During the last trimester of pregnancy, there is active collagen and protein synthesis in the uterus and foetus. HAGBERG et al (1980) suggest that the decreased synthesis observed in other tissues, including wounds, may serve in energy and protein conservation, the pregnancy dominating the function of other systems. Experimental investigations often use similar levels as found during this trimester.

That this dose-dependent effect is significant can be seen from other investigations on wounds in oophorectomized rats with and without oestrogen supplementation. Ovariectomy itself caused delayed healing; physiologic supplementation almost completely restored the normal rate of healing. On the other hand, high doses of oestrogen were inhibitory. None of these effects were seen in old female rats which were mostly anestrus and exhibiting markedly altered ovarian function. Such animals are supposedly less responsive to oestrogen or progesterone. Potentiation of cutaneous healing here is suggested to follow increased production of fibrinous substances, dilatation of blood vessels and accelerated angiogenesis in response to oestrogen, progesterone and androgen (ROTH et al 1981).

It is additionally noted that salivary and neutrophil peroxidase activity peaked in mid-menstrual cycle (coinciding with the ovulatory

oestrogen peak). While the cause of the fluctuation in activity of salivary factors (see 6.2.4) is unknown, this neutrophil effect may result either from increased cell numbers in saliva, or from their increased activity at this time (COCKLE & HARKNESS 1978).

From these observations it is theoretically reasonable to assume that women with peak serum oestrogen and progesterone levels (that is at ovulation, puberty and in the last trimester of pregnancy) are likely to develop enhanced inflammatory responses to surgery. The work on the gingival crevice suggests that this enhancement is likely to depend upon the severity of the injury and upon the extent of bacterial contamination. A carefully documented study of menstrual history and measurements of oedema are required before the clinical significance of these factors could be determined.

5.2.3.2 Other hormones.

Of the vast gamut of hormones which have been implicated in modifying wound healing, the thyroid hormones are reported to come a little behind the glucocorticoids and female sex hormones in significance. Thyroid hormones (particularly thyroxine) are involved in general metabolic management and thus influence a variety of body systems.

SENA et al (1981) report that the experimentally-induced hyperthyroid state leads to significant inhibition in the development of carrageenin-provoked rat paw oedema; hypothyroidism had no effect. On the other hand, the hyperthyroid state slightly inhibited the chronic response to the cotton pellet, whereas hypothyroidism enhanced it. These differences and various contradictory results in the literature (see SENA et al 1981) are suggested to relate to the mediators

involved. Thyroid hormones are thought to regulate the inactivation of the prostaglandins and are thus important in inflammatory models where prostaglandins are released, for example, carrageenin (SENA et al 1981). They are also reported to increase rates of cell proliferation and anabolic processes such as synthesis (ROTH et al 1981).

It is probably unlikely that these modifications are significant to oedema formation unless thyroid hormone levels move outside the normal range, for example in hyperthyroidism when the inflammatory oedema is likely to be suppressed. Further work is required to clarify the effect of these hormones on wound healing. Some effect is likely, at least in theory, because of their wide-ranging metabolic function.

Pituitary growth hormone is also implicated here. Similar to the thyroid hormones, its functions are widespread. Excess growth hormone (as seen in acromegaly) is reported to produce increased albumin synthesis and catabolism. The plasma protein transcapillary escape rate is also reportedly increased. The mechanisms for these changes are not known (ROSSING et al 1974).

Again, it is unlikely that growth hormone mediates significant effects on inflammation at physiological levels in the adult. Whether there is any effect during periods of active growth in children, or in conditions such as acromegaly is a matter for speculation. If so, the above observations would suggest enhanced responses.

5.2.4 Personality/Psychological Factors.

The widely reported psychosomatic linkage through which psychological trauma and stress may produce organic change and disease is, in theory, also applicable in wound repair. The problem is that the

effector mechanisms of this link are largely unknown.

"Stressors" are stimuli which create a certain pattern of responses to stressful stimulation. These responses include:

- i) increased adrenocorticotrophic output by the pituitary,
- ii) increased output of adrenal cortical and medullary hormones,
- iii) changes in protein, glucose and lipid metabolism, and
- iv) thymico-lymphatic involution (COHEN 1979).

Of these factors, the increased secretion of the adrenals is the best described. Again, conflicting data are reported as to the effect of this secretion. SHERWOOD & SMITH (1979), for example, observed no alteration in the healing rate of incision wounds in animals which had also been subjected to the stress of moderately severe burns. Generally however, stress is reported to cause suppression of repair processes in a similar manner to therapeutic useage of steroids (see 8.3.2.5).

Long-term stress is reported to induce a wide variety of changes from enhanced periodontal disease and dental caries (MANHOLD 1978) to connective tissue atrophy and premature ageing (COHEN 1979). The aetiology of atrophic mucosal alterations such as lichen planus is also suggested to be partially stress-related (MANHOLD 1978; JOLLY 1983).

These aspects may in fact represent part of the same response. MANHOLD (1978) postulated that chronic stress may be associated with altered tissue oxygen metabolism. In connective tissue, the continued stimulation of the adrenal cortices seen in chronic stress produces collagen atrophy due to retardation of fibroblast synthesis (COHEN 1979). The critical dependence of fibroblasts on oxygen supply for synthetic function (NIINIKOSKI 1980b; SILVER 1982 - Chapter 1) could explain these connective tissue changes. Though Manhold postulates

rather complex mechanisms for the impairment of oxygen metabolism, the long-term effects of raised serum adrenaline must include increased peripheral microvascular tone, notwithstanding other metabolic alterations.

As will be seen in Chapter 8, adrenal secretions, particularly the steroids, are considered to act in the initial stages of repair. Thus, the prolonged stress associated with the restraint of rats in a fixed box produced significantly smaller inflammatory responses to paw injections of carrageenin and other irritants compared to non-restrained rats. Particularly obvious was the reduction (about 70%) in maximum paw swelling in restrained animals. Although the physical inactivity and compression of limb vessels (with partial limitation of flow) contributed to this reduction, restraint was seen as a major factor. Stress produced by this physical restraint was suggested to lead to increased release of "antiphlogistic catecholamines and adrenal corticosteroids" (BEKEMEIER et al 1982).

The question of corticosteroids and their mechanisms of action is covered in more detail in Chapter 8.

Concerning personality factors, MCKENZIE et al (1967) found that no significant correlation was evident between the patients' psychological test scores/personality profiles and the post-surgical phase.

5.2.5 Diurnal Cycles/Time of Day.

5.2.5.1 Diurnal cycles.

COOPER (1939) was among the first to draw attention to the existence of biological rhythms. To quote from this author, "the

existence of biological rhythms, the cardiac and respiratory rhythms, the longer rhythms of the female sexual cycle is so much a part of common knowledge that, for a relatively long period, biological rhythms were taken for granted". Cooper postulated that since the basis of life was the cell, the source of many of the systemic biological rhythms may be metabolic or growth processes within the cell itself, particularly the fundamental rhythm of cell division.

This author went on to demonstrate a consistently occurring mitotic rhythm in tissue from human prepuce; activity was greatest between 2100 and 2200 hours and least between 0500 and 1000 hours. Since these observations, rhythms have been observed in enzymic activity, organ secretions (for example, bile, gastric acids), hormone secretion, body temperature, and in physical and psychic performance (DRESCHER 1969). They have also been described in counts of salivary microflora (particularly Candida albicans, WILLIAMSON 1972a;b), in the volume of the hand (WARD 1976) and in sleep/wake patterns (AKERSTEDT 1979). The list goes on ...

Of particular relevance to this thesis is that the periodic rhythmicity of mitosis is altered in the healing wound and that rhythmical variation has also been described in post-surgical oedema. Before these factors can be examined, it is necessary to outline the observed patterns of mitotic activity and the supposed mechanism of their control. Unless specified, this outline is adapted from the review by AKERSTEDT (1979).

The concept.

The rhythmical variation in the activity level of many aspects of living matter forms repeated cycles in which it rises from a minimum ("trough") to a maximum ("peak") and then falls back to a minimum

and so on. The length of one cycle ("period") may vary from milliseconds to months or years. This thesis focuses on rhythms with a period of approximately 24 hours (so called "circadian" or "diurnal rhythms").

Origins.

The source of a rhythm may be either exogenous (factors such as the alternation of light/dark) or endogenous (the "biological clock"). While the former may be quite obvious, many aspects of endogenous periodicity are poorly clarified. The criteria which define such factors to be endogenous are described by AKERSTEDT (1979).

As stated by DRESCHER (1969), the mechanisms of regulation of endogenous rhythms may be cellular or central. The bulk of evidence would suggest that central control is at least partially operative. Hypothalamic structures are most likely implicated here, perhaps operating as part of a hypothalamo-adrenal medullary axis (DRESCHER 1969; AKERSTEDT 1979).

Sympathoadrenomedullary activity.

The hypothalamo-adrenal medullary axis functions as a neuroendocrine transducer, integrating the sympathetic and endocrine systems. The main hormone effectors of this axis are the catecholamines, adrenaline and noradrenaline. Both have been clearly demonstrated to have endogenous circadian rhythms with an early afternoon peak and a night (sleep) trough in light-active animals. Cortisol (hydrocortisone) demonstrates a similar periodicity.

Following intense research, adrenaline has been established as a central factor in psychological arousal. Thus, circulating adrenaline levels are well correlated with various indices of performance

capacity, in addition to spontaneous adjustment to changing circumstances. It would appear that these rhythms in the level of adrenaline are quite strong, since studies demonstrated that inducing severe anxiety in psychiatric patients at the cortisol trough demonstrated no cortisol response in spite of the strong emotions induced. This and other studies suggest that for some functions, the response is potentiated when the cortisol base is approaching its peak, while the system is apparently refractory to stimuli while approaching the trough. No information is available on the catecholamine responses in this regard (AKERSTEDT 1979).

At intermediate levels, stress may impose significantly upon the base-line cycle, so disrupting the normal metabolic balance (DRESCHER 1969). In the short-term, such "disruption" is of no consequence. The effect of chronically elevated secretions, however, is quite different, as has been mentioned (5.2.4).

These rhythms are very important in that they establish a favourable climate for cell metabolism. This is particularly evident with respect to cell mitosis on which a large volume of literature has been published.

It should also be mentioned that breakdown of the periodicity is thought to be the basis of many stress-related diseases, including many psychiatric disorders in which a circadian rhythm of hormone secretion is frequently not detectable, or is grossly desynchronized (LUCE 1971). Similarly, refractoriness to epidermal chalone (see below) is suggested to lead to the formation of hyperplastic tissue (BERTSCH & MARKS 1982).

5.2.5.2 Diurnal variation of epidermal proliferation.

It is well known that the proliferative activity in oral and other epithelia is subject to diurnal variation in rodents (see MANTELL 1973; HAMILTON & BLACKWOOD 1974; REEVE 1975; SCRAGG & JOHNSON 1982). Similar variations are also reported in other tissues, for example, kidney (SAETREN 1972). Significant inter-animal variability is usually evident in the timing of the "troughs" and peaks". Generally however, the period of the lowest proliferative activity normally occurs during the animals' waking period. Since most experimental animals are nocturnal, proliferative activity is usually lowest in the evening and night, and highest in the morning (SCRAGG & JOHNSON 1982).

The situation in man would be expected to be reversed. However, information from human studies has been less conclusive, with some authors observing little or no diurnal rhythm (see SCRAGG & JOHNSON 1982). Most however, suggest that some rhythm is likely to be operating (see SCHELL et al 1981a;b). Of note is that some of the studies in which no rhythm was apparent were performed on neoplastic or pathologic tissue in which proliferative function is notably distorted (SCRAGG & JOHNSON 1982).

5.2.5.3 Epidermal homeostasis.

Taking the oral mucosa as a representative sample, this epithelial tissue maintains itself by a system of cell renewal. Cells are produced by mitosis in the basal layer and they subsequently move up to the surface where they are desquamated (MARKS 1972; HAMILTON & BLACKWOOD 1974). Under normal circumstances cell production and cell loss balance, and the tissue thickness remains constant within fairly narrow limits. This dynamic equilibrium - homeostasis - is suggested to

be self-regulatory via feed-back mechanisms (HAMILTON & BLACKWOOD 1974).

Bullough and Laurence have been foremost in the investigation of these control mechanisms (BULLOUGH & LAURENCE 1960 (cited by BULLOUGH 1971); 1961; 1964; 1968). These investigators discovered a group of locally produced physiologically active substances - chalones - which only act on the tissue in which they are synthesised. Each chalone is thought to form part of a "tissue-specific homeostatic mechanism, which regulates the mass of that tissue, and, when necessary, directs its regeneration" (BULLOUGH 1971). Other chalones have also been described for granulocytes, erythrocytes, liver, kidney, and so on (BULLOUGH 1971). The epidermal chalone has been most fully investigated, and is the basis of this discussion.

Further investigation demonstrated that these mitotic inhibitors exerted their most powerful action only in the presence of adrenaline, and to a lesser extent, cortisol. The concept of the "chalone-adrenaline complex" was thus developed (BULLOUGH & LAURENCE 1964; MARKS 1972). The observed diurnal cycle of epidermal mitosis is suggested to be explained by the periodicity of levels of circulating adrenaline, and thus of the formation of this loose complex (MARKS 1972). Hydrocortisone is suggested to limit the rate of loss of adrenaline from epidermal cells, thus prolonging the association of chalone and adrenaline (BULLOUGH & LAURENCE 1968).

5.2.5.4 The diurnal mitotic cycle and wounding.

The most significant observation concerning mitosis in wounded tissue is that it loses its diurnal cycle in many tissues. Under the chalone-adrenaline hypothesis, this is essential since wounding causes

loss of chalone from the cells allowing unrestricted mitosis to occur with restitution of epidermal integrity and volume (BULLOUGH & LAURENCE 1964; BULLOUGH 1969; REEVE 1977).

The tissue under consideration is highly significant. Some tissues, for example, hair follicles, do not demonstrate normal diurnal variation of mitotic activity (BULLOUGH & LAURENCE 1964). In others, such as rectal mucosa, wounding does not abolish the normal diurnal cycle (REEVE 1975). Differences in basal metabolic rate may explain some of these inconsistencies. Hair follicles, for example, having very high rates of mitosis, are obviously subjected to only minimal suppression of proliferation - the chalone system is therefore of diminished importance here (BULLOUGH & LAURENCE 1964). The situation in rectal mucosa however, cannot be so simply explained. Further work is thus required to clarify these questions. REEVE (1975) suggests that the factors responsible for increased mitotic rate after wounding are probably different from those responsible for the diurnal cycle.

5.2.5.5 Diurnal cycles and inflammation: timing of surgery.

The above information is chiefly included to provide understanding of the effect of the timing of surgery on cellular homeostasis. However, on this point experimental investigation is almost non-existent.

REEVE (1977) gives detailed observations on wounded guinea-pig tympanic membrane (supposedly having similar features to oral mucosa). This investigator found no difference in the time-pattern of mitosis found in wounds inflicted at 0900 hours and at 2100 hours. This suggests that the biological rhythms are not of great importance in healing.

Perhaps the relevance of these diurnal rhythms relates more to the person's ability to cope with surgical stress (that is, when circulating catecholamine levels are approaching their peak) than to wound healing itself. This concept is proposed by DRESCHER (1969), who strongly advises that the timing of elective surgery must take such rhythms into account.

The studies of POLLMANN (1982) and of POLLMANN and HILDEBRANDT (1981) would tend to confirm such recommendations. These authors reported that patients were better able to cope with oral surgery in the morning, this effect being attributed to a diurnal variation in the duration of anaesthesia provided by an injection of mepivacaine. The longest duration was found to be around 1500 hours with the shortest in early morning and at night.

The effect of these circadian changes in metabolic function on the formation of oedema is not clear. One study reported some circadian periodicity of oedema following removal of third molar teeth, the greatest oedema occurring at night and early morning (POLLMANN & HILDEBRANDT 1982). Such results in fact tend to contradict the expectation that the higher nocturnal catecholamine levels would result in reduced oedema. Investigation of this is understandably very difficult, particularly on human subjects, because of the frequency of measurements which are required. The question remains unresolved.

5.2.6 Tissue Specificity.

The rate of healing shows marked differences between various tissue systems. For example, visceral wounds demonstrate significantly higher metabolic rates than do skin wounds. In particular, the rate of collagen synthesis is higher in these internal wounds. Thus, after.

three months, the stomach has regained 75% of the original breaking strength of intact unwounded tissue, and the urinary bladder has regained 120% of the unwounded strength. By contrast, wounded skin has regained only around 50% of its original strength after this time (CHVAPIL & KOOPMAN 1982).

Whilst these observations are not explained, blood flow is likely to be significant. Thus in view of the various studies reported in 2.7.3, the rate of healing in oral mucosa may fall somewhere between skin and visceral rates. The effect of the rate of healing on oedema is discussed in Chapter 6, particularly 6.2.5.5.

The enormous inter-patient variability in the pattern of healing is known to all practising surgeons. Some explanation of these differences may lie in the presence of different, genetically-determined connective tissue constitutions. So far, attempts to classify these constitutions (somatotypes) have been unsuccessful, however in theory such somatotypes may modify the types and proportions of the various connective tissue components (collagen and proteoglycans) in both normal tissue and in healing wounds (CHVAPIL & KOOPMAN 1982). The degree of collagen cross-linkage and the rate of age-related degeneration might also be influenced by cellular DNA.

Though this concept is only hypothetical, the fundamental nature of the differences which are observed requires a concept which originates at a very basic level of cell function. The genetic code would certainly provide such a base. Any genetically-determined alterations in the composition and structure of connective tissue could conceivably produce significant variations in the expression and formation of oedema.

5.3 PATHOLOGICAL FACTORS.

It will be observed from the outline at the beginning of this chapter that a wide range of pathosis is included for discussion here. In reviewing this field of the literature, attention is focused on pathological factors/conditions which have been suggested to produce either vascular alterations or defective function of the cells involved with repair. The hypothetical effect of each of these alterations on inflammation and oedema is considered in 5.4.

5.3.1 Diabetes.

Perhaps one of the most noted features of diabetes is the high propensity to failure of the healing process with subsequent dehiscence and infection. In reviewing the effect of diabetes on healing, it is first essential to grasp some understanding of the metabolic dysfunction which is involved and then to consider the ultrastructural changes (particularly vascular) which result from this altered metabolism.

5.3.1.1 The diabetic metabolic disorders.

Closer examination of diabetes reveals that it is a syndrome rather than a single disease entity (GOODSON & HUNT 1979a). Two basic and quite different metabolic disorders may be differentiated: insulin-deficient (juvenile) and insulin-resistant (mature onset) (GOODSON & HUNT 1979b). Other clinical forms exist but these are poorly clarified (GOODSON et al 1980). In all forms, altered carbohydrate metabolism is only one aspect.

Insulin-deficient diabetes.

Most experimental investigations use streptozotocin or alloxan to destroy beta cells of the pancreas so producing pathophysiologic characteristics which are similar to those seen in juvenile diabetes (GOODSON & HUNT 1979a). These are characterised (in summary) by insulin deficiency, decreased basal insulin levels, increased insulin receptors, increased response to insulin, and hyperglycaemia (GOODSON & HUNT 1979b).

The clinical expression of this form of diabetes is relatively simple. Pancreatic production of insulin is insufficient to meet metabolic requirements. Cells compensate for this deficiency by increasing the numbers of plasmalemmal insulin receptors and increasing the response to insulin. However, the effective insulin deficiency remains and hyperglycaemia develops (GOODSON & HUNT 1979a; GOODSON et al 1980).

Insulin-resistant diabetes.

Although the majority of diabetics are resistant to insulin, little experimental investigation has been performed which relates to this model. Insulin resistance is usually associated with obesity. These patients have increased fasting levels of insulin (hyperinsulinaemia) which leads to the overstimulation of fat and liver cells. Overstimulation causes these target cells to gradually become more resistant to insulin through a reduction in the number of insulin receptors, and a diminished response of intracellular metabolism to insulin (GOODSON & HUNT 1979a;c). Further, the pancreas of such individuals, being continually stimulated to produce insulin, becomes less able to cope with sudden glucose loads. Thus, though there is a fasting hyperinsulinaemia, the inability of the pancreas to cope

with such glucose loads again leads to hyperglycaemia (GOODSON & HUNT 1979a).

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From this point in the discussion, the above demarcation is relatively arbitrary. In both forms of diabetes there is a functional deficiency of insulin (either through actual lack or diminished response), and there is hyperglycaemia. Most of the clinically-observed abnormalities can be explained by these factors, as will be seen.

5.3.1.2 Ultrastructural changes in diabetes.

The ultrastructural changes which result from the altered metabolism of diabetes primarily involve the vasculature. Both small and large vessel disease is described. Throughout, there is little understanding of cause and effect relationships.

Large vessel atherosclerosis.

It is not known whether the association of increased large vessel disease with diabetes is a result of diabetes itself, or merely that it simply reflects a higher incidence of other risk factors such as hypertension and obesity. Other than that it develops more in peripheral than central vessels, the changes are of similar appearance to non-diabetic arteriosclerosis (GOODSON et al 1980).

The effects of such large vessel obstructive disease can be very significant, leading to compromise of supply by the same mechanisms as non-diabetic vessel disease. Impaired flow produces impaired perfusion and hypoxia in the tissues which such vessels supply (GOODSON et al 1980).

Diabetic microangiopathy.

Considerable debate has occurred in the literature as to the nature of the basic vascular lesion in diabetes. The so-called characteristic picture of diabetics and "prediabetics" is endothelial thickening of the small vessel wall which strongly reacts with periodic-acid Schiff staining - "PAS-positive thickening" (McMULLEN et al 1967). ("Prediabetics" are individuals who are chemical diabetics without clinical signs or close relatives of diabetics who therefore have a high risk of developing the disease). Not all vessels are affected by these changes, but vessels in all body tissues, including gingival mucosa (RUSSELL 1966; McMULLEN et al 1967; CAMPBELL 1974) may demonstrate PAS-positive thickening.

Electronmicroscopic examination of diabetic microangiopathy has demonstrated that PAS-positive thickening is represented by marked irregularities in the capillary basement membrane, increased perivascular collagenisation, and endothelial thickening (KARASEK et al 1973; LIN et al 1975). Basement membrane (BM) thickening is frequently described in such vessels, and often with marked irregular lamellations (CAMPBELL 1974; CIMASONI 1974; LIN et al 1975). It is vessels at or proximal to true capillaries (particularly the terminal arterioles), which are most affected (LIN et al 1975). These changes may progress to produce occlusion of the vessel lumen (LIN et al 1975; WINTERS 1982).

There is some confusion, however, concerning the significance of these alterations. Some studies have reported the presence of similar changes in association with the vessels of "healthy" individuals (CAMPBELL 1974; KEENE 1975). The finding of biological and physiological variations in these vessels and of focal and segmental

variations in the BM has further complicated this issue (LIN et al 1975).

A carefully controlled study by FRIEDERICI et al (1966) demonstrated that there was in fact no correlation with the presence of severe basement membrane changes and diabetes. In this study, some "control" patients demonstrated marked BM alterations, whilst some severe diabetics with nephropathy and retinopathy showed relatively normal BM. This is also supported by FRANTZIS et al (1971).

Most studies however, do suggest that capillary BM thickening is clearly associated with the manifestation of this disease (RUSSELL 1966; LIN et al 1975; GOODSON et al 1980) though perhaps as an acceleration of ageing (GOODSON & HUNT 1979a). Some suggest that this effect increases with the duration of diabetes (CAMPBELL 1974; GOODSON et al 1980).

It has been suggested that capillary BM thickening results from increased vessel permeability (PARVING et al 1976; ROSSING et al 1976; GOODSON & HUNT 1979a; YUKIHIIDE et al 1981). PARVING et al (1976) and ROSSING et al (1976) demonstrated that the transcapillary escape rate of albumin and the capillary filtration coefficient were both increased in diabetics during periods of poor metabolic regulation, reverting to normal when control is achieved. This may explain how DVORAKOV et al (1977) found neither function of filtration to be increased in diabetes.

These results support the early concepts of Ditzel (1968) that a functional microangiopathy precedes the anatomical changes. The mechanisms for such increased filtration may be increased surface area and/or increased hydrostatic pressure and/or increased "pore" diameter. Thus the increased permeability of the microvessels to plasma proteins,

is suggested to be an initial event in the morphogenesis of diabetic microangiopathy. Deposition of these macromolecules in the wall and BM is thought to result in the ultrastructural features described above (PARVING 1975a; PARVING et al 1976; ROSSING et al 1976; YUKIHIDE et al 1981).

The cause of the permeability changes is unknown. Perhaps the impaired metabolism resulting from insulin "deficiency" may affect the endothelial cell membranes allowing disruption (LUND-ANDERSON & LASSEN 1981; YUKIHIDE et al 1981). It is certainly likely that the metabolic failure proceeding from insulin deficiency is the primary breakdown of diabetes. Any vascular lesions which develop are most likely secondary effects (GOODSON et al 1980; YUKIHIDE et al 1981).

5.3.1.3 Insulin and wound repair.

An important observation in the complex debate regarding insulin and wound repair is that the tendency towards deficient healing in diabetes is significantly reversed by the administration of insulin. The disordered functioning of this hormone is thus critical to the development of the impaired healing responses, and probably also to the other clinical features of diabetes (GOODSON et al 1980).

Insulin binds to specific receptor sites on hepatocytes, adipocytes, leucocytes and fibroblasts. Depending upon the form of diabetes, these receptors either decrease in number or tend to become refractory to the action of insulin. These changes are thought to produce widespread metabolic consequences (GOODSON et al 1980).

The ability of insulin to correct the deficient healing response in diabetes is time-dependent. Thus, insulin given in the first 10 days of healing gives more benefit than insulin given later. Along with the

observation that insulin catabolism is greatest in the early phase of repair, these findings have led to the hypothesis that the major effect of diabetes on healing is in its impairment of the inflammatory response (GOODSON et al 1980).

The role of insulin in repair is further emphasised by the observation that healing in poorly-controlled diabetics shows decreased collagen formation (both quantity and quality), faulty collagen remodelling, and decreased capillary ingrowth, DNA synthesis and cell infiltration (GOODSON & HUNT 1979a; WERINGER et al 1982).

Early supplementation with insulin restores normal healing, even if insulin therapy is later discontinued (GOODSON & HUNT 1979a). Thus insulin must primarily act on the cells (for example, fibroblasts), rather than on the products of their synthesis (collagen, DNA ...). (GOODSON & HUNT 1979a). Its importance, however, does not exclude the effects of other factors including small vessel disease and hyperglycaemia (GOODSON & HUNT 1979b). The effects of insulin on cell growth are reported to occur at concentrations lower than those required to control hyperglycaemia (WERINGER et al 1982).

5.3.1.4 Glucose and wound repair.

The effects of hyperglycaemia are not so well understood. Unlike insulin deficiency, restoration of the blood sugar level (BSL) to normal does not produce significant alleviation of the inhibited responses of diabetic wounds (GARCIA-LEME et al 1973; LLORACH et al 1976). Thus the effect of glucose is less direct than that of insulin.

Some suggest that wound fluid is itself hyperglycaemic. This is reported to provide an ideal environment for incubating microorganisms. Some state that bacterial infection increases proportionally to the

amount of extracellular glucose (GLASSER & BARTH 1982). Other reports however, demonstrated little evidence of this, with only fungi such as Candida albicans growing more readily in tissues with high glucose content (WINTERS 1982).

Hyperglycaemia is also reported to affect leucocyte function. Thus, chemotaxis, vessel wall adherence, phagocytosis, and intracellular kill are impaired where the BSL is over 200 mg% - a common occurrence in poorly controlled diabetics (GOODSON & HUNT 1979a; GOODSON et al 1980; GLASSER & BARTH 1982). GLASSER & BARTH (1982), in fact, found a linear relationship between leucocyte function and fasting glucose levels.

In view of this depression of leucocyte function at levels over 200 mg%, GOODSON et al (1980) suggest that the current practice of using free glucose solutions in the 5 to 20% range following surgery may tend to create hyperglycaemia in normal individuals. This will require further investigation.

The proposed mechanisms of action of such raised BSL's are varied. Firstly, most of the energy in leucocytes is derived from glucose through anaerobic glycolysis. While the entry of glucose into leucocytes does not appear to be insulin-dependent, leucocytes in diabetic patients are seen to accumulate glucose-6-phosphate and fructose-6-phosphate, suggesting a blockage at this point, so limiting energy production (GOODSON et al 1980). Secondly, hyperglycaemia produces a hyperosmotic environment which is also thought to retard leucocyte function (GLASSER & BARTH 1982).

On a similar metabolic level, the ketoacidotic state which may develop in poorly-controlled diabetes probably also exerts negative

influences on many stages of healing. The decreased pH and increased serum osmolarity associated with ketoacidosis are suggested to inhibit chemotactic, phagocytic and bacteriocidal functions of granulocytes. Diminished tissue oxygenation and poor cellular nutrition (which also characterise this state) affect wound reconstruction by delaying epithelial and fibroblastic proliferation (WINTERS 1982).

Plasma features (such as hypoinsulinaemia, hyperglycaemia or both) are probably also responsible for the marked changes which occur in blood rheology in diabetes. DINTENFASS and DAVIS (1977) demonstrated significant correlation between increased severity of diabetes (particularly BSL) and increased rheological alterations in areas such as plasma viscosity, blood viscosity (female only), capillary pressure and red cell (RC) aggregation.

Further studies (YUKIHide et al 1981) correlated factors such as plasma viscosity, plasma fibrinogen, the dynamic rigidity modulus and platelet aggregation with the degree of RC aggregation. With increased aggregation, these changes all act to impair blood flow and thus tissue perfusion. Changes in platelet adhesion and aggregation may be particularly significant in increasing vessel occlusion and subsequent tissue ischaemia (WINTERS 1982). They may therefore further impair the nutrient supply of an already depressed wound.

Also significant is the observation that red cells in diabetic patients contain increased proportions of glycohaemoglobins, which have increased oxygen affinity. In addition, diphosphoglycerate (DPG) levels are altered, progressively decreasing as the control of diabetes deteriorates. (DPG has significant effects on the form of the oxyhaemoglobin dissociation curve) (DITZEL 1977). Together these alterations may significantly impair oxygen transfer to the tissues.

Thus, in the acidotic phase, oxygen transport is relatively normal since the loss of DPG is virtually counterbalanced by the effect of enhanced dissociation in acidic pH. When the acidosis is corrected, however, there is a fall in the oxyhaemoglobin dissociation curve. That is, this curve undergoes a "shift to the left", thus greatly decreasing the tissue availability of oxygen (DITZEL 1977).

5.3.1.5 Diabetes and inflammation.

Although CIMASONI (1974) did not observe any alterations in the inflammatory response to dental plaque in the gingival sulcus of diabetics compared with non-diabetics, suppression of inflammation is generally described. For example, LLORACH et al (1976) demonstrated significantly reduced oedematous responses to noxious stimuli in experimental paw oedema. The vascular response to intradermally injected histamine, serotonin and bradykinin, and to physical stimuli was also found to be depressed. GARCIA-LEME et al (1973) also supported these findings with the exception that they found no significant difference in the formation of bradykinin between diabetic and non-diabetic rats in response to exogenous inflammatory stimuli.

These reports suggest that diabetes impairs cellular responses more than fluid-phase responses. (Note the reports of diminished leucocyte function mentioned above). These cellular defects are suggested to be at least partly responsible for subsequent poor healing (GOODSON & HUNT 1979a).

Particularly evident in the diabetic wound is the delayed onset of migration and the significantly lower density of the leucocyte infiltrate (GOODSON et al 1980; WERINGER & ARQUILLIA 1981; GLASSER & BARTH 1982; WERINGER et al 1982). Diabetic wounds at 8 hours consist

predominantly of disrupted capillaries with considerable interstitial oedema. Minimal vascular, cellular or fibrous elements are evident (WERINGER & ARQUILLA 1981). Both hypoinsulinaemia and hyperglycaemia are important causal agents here as has been shown.

The effect of the ultrastructural changes on the inflammatory response is unknown. Some suggest that the diabetic microangiopathy is important in delaying leucocyte diapedesis and migration and in hindering increased capillary permeability (GLASSER & BARTH 1982). It is unlikely however, that this mechanical interference is the sole limiting factor. Further, the description of WERINGER and ARQUILLA (1981) of marked interstitial oedema and disruption of tissue and vessel structure in the early diabetic wound suggests a relatively superficial role for any such mechanical effect.

In summary, diabetes is a multifaceted defect in which vascular damage and carbohydrate intolerance are only a part. The metabolic defect, in severe cases, is seen to produce extensive depression of all aspects of the early phases of wound healing. These wounds are thus predisposed to a very drawn out course of healing at best, and severe sepsis and wound breakdown at worst. Suppression of the inflammatory phase seems to be a critical component in these complications.

The extent to which these factors develop is thought to depend on the duration of the absolute or relative lack of insulin, and upon the type and site of the wound (GLASSER & BARTH 1982). It has been shown that the difficulties seen in diabetic healing reflect a variety of aetiological factors. These include:

- (1) ischaemia caused by large- and small-vessel occlusive disease;
- (2) decreased inflammatory response (both fluid and cellular) caused by hyperglycaemia together with the effects of defective insulin

metabolism;

- (3) possible effects of capillary basement-membrane thickening;
- (4) possible direct effects of insulin deficiency on fibroblasts; and
- (5) possible genetic defects in diabetic cells.

(adapted from GOODSON et al 1980).

The clinically important point of this discussion is that well-controlled diabetes probably exerts only minimal depression of the repair processes. It is thus essential to provide as complete control as possible, since the pathophysiology of diabetes implies that very sudden balance shifts may occur.

5.3.2 Haematological Disorders.

The haematological disorders which relate to inflammation may be classified into three groups according to cell type: leucocyte disorders, anaemias, thrombocyte disorders. The effect of these disorders on post-surgical oedema is considered in 5.4.

5.3.2.1 Leucocyte disorders.

Three types of disorders may be identified. The cells might not migrate normally; they may show poor chemotactic responsiveness or phagocytic ability; or they may have diminished microbiocidal activity (RYAN & MAJNO 1977). These defects may occur singly or concurrently with others, as in the case of diabetes. In addition to these functional defects, a numerical deficiency may also be present. RYAN and MAJNO (1977) identify a large number of disorders in these areas, many of which are obscure syndromes. Only the major leucocyte disorders are discussed here.

SIMPSON and ROSS (1972, cited by ROSS 1980), using anti-neutrophil serum, were able to examine the process of wound repair in the absence of neutrophils. The conclusion of these investigators was that the absence of neutrophils was of no significance on the subsequent pattern of cell migration and fibrogenesis. This idea supported earlier observations of DALE and WOLFF (1971) using the "skin window" technique. ROSS (1980) adds the qualification that these conclusions are reasonable in the absence of overt infection. In the presence of large numbers of microorganisms, neutropenic wounds rapidly became infected and the animals died of septicaemia.

These conclusions are in contrast with those of BAMBARA et al (1979). Also using the skin window technique in patients with a variety of leucocyte disorders, these investigators observed significant alteration of the inflammatory process, with delayed migration and reduced phagocytosis. Further they found that depression of the PMN response in these disorders did lead to depressed monocyte accumulation in the skin window. This interrelationship is suggested to centre in the secretion of monocyte chemotactic factors by neutrophils (RYAN & MAJNO 1977; BAMBARA et al 1979).

Impaired neutrophil function and/or depleted numbers must generally be expected to cause significant depression of the inflammatory response. Some of the discrepancies between experimental results may be due to differing sensitivities between the experimental models (see VINEGAR et al 1974). Such depression is likely to delay subsequent phases of healing as seen in diabetes. The presence of bacterial contamination may be critical in determining the outcome of the injury in such individuals.

Should monocyte function be impaired, both inflammation itself and later phases of healing (fibroblast and endothelial cell proliferation, and connective tissue synthesis) may show marked disruption since many aspects of repair appear to be orchestrated by the macrophage (See Chapter 1).

The degree of leucocyte suppression closely correlates with the relative disruption observed in the healing response. The propensity to the development of infection is well illustrated in the case of cyclic neutropenia. The period of leucocyte depression is marked by reduced gingival resistance to dental plaque. The entry of bacterial toxins is thus greatly facilitated and gross inflammation and infection commonly result.

The alterations in healing are likely to be the same, irrespective of the aetiology of leucocyte dysfunction, though the mediating agents themselves may produce additional affects (for example where leucocyte dysfunction results from the administration of steroids or chemotherapeutic agents).

5.3.2.2 The anaemias.

The effects of anaemia on repair are largely due to their prior alteration of tissue metabolism and structure.

Iron-deficiency anaemia, for example, is reported to produce marked reductions in the thickness of the buccal oral epithelium (the iron-deficiency itself is possibly a major aetiological factor in this) (RENNIE et al 1982). Hypoplastic anaemia demonstrated destructive changes in the connective tissue ground substance, increased deposition of mucopolysaccharides in the wall of microvessels and increased vascular permeability (LAGUTINA et al 1971). These changes

confer decreased resistance to injury upon the affected tissues, and thus the response to injury tends to be exaggerated.

Probably the most significant feature of most anaemias is their reduction of tissue oxygenation. As will be seen in some detail in Chapter 6, wound oxygenation is the governing factor of the healing processes. Thus the tendency of anaemia to cause hypoxia is critical, since this may prolong the inflammatory reaction and delay cell proliferation and synthesis.

Anaemia is also commonly associated with malnutrition and aberrations in blood volume which further compromise healing. It is noted that normovolaemic anaemia does not normally reduce oxygenation and therefore has little effect on wound healing in the absence of other compromising factors (CHVAPIL & KOOPMAN 1982).

5.3.2.3 Thrombocyte disorders.

The studies of AURSNES (1974) clearly demonstrated that a major effect of thrombocytopenia was the altered integrity of the vascular wall with subsequent increased permeability to red cells and plasma proteins. This permeability increase leads to a transient increase in whole body weight, probably as a result of a secondarily increased interstitial fluid volume. Restoration of platelet numbers via transfusion tended to restore normal vascular conditions, depending upon the cause of the thrombocytopenia.

These results are expanded by the recent work of JORIS and colleagues (1980) who demonstrated that continued leakage of directly injured vessels was the major effect of thrombocytopenia on wound healing. This effect was chiefly located at the wound margins where the

degree of vessel sectioning (to produce the experimental wound) was greatest. At this site, there was a slight but significant inhibition of the proliferative phase accompanied by oedema, fibrin exudate and scattered macrophages. The reported effects of platelets in stimulating fibroblast proliferation and synthesis (see 3.2.2.5) were not evident here (JORIS et al 1980).

The role of platelets in the maintenance of endothelial integrity is a subject of considerable current debate. This role is clinically evident in the petechiae and purpura which commonly accompany thrombocytopenia. Microscopically it is seen in the attenuation of normal continuous endothelium and its "conversion" in some sites to the fenestrated form. Rapid passage of erythrocytes is thus facilitated (KITCHENS & WEISS 1975). The mechanisms involved in these aspects are unknown though an altered balance between the various prostaglandins has been implicated.

These alterations, particularly the persistence of haemorrhagic ooze from sectioned vessels, must produce increased oedema. From the above description, they would appear to also delay fibrogenesis. These effects probably occur as a result of the obviously delayed resolution of oedematous fluid, and possibly from the continued release of inflammatory mediators through the maintenance of plasma/tissue interaction.

It is therefore essential to ensure that adequate serum levels of functioning platelets are present before surgery, both for haemostasis and for subsequent wound healing.

5.3.3 Cardiovascular Disease.

Cardiovascular (CV) diseases are generally associated with impaired blood flow conditions. These may follow either vessel stenosis or pump failure following myocardial ischaemia or infarction. Under conditions of chronic hypoperfusion, the flow forces may not be high enough to guarantee an ideal flow adaption of red cells (RC) (VOLGER et al 1981). The rheology of blood in vascular diseases is therefore affected by:

- i) the highly shear-dependent blood viscosity (see 2.6), and
- ii) the metabolic changes associated with hypoxia, which influence RC deformability and the coagulation system.

Apart from these flow-induced reactions, there are inherent changes in the flow properties of blood, for example, increased plasma viscosity, and decreased RC deformability (VOLGER et al 1981).

In reviewing a number of cardiovascular risk factors and their correlation with flow properties, VOLGER et al (1981) found that only in those individuals who were merely overweight with no other risk factors was the macro- and micro-rheology normal. Further, the impairment of blood flow was apparent in these situations before the manifestation of vasculopathy.

These findings may provide partial explanation of the observation that the albumin transcapillary escape rate (TER_{alb}) is elevated in hypertensive patients (PARVING 1975b; ROSSING et al 1976), since locally impaired flow may further increase intravascular pressure producing increased filtration. Further, PARVING (1975a) demonstrated that acute hypertension causes small blood vessels to react with dilatation in some areas and constriction in others, further compromising flow.

As in diabetes, these changes in vascular permeability are thought to constitute the initial phenomena in the morphogenesis of hypertensive vascular lesions with plasma protein deposition within the wall (PARVING 1975a;b). VOLGER et al (1981) note that although the vascular disease does not cause the rheological changes, the latter are mostly found in conjunction with vascular lesions.

An additional feature of CV disease and its risk factors is that plasma viscosity itself increases. This appears to result from either increased total plasma protein concentration and/or by increases in component plasma proteins such as fibrinogen and alpha globulins (VOLGER et al 1981).

Thus CV disease and its associated risk factors interfere with the vascular wall, and alter vessel content. Platelets are stimulated to increased aggregation, plasma viscosity is increased and RC deformability is disturbed.

In the wound, these changes principally effect wound oxygenation, impairing fibroblast proliferation and function. Hypertension itself, if severe, may prolong haemorrhagic oozing (despite the increased tendency to thrombus formation), and thus prolong the formation of oedema. As in diabetes, the vasculopathy may impede leucocyte diapedesis, further delaying repair. These changes (other than the presence of vasculopathy) are at least partially reversed if the hypertension is controlled (see ROSSING et al 1976).

5.3.4 Renal, Hepatic and Other Systemic Disease.

SCHILLING (1976) reports that hepatic disease and failure has a profound influence on protein synthesis with a subsequent marked increase in wound complications (particularly wound sepsis and

dehiscence). Hepatic disease is also said to lower serum albumin (because of decreased synthesis) (CHVAPIL & KOOPMAN 1982). In combination with a loss of albumin via haemorrhage, such a fall in serum concentration may allow increased fluid shift out of the vasculature with generalised oedema being the result (SCHUMANN 1979).

In the context of surgical healing, liver disease may produce the more acutely evident problem of excessive haemorrhage due to decreased synthesis of clotting factors. In the extreme case, haemorrhage may be severe. With low-grade disease, persistent oozing is likely, again leading to prolonged inflammation and increasing the resultant oedema and haematoma.

This picture is somewhat contradicted by the observation that liver disease also affects the balance of essential fatty acids, the unsaturated fatty acids (for example, linoleic and arachidonic) being synthesised in the liver. While this must undoubtedly diminish the availability of prostaglandins and related inflammatory substances, the impaired haemostatic function (possibly also including platelet function) is the overwhelming effect where direct vascular injury is a feature.

It is also observed that bilirubin and jaundiced sera caused morphological changes in fibroblasts in culture and impaired their growth. This suggests that jaundice per se also has an adverse effect on wound healing (TAUBE et al 1981).

Thus hepatic disease may impair many aspects of the early repair process.

Renal failure is reported to increase the incidence of wound complications and delayed healing primarily because of uraemia and

renal acidosis (SCHILLING 1976). SHINDO and KOSAKI (1982b) demonstrated that the degree of endothelial and fibroblastic proliferation was inversely correlated with the severity of the uraemia. This explains the slight delay in the increase of wound strength seen in uraemic wounds. It appears that uraemia results in a diminished response to insulin and that this is the basis of these disruptions (SHINDO & KOSAKI 1982a).

Wounds in uraemic animals demonstrate increased grade of oedema and increased numbers of inflammatory cells. The zone of granulation is also larger and less hydroxyproline is evident. In other words, there is a greater tendency towards inflammatory processes and less collagen-forming tissue in such wounds (SHINDO & KOSAKI 1982b).

RILEY (1981) suggests that the disruption of healing which occurs in jaundiced and uraemic tissues is probably further complicated by the deleterious effects of the malnutrition which usually accompanies these conditions.

VERNIGORODSKY et al (1979) found pronounced changes of haemodynamics in Addison's disease. Arterial pressure was reduced and a compensatory increase was found in the minute volume and tone of the major blood vessels. Capillary permeability was also increased. These changes were reversed following institution of hormonal therapy.

In summary, these reports demonstrate the extent of conditions which may alter the structure and function of the microcirculation. With normal physiological function being optimal, any such changes are deleterious. Generally, prolongation of the inflammatory response and delayed fibrogenesis may be expected as a result of impaired tissue metabolism.

5.3.5 Connective Tissue/Skin Disorders.

As has been shown, an abnormal or diseased state within the body often reflects itself by structural or dynamic changes in the skin microcirculation. Specific changes of the capillaries of the nailfold, lip and conjunctiva have been reported in such systemic diseases as diabetes, arteriosclerosis, hypertension and even schizophrenia. Thus, vital microscopy of the skin microcirculation is able to provide useful information on the state of the vessels (FAGRELL & INTAGLIETTA 1977).

The skin microcirculation may also show marked changes in response to connective tissue/skin disorders. For example, abnormally dilated capillaries have been observed in scleroderma, dermatomyositis and Raynaud's syndrome. Observations are most commonly performed on nailfold capillaries, however, they are also seen in other areas (MARICQ et al 1977).

Skin disorders.

The anatomy and function of blood vessels in psoriasis (the most commonly examined skin disease) are characteristic of hyperplastic tissue, and are similar to that in skin wounds, neoplasia and other such tissue. They typically appear elongated and coiled, and often oriented towards a point of maximal epidermal activity (RYAN 1980). See FIGURE 5.1.

Further, whereas in normal skin the papillary vessels may slightly indent the epidermis, in established chronic psoriasis, indentation may occur to such an extent that these vessels are closely enveloped by epidermal ridges. (There is a gradation from absence of papillary vasculature altogether, to budding of the horizontal subpapillary plexus, to an elongated and subsequently fully coiled loop (RYAN 1980)).

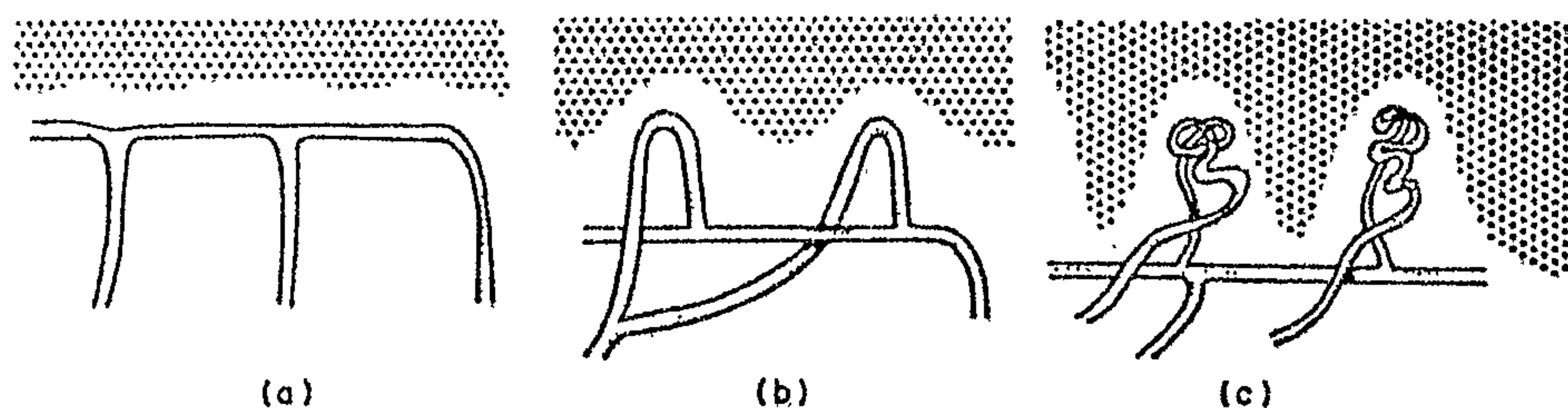


FIGURE 5.1a Three patterns of blood supply to the epidermis: a) atrophy - the horizontal subpapillary venous plexus, b) the normal papillary loop of healthy skin, and c) the coiled papillary loop in hyperplastic conditions such as psoriasis and healing wounds (from RYAN 1980, p30).

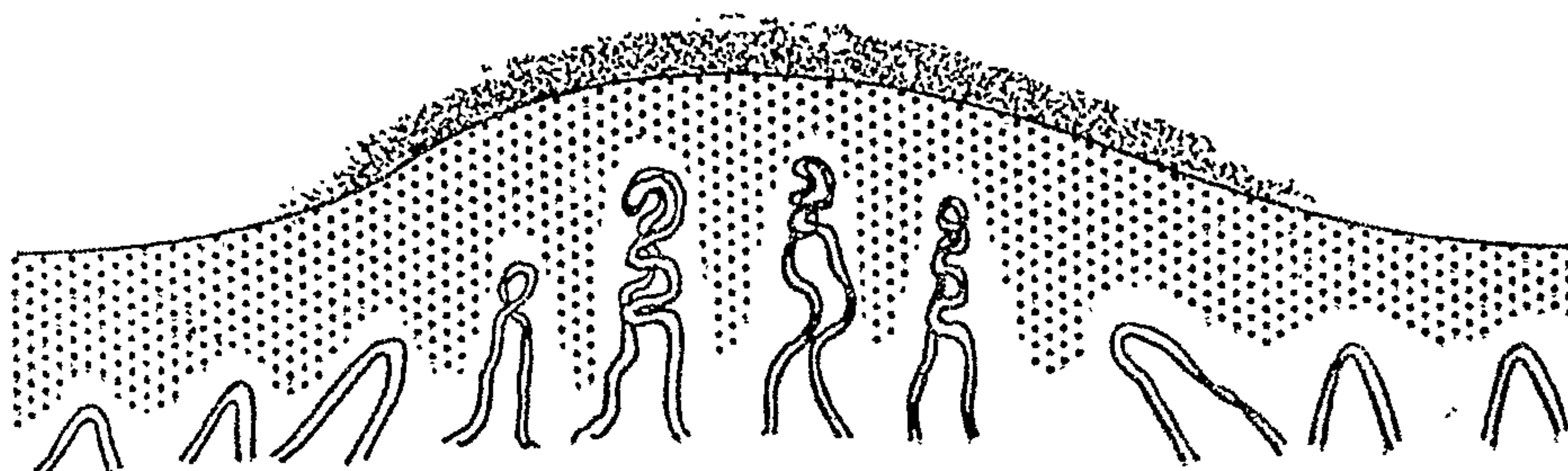


FIGURE 5.1b The typical pattern of papillary vessels in psoriasis: the central vessels are enveloped by the epidermis in the established part of the lesion. Vessels at the edge of the plaque tend to slope towards the site of maximal activity (from RYAN 1980, p31).

This configuration is seen to alter the pattern of blood flow. While there is no significant obstruction to inflow, flow through the papillary capillary (and especially at its junction with the subpapillary venous plexus) shows intermittent obstruction. This is an important feature of blood flow in hypertrophic epidermis and is characteristic of vessels associated with tissue growth (RYAN 1980).

The importance of this intermittent obstruction lies in its contribution to raised intracapillary pressure and thence to increased permeability. (See FIGURE 5.2). These changes also cause viscosity, haematocrit and RC aggregation to increase. Hence, flow is more frequently interrupted and long periods of stasis may occur (RYAN 1980).

In addition to these morphological changes, the most significant functional alteration is the presence of a marked increase in vessel leakiness to albumin and larger molecules such as IgG (RYAN 1980; WORM & ROSSING 1980). In many inflammatory skin diseases, and particularly

psoriasis, there is an increase in the number of endothelial gaps seen in post-capillary venules. This is suggested to represent either an inherent weakness of the endothelial intercellular junctions in these conditions or the presence of an unidentified stimulus producing a histamine-like response (WORM & ROSSING 1980).

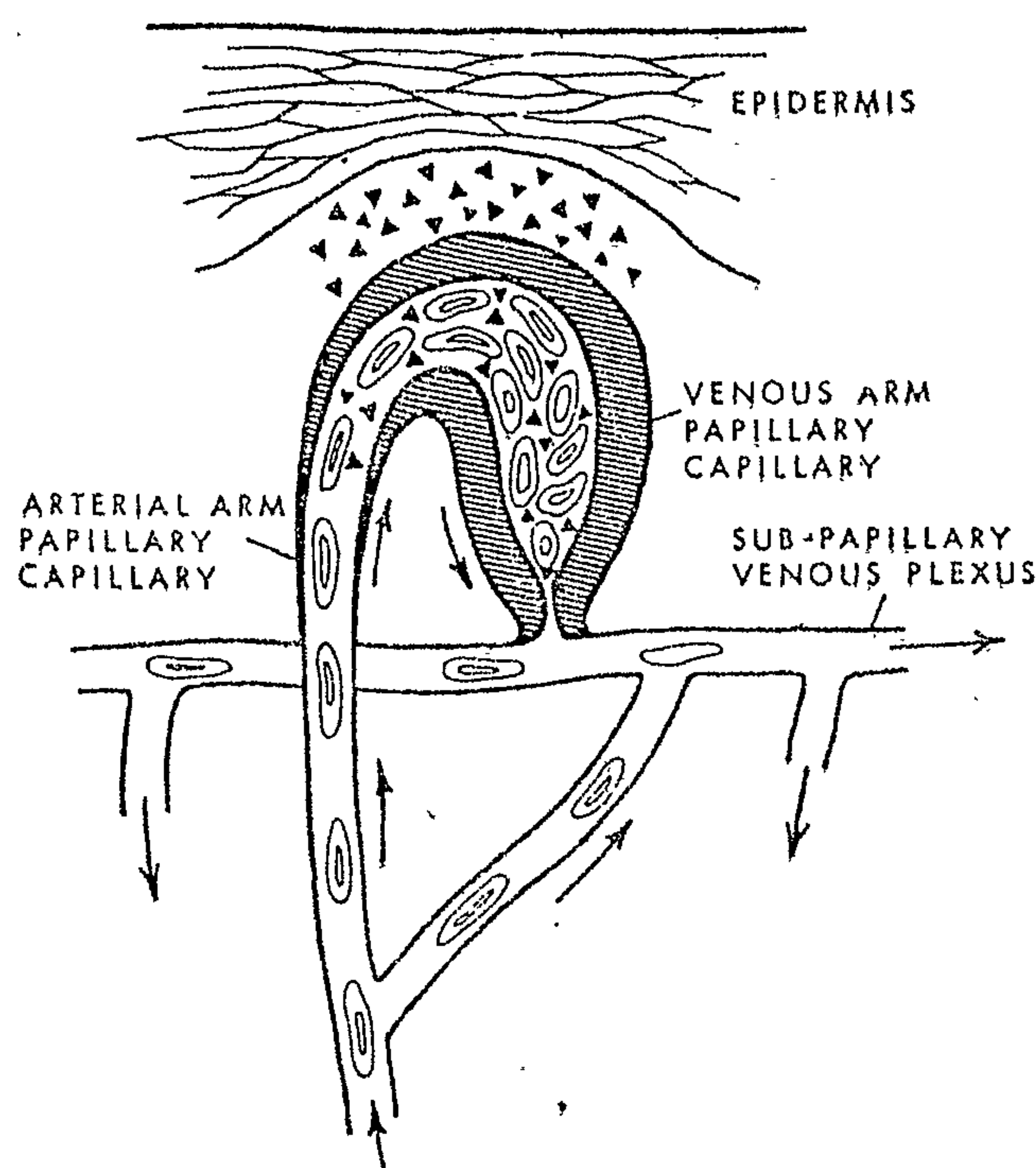


FIGURE 5.2 Haemoconcentration and hyperreactivity of the endothelium at the distal end of the papillary vessel encourages intermittent obstruction of outflow (from RYAN 1980, p32).

Hypoalbuminaemia and a slightly reduced plasma volume are also common findings in patients with extensive skin disease. The hypoalbuminaemia was thought to have resulted from increased extravasation; however, the interstitial levels of albumin were not found to be elevated and the true cause remains unknown (WORM & ROSSING 1980).

The cause of reduced plasma volume is clearer. It probably occurs as part of a generalised extracellular dehydration and/or an abnormal fluid distribution between the intravascular and extravascular compartments. The demonstration of oedema in skin with extensive

disease (MARKS & SHUSTER 1973) is evidence that the later does occur. The reason for this altered fluid distribution is however unknown (WORM & PARVING 1981).

In summary, the vascular pattern associated with skin diseases such as psoriasis closely resembles that associated with inflammation. It demonstrates increased leakiness to plasma proteins and aberrant rheological features so that interstitial oedema is common. A surgical wound in such tissues must exacerbate these abnormalities so that oedema is greater and more slowly resolving than in normal tissues. Resolution of oedema is further delayed because the lymphatic and proteolytic systems are approaching overload in unwounded, diseased tissue. Thus fibrogenesis and wound repair as a whole are significantly delayed.

Connective tissue disorders.

Oedema may also be associated with the collagen vascular diseases such as disseminated lupus erythematosus, systemic sclerosis, dermatomyositis, and rheumatoid arthritis. Again there is increased permeability to plasma proteins which is suggested to cause increased fluid outflow. Where the permeability increase is relatively minor, oedema does not occur, since the lymphatic and proteolytic systems are able to keep pace with the leakage and maintain equilibrium. More significant changes result in increased levels of interstitial fluid. (MARKS & SHUSTER 1973).

Significant ultrastructural alterations may also occur in these conditions. Lesions of chronic lupus erythematosus, for example, demonstrated marked endothelial stimulation and proliferation, resulting in varying degrees of lumen stricture. Also, the capillary BM was frequently thickened, focally disconnected or fragmented. On

occasions, collagen fibres were directly apposed to endothelial cells. The collagen surrounding such vessels demonstrated short, uneven fibres. Increased elastic fibres were also present. Thus the ultrastructural changes here are quite different from those of other microangiopathies such as diabetes (KOZAKIEWICZ & WRZOLKOWA 1976).

In all these conditions, the vascular changes may be local or generalised. Where present, the tissue already demonstrates increased lymphatic/proteolytic load and sometimes frank interstitial oedema. Surgery in such areas would produce rapid overloading and marked oedema formation.

As has been mentioned, their vasculature has the same appearance as that in the healing wound, which may indicate maintenance of the inflamed state in vessels sustaining surgical injury. The deposition of collagen and ground substance is also likely to be aberrant. Surgical trauma itself may in fact initiate the development of a local lesion, prolonging the course of healing.

Functionally there is increased vessel fragility which is likely to lead to inadequate early endothelial repair, contributing to further leakage. If the collagen aberrancy also includes that surrounding the lymphatics, as is likely, weakened abluminal fibres may lead to vessel collapse in oedematous circumstances, further compromising the fluid balance.

5.3.6 Obesity.

Contrary to their appearance, obese patients are often malnourished since the food which they select and consume in large quantities is frequently high energy, but has low nutrient value. Thus nutritional deficiencies may be present (see Chapter 6).

From a physiological viewpoint, obesity frequently presents problems of hypoventilation as a result of restricted diaphragm/chest wall movement. This leads to problems in achieving a satisfactory oxygen saturation of arterial blood and may produce a chronic hypoxic state within the tissues (WALTER 1976; WINTERS 1982).

Obesity at the operating site will also create difficulty because of increased operator inconvenience (hence increased surgical time), and because adipose tissue is relatively avascular. Thus the wound oxygen tension is further compromised (GROSZEK 1982; SCHUMANN 1982) and infection and wound breakdown are more common (GOODSON & HUNT 1979c).

Whilst much speculation has been made in relation to the effect of obesity on oedema (with opinions being forwarded on both sides), the answer has yet to be elucidated with careful scientific study. Relative tissue avascularity and hypoxia must delay progression from the inflammatory phase to proliferation. This may contribute to tissue infarction in some areas and thus to wound breakdown.

5.4 CONCLUSION.

This chapter has reviewed a wide range of literature in an effort to clarify the effect of various physiological and pathological factors and disease states on early wound healing particularly relating to oedema formation. While it is not possible to cover all factors, the most significant are outlined here. Usually some explanation of the basic disease processes involved has been necessary to enable the drawing of reasonable conclusions.

In general it can be stated that the normal physiological function is optimal. Any alterations more than likely impair tissue function, thus altering the repair process. These changes may be relatively insignificant (producing little clinically obvious effect) or they may be gross.

As has been noted, the response of normal tissue to physical trauma may show considerable variation. As suggested by REEVE (1977), the degree of the response depends chiefly upon the extent of the vascular injury. The larger the injury, the greater the extravasation and the greater the inflammatory response.

Some inter-individual variability in this response to surgical injury is likely to occur through basic, (perhaps genotypical) differences in connective tissue composition and arrangement. Age- and sex-related differences also contribute to this variability though perhaps only slightly on their own. The effect of the various biological rhythms presents an interesting hypothesis which needs further investigation but certainly is likely to provide some definition of a patient's ability to cope with surgical stress. Oedema has been shown to exhibit some circadian periodicity. However, as yet, research has not shown that the time of day of surgery exerts any influence on the level of maximum oedema, or on other parameters associated with surgical healing.

It is again reiterated that the process of inflammation forms an integral part of early wound healing. It has therefore been considered appropriate to include discussion of other aspects of repair, thereby maintaining inflammation and oedema in context.

The benefit of this approach can be seen in the various states which have been discussed. In most, the basic pathosis concerns abnormalities of tissue and vascular metabolism. These abnormalities generally predispose the tissue either to increased protein-rich fluid transudation or to delayed endothelial and fibroblastic proliferation and synthesis following surgical wounding. In the former, a significant defect is likely to increase the level of maximum oedema. In the latter, resolution of oedema is prolonged, and the associated delay in gaining wound strength must precipitate further vascular disruption, so maintaining oedema formation. See also 6.2.5.5 and Chapter 10.

The question remaining to the clinician at the conclusion of the presentation of these aspects must be, "to what extent do these factors operate in the in vivo clinical setting?" Because of the wide range of surgical procedures and the large inter-individual variability, clinical comparison is not possible outside carefully constructed studies. The belief of this author is that, in many cases, "maximum oedema" is limited by tissue factors such as tissue compliance (4.2.2.1). Perhaps this tendency to maintain normal dimensions through tissue tension provides a "pressure bandage" which tends to limit the spread of interstitial fluid. Thus the resilience of the connective tissue and fascial planes may play some role in defining oedema, thereby limiting its progress. The exception to such a hypothesis would be those connective tissue diseases in which the collagen framework is weakened or disrupted.

Significant omissions from this chapter are the effects of nutritional disorders and irradiation on wound repair. These, being relatively external determinants of the wound environment are considered next, along with intra-operative factors which may also alter oedema.

CHAPTER 6 THE EFFECT OF EXOGENOUS FACTORS ON OEDEMA.

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6.1 INTRODUCTION.

In the previous chapter, it was observed that marked variations may be evident clinically in the development of post-surgical oedema. It was also demonstrated that many physiological and pathological factors may modify the early processes of wound repair and thus alter the oedematous response. A variety of extrinsic factors are also highly significant in defining the wound microenvironment.

Of these, the supply of nutritional substrates including vitamins, trace elements and molecular oxygen, is perhaps the most critical. Other factors such as irradiation and tobacco smoking are also important since they may alter the vasculature, thus limiting nutrient supply.

Of particular oral importance is the microbiology of the wound. While infection per se does not form a part of this thesis, the presence of bacterial contamination in the wound may produce significant alteration of the tissue response. The effect of saliva is closely allied to this. Saliva is important both in establishing the unique environment of the mouth, and in its interactions with microorganisms and cells.

Macroscopically, the most obvious exogenous factors are those which result from infliction of the wound itself. Though this is obvious, this aspect of the injury is often ignored. It is important to gain understanding of the particular effects of each aspect of surgery; the central agents have yet to be fully identified (see 6.3).

This chapter reviews these and other factors with the aim of clarifying the relative contributions of each in the early tissue response to a surgical wound in the mouth. Discussion is divided

according to those factors which affect the environment of the wound and those which relate directly to the wound itself.

As was seen in the previous chapter, it is not possible to divorce post-surgical inflammatory oedema from the initial tissue response to injury as a whole. Thus, whilst discussion here centres on oedema and factors which may increase or decrease its formation, the context of wound repair is maintained.

6.2 ENVIRONMENTAL FACTORS.

6.2.1 Nutrition.

As stated by POLLACK (1979c), "wound healing proceeds more efficiently and quickly in well-nourished, (healthy) individuals". By contrast, "individuals who are malnourished and chronically ill heal less well and are at greater risk of complications during and after surgery". This is so because the process of healing is essentially anabolic. The wound therefore depends heavily on the availability of the nutrients required to form new tissue, but which cells are unable to produce de novo.

The delivery rates of nutritional substances depend upon their availability in the blood (intake), their diffusion constants, capillary permeability, and the distance which they must diffuse. The supply of rapidly utilized substances such as oxygen also depends on their rate of consumption, their concentration in capillary blood and the capillary blood flow (NIINIKOSKI 1980a).

These factors are highly significant when considering the size of the wound dead space. A large dead space dramatically impairs nutrient supply, leading to a prolonged inflammatory response with

reduced collagen deposition. If nutrient supply is extensively curtailed, necrosis of the overlying tissue may occur (NIINIKOSKI 1980a).

In addition to these local factors, it is important to note that any significant physical, physiological or emotional stress instigates a variety of hormonal reactions which increase nutrient mobilisation and excretion throughout the body. Thus, wounding leads to both local and systemic metabolic changes, the sum of which dramatically increases nutrient needs (NAVIA & MENAKER 1976).

In the case of the dental patient, not only do nutritional needs increase following surgery, but also nutrient intake may be compromised by impaired masticatory function due to oral pain, missing teeth or trismus (NAVIA & MENAKER 1976). Thus, nutritional problems may result from (1) failure to consume a diet which meets normal daily needs, (2) increased physiological requirements for certain nutrients which are not met, and (3) increased loss of tissue components. (For example, protein losses may occur during the catabolic phase of healing; water, electrolytes, vitamins and protein may be lost in blood and exudates, or through vomiting and diarrhoea which may follow surgery, NAVIA & MENAKER 1976). It is also noted that every patient having general anaesthesia is fasted for variable periods, a further factor which may further compromise the nutrient balance without appropriate post-operative management (SCHILLING 1976).

In understanding the magnitude and time sequence of these nutritional demands, it is essential to first examine the biochemical and metabolic events which occur following trauma or surgery. NAVIA and MENAKER (1976) outline three main phases in these events (see below). While there is considerable individual variability in the

characteristics and duration of these phases, the response described by this outline aids in clarification of the nutritional picture.

i) The early phase (or immediate response) may be termed the "ebb period" which lasts approximately 24 hours. General metabolism and heat production is depressed and hyperglycaemia and glycosuria may develop.

ii) The second phase follows the early and generally lasts from 4 to 10 days, depending upon the severity of the injury. It is accompanied by considerable losses of nitrogen, sulphur, ascorbic acid and creatinine in the urine. Phosphorus, magnesium and zinc are also lost, both in the urine and faeces. Termed by some the "flow period", there is a characteristic rise in body temperature which is probably due to increased oxidation of body protein. Fluid and protein are lost from the wound as a result of cell and vessel damage and increased tissue breakdown. The circulating levels of many nutrients, as well as their excretion levels, are temporarily increased during this stage.

iii) The third phase follows and is characterised by an increased anabolic activity of the body, allowing repair of the damaged tissue and restitution of the individual to normal life. This phase shows considerable variability in duration and course. Successful convalescence is chiefly dependent on nutritional management.

The systemic effects of these stages are fully discussed by NAVIA and MENAKER (1976).

When the wound itself is considered, the need for careful management of post-operative nutrition becomes even more apparent. It was stated above that a healthy vasculature was a critical requirement for normal healing. However, the wound itself disrupts the vessels in the local area, and together with subsequent haemostasis, the edge of the wound is essentially devascularised (HUNT et al 1969). The size of

the dead space so created is dependent upon the size of the wound and the accuracy with which closure apposes the wound margins.

In any case, a significant functional defect is produced by the disruption of vascular flow. As a result, large nutrient gradients occur across the wound from the point of injury to the nearest functioning circulation. This particularly applies to substances which are rapidly metabolised (such as oxygen) (HUNT et al 1969).

These gradients become highly significant in the formation of granulation tissue, since proliferating cells (particularly fibroblasts) are more sensitive than resting cells to nutrient deficiencies. The delivery of nutritive substances to the healing edge may thus be extremely precarious (NIINIKOSKI 1980a;b).

Clarification of the exact effects of nutritional deficiencies is difficult since all result in suppressed metabolism and weight loss, and these effects in themselves inhibit all aspects of healing (CHVAPIL & KOOPMANN 1982). The suggested involvement of nutrients in wound healing is outlined in TABLE 6.1 and TEXT-FIGURE 1.4.

TABLE 6.1 Nutrients affecting Wound Repair.

Nutrient	Specific component	Contribution to Wound Healing
Proteins	Amino acids	Needed for vascularisation, lymphocyte formation, fibroblast proliferation collagen synthesis, and wound remodelling. Required for certain cell-mediated responses including phagocytosis and intracellular killing of bacteria.
Carbo- hydrates	Glucose	Needed for energy requirement of leucocytes and fibroblasts to function in inhibiting activities of wound infection.
Fats	Essential unsaturated fatty acids: a) linoleic b) linolenic c) arachidonic	Serve as building blocks for prostaglandins that regulate cellular metabolism, inflammation and circulation. Are constituents of triglycerides and fatty acids contained in cellular and subcellular membranes.
Vitamins	Ascorbic acid	Hydroxylates proline and lysine in collagen synthesis. Enhances capillary formation and decreases capillary fragility. Is a necessary component of complement which functions in immune reactions and increases host defences against infection.
	B complex	Serve as co-factors of enzyme systems.
	Pyridone, pantothenic and folic acids	Required for antibody formation and leucocyte function.
	A	Enhances epithelialisation. Labilises membranes. Enhances collagen synthesis and crosslinking.
	D	Necessary for absorption, transport and metabolism of calcium. Indirectly affects phosphorus metabolism.
	E	May stabilise membranes thereby limiting inflammation and healing.
	K	Needed for synthesis of prothrombin and clotting factors VII, IX & X. Required for synthesis of calcium-binding protein.
Minerals	Zinc	Stabilises cell membranes. Needed for cell mitosis and proliferation.
	Iron	Needed for hydroxylation of proline and lysine in collagen synthesis. Enhances bacteriocidal activity of leucocytes, Secondarily, deficiency may cause decrease in oxygen transport.
	Copper	Is an integral part of the enzyme, lysyloxidase, which catalyses formation of stable collagen crosslinks.

(Adapted from SCHUMANN, 1979, p696).

6.2.1.1 Proteins, carbohydrates and fats.

Protein.

The effect of severe trauma or major surgery on protein metabolism is evidenced by the marked urinary excretion of nitrogen which follows. In fact, a negative nitrogen balance may occur in such situations. Such a severe change is unlikely to occur following minor oral surgical procedures. Nevertheless, adequate essential amino acids are required to ensure optimum healing (POLLACK 1979c; CHVAPIL & KOOPMANN 1982).

Prolonged protein deficiency is generally described as delaying the proliferative phase of healing and thereby impairing wound strength (NEWCOMBE 1972; SCHILLING 1976; HARRIS 1979b; RILEY 1981). POLLACK (1979c) reports that neogenesis, fibroblast proliferation, proteoglycan and collagen synthesis, and collagen remodelling are impaired by amino acid deficiency. Some cell functions, such as phagocytosis, may also be impaired by protein deficiency and caloric undernutrition.

However, such deficiencies probably need to be quite severe and prolonged for these effects to be evident, since short-term starvation (10 days) was without effect in adult rats. Such starvation was however significant in growing animals. Elderly patients too are more likely to suffer ill effects from deficiencies of protein because of reduced metabolic efficiency. Restoration of adequate protein intake is seen to restore normal healing (NEWCOMBE 1972).

As with other nutritional deficiencies, the delayed repair evident here (as expressed by the delayed collagen synthesis and so on) may prolong the inflammatory state and also possibly increase the amount of tissue which becomes non-vital due to delayed capillary ingrowth. In the chronic deficiency state, tissues are likely to become more susceptible to the effects of trauma. These effects will prolong and exacerbate the oedematous response in extreme cases. Minor deficiencies of protein are probably of little effect. The systemic effects of chronic protein deficiency may also prolong the patient's feeling of malaise following surgery.

Carbohydrates.

Disturbances in the metabolism of carbohydrates may give rise to direct and indirect effects. These substances, such as glucose and other sugars, are critical for energy production by cells via

glycolysis, the pentose phosphate shunt, Krebs cycle and oxidative phosphorylation (POLLACK 1979c; NIINIKOSKI 1980b). All the cells affected by such a deficiency will be less efficient in proliferation and synthesis.

An indirect effect of carbohydrate deficiency is the replacement of these substances by the less efficient use of amino acids. This may lead to a secondary protein deficiency.

All aspects of healing may thus be delayed and less efficient when carbohydrate supply is deficient. While it would be expected that oedema would therefore be prolonged, the concomitant suppression of leucocyte activity may reduce the degree of tissue lysis caused by these cells, thereby decreasing the response. Overall, however, wounds in severe chronic carbohydrate deficiency are likely to show exacerbated oedema.

Fats.

The role of fatty acids in wound repair is largely unexplored (POLLACK 1979c). CAFFREY and JONSSON (1981) suggest that essential fatty acid deficiency (EFAD) chiefly effects wound healing through diminished prostaglandin synthesis due to a lack of substrate. Rats with EFAD demonstrated retarded wound closure. This retardation was reversed by topical applications of arachidonic acid (AA) (CAFFREY & JONSSON 1981).

Linoleic acid also appears to be significant since it alone (not AA) reduced the trans-epidermal water loss which characterises EFAD (CAFFREY & JONSSON 1981). The postulated effects of linoleic acid derivatives (series 1 PGs) in negative feedback mechanisms may lead to prolongation of the healing response though the effect is probably more

significant in chronic inflammation (see Chapter 3).

These results are only preliminary and further work is required to clarify the role of fatty acids in repair. Many effects could be ascribed to the diminished prostaglandin synthesis. Not only is there diminished PG potentiation of vascular permeability responses. Significant depression of platelet aggregation (due to decreased thromboxane generation) may also be expected from in vitro studies.

However, as with many aspects of inflammation and repair, so many processes are duplicated and interconnected that deficiencies of single components are often not clinically significant, especially if only minor.

6.2.1.2 Vitamins.

Vitamin C

The best understood of the nutritional deficiencies is the deficiency of vitamin C (ascorbic acid) or scurvy. The effect of such deficiency on wound healing is well established with a plethora of published data.

Vitamin C is unique among the vitamins in that it regulates the formation and maintenance of both intercellular cement and collagen. Thus, the structural integrity of all body tissues is dependent upon this vitamin (RINGSDORF & CHERASKIN 1982).

A principal flaw which results from ascorbic acid deficiency is the depression of collagen synthesis, vitamin C being an essential co-factor (RILEY 1981). The collagen formed under such circumstances is underhydroxylated, coarse and irregularly deposited (see 1.5.2). Thus wound strength is decreased and a higher incidence of dehiscence

results (NEWCOMBE 1972; SCHILLING 1976; RINGSDORF & CHERASKIN 1982) since this collagen is more easily lysed by proteolytic enzymes (POLLACK 1979c).

Neutrophil function is also impaired by vitamin C deficiency. In these cells, the vitamin C is required in the reduction of oxygen to the superoxide anion used in intracellular microbiocidal functioning. Thus scorbutic wounds exhibit decreased resistance to infection (POLLACK 1979c; CHVAPIL & KOOPMANN 1982). Synthesis of components of the complement sequence is also impaired where ascorbic acid is deficient (POLLACK 1979c).

Whilst scurvy is now fairly rare in the Western world, vitamin C is not stored in large amounts and serious deficiencies can occur following major surgery in the absence of supplementation (SCHILLING 1976; BERLINGER 1982). Surgery itself produces an immediate depression of plasma levels by about 20%, and one study of surgical patients found 30% to be vitamin C-deficient (NEWCOMBE 1972).

In experimental studies, the effect of administering ascorbic acid to scorbutic animals depends on the relationship between the time of administration and the time of wounding. If replacement therapy was given to guinea pigs on the day of wounding, no collagen appeared until the sixth day. However, if administration was delayed until the tenth day, hydroxyproline was detectable within 24 hours (NEWCOMBE 1972). Thus the effect of vitamin C is not seen until the wound is prepared for collagen synthesis.

Some debate still exists in the literature as to whether any benefit is gained from the administration of vitamin C supplements in healthy individuals. RINGSDORF and CHERASKIN (1982) in a small study on

human gingival wounds reported marked acceleration of healing where such supplementation was performed. These authors report a number of other studies describing similar results. POLLACK (1979c) however, states that "there is no convincing evidence that wound healing is accelerated by the administration of vitamin C when its tissue levels are normal. One possible resolution of these opposing views is that individuals where benefit was observed were bordering on deficiency states, though this was not apparent clinically.

A very significant effect of the altered collagen metabolism seen with these deficiencies is that fibrous support for new blood vessels is poor (RINGS DORF & CHERASKIN 1982). Thus, neovascularisation is delayed (CHVAPIL & KOOPMAN 1982) and the high permeability levels which characterise new vessels are maintained (RINGS DORF & CHERASKIN 1982). This effect is also the result of impaired endothelial cell synthesis of vascular basement membrane.

Hence, the inflammatory phase is prolonged, vascular leakage persists (see 6.2.5.6), and contaminating microorganisms are less efficiently destroyed where ascorbic acid deficiency is present. The formation of oedema thus persists for considerably longer and is generally exacerbated in this state. The repair process may be so impaired that an acute non-healing wound becomes a site of chronic inflammation.

Vitamin A

In recent years, the role of vitamin A in wound healing has been intensely examined. Many questions however remain.

Some earlier studies (JOLLY 1964) demonstrated markedly reduced epithelial migration in palatal wounds of vitamin A-deficient rats. The

most severely deficient animals demonstrated no migration at all 24 hours after wounding. The numerical intensity of mitoses was also markedly depressed.

Several other workers have more recently confirmed this effect on epithelialization (PECK et al 1977; POLLACK 1979c; CHVAPIL & KOOPMAN 1982). These authors also report markedly delayed wound closure in vitamin A-deficient wounds. This apparently results from a decreased rate of collagen synthesis and cross-linkage (POLLACK 1979c; CHVAPIL & KOOPMAN 1982). This depression of collagen metabolism may simply follow the observation that vitamin A deficiency in laboratory animals causes depletion of vitamin C reserves, reproducing the effects of ascorbic acid deficiency described above (GOLAN et al 1980).

Other mechanisms whereby vitamin A affects wound healing are poorly understood. It is known that this vitamin exerts a labilizing effect on lysosomal enzymes (POLLACK 1979c; RILEY 1981). This effect appears to be dose-dependent so that at low doses, vitamin A incorporates into biological membranes, dissolving the lipid moiety and contributing to mild membrane lability. At higher doses, it appears to contribute to membrane disintegration (CHVAPIL 1980; CHVAPIL & KOOPMAN 1982). The release of fibroblast stimulating factor from macrophage lysosomes as a result of this labilization may partially explain the stimulatory effects of this vitamin (CHVAPIL 1980), but this cannot be the whole picture.

These membrane effects may also explain the deficient epithelialization which vitamin A-deficient wounds exhibit. Binding of this vitamin to epidermal cell membranes may alter the surface morphology of undifferentiated epidermis (the basal layer) (PECK et al 1977), allowing migration and mitotic division (HARRIS 1979b). It is

also suggested that vitamin A potentiates epidermal growth factor-induced mitogenesis (GERBER & ERDMAN 1982). Enhanced macrophage migration to the site of injury which SCHILLING (1976) ascribes to this vitamin, may similarly relate to membrane effects (such as adherence to endothelium).

Reports of the clinical effects of vitamin A administration are frequently contradictory. GERBER and ERDMAN (1982) report increased wound strength following administration of small dietary supplements of vitamin A or its components in rats with marginal vitamin A status. This was limited to the initial phase; later supplementation appeared to be of no effect.

However, other authors (SALMELA 1981b; CHVAPIL & KOOPMAN 1982) report that supplements of this vitamin had no effect on wound healing, and GOLAN et al (1980) reported an inhibitory effect when vitamin A was administered both topically and systemically. HARRIS (1979b) also reported inhibition, although high doses were required. These differences may in fact relate to differences in nutritional status of the animals, and to differences in dosage.

Another clinical effect which has been reported is that administration of supplemental vitamin A at least partially reverses the inhibited healing in cortisone-impaired wounds (HUNT 1976b; CHVAPIL & KOOPMAN 1982). This concept attracted considerable interest since it suggested that a combination of vitamin A and corticosteroid may remove the deleterious components of the healing process without compromising the repair.

The reversal effect is postulated to result from the opposite effects of vitamin A and the steroids on lysosomal membranes. However,

studies of GOLAN et al (1980) and SALMELA (1981b), and SALMELA and AHONEN (1981) found that toxic doses of vitamin A were required to achieve these results, lower doses being ineffectual.

It is thus apparent that considerable debate remains concerning many of the actions of this vitamin in wound healing. Further investigations may clarify these questions.

It may be summarised that vitamin A deficiency depresses proteoglycan and collagen synthesis, collagen remodelling and cross-linking, epithelialization, and perhaps restricts lysosomal release from inflammatory cells. As with scorbutic wounds, the overall effect of vitamin A deficiency is prolongation of the inflammatory response and hence delayed proliferation. Some persistent vascular leakage must again be expected with concomitant exacerbation of oedema.

Vitamin B-complex

B vitamins are reported to act as co-factors in a variety of enzyme systems. Disturbances of protein, carbohydrate and fat metabolism may thus occur in their absence (SCHILLING 1976; POLLACK 1979c). Inhibition of antibody formation and other leucocyte bacteriocidal functions is also likely (POLLACK 1979c; RILEY 1981).

These proposals may, in part, be supported by the studies of ALVAREZ and GILBREATH (1982) who found that a deficiency of thiamine (vitamin B1) significantly depressed collagen synthesis. These authors put forward the view that cell functions such as synthesis, mitosis, and migration are depressed in thiamine deficiency because of a reduced capacity of cells to generate sufficient ATP.

Such a depression of cellular functions would be likely to delay macrophage proteolysis of inflammatory oedema, causing clinically

evident swelling to persist for longer. Combined with a reduced synthetic capacity, and hence suppressed formation of granulation tissue, including stable new vessels, some continued leakage might also be expected. So would an increased risk of infection. These effects however, must remain speculation at this stage.

Vitamin E

Vitamin E has recently been the subject of intense investigation. This vitamin is believed to stabilise cell and lysosomal membranes. Particularly with respect to macrophage membranes, vitamin E might therefore inhibit repair. It has also been found to be an antioxidant, scavenging free radicals (HARRIS 1979b; POLLACK 1979c; CHVAPIL & KOOPMAN 1982). As such it may limit the effect of PMN-induced tissue lysis and accelerate prostaglandin biosynthesis towards the formation of stable, relatively inactive end-products, also inhibiting inflammation. However, the precise role (if any) that this vitamin plays in repair must remain conjecture at this stage (POLLACK 1979c).

Vitamin K

This vitamin is of critical importance for the synthesis of prothrombin and clotting factors II, VII, IX, and X. A deficiency of vitamin K may thus indirectly influence healing as a result of inadequate haemostasis and haematoma formation (POLLACK 1979c; RILEY 1981; SCHUMANN 1982). The clinical consequences of haemorrhage following minor deficiency states are discussed in 6.3.5. In more severe circumstances, the control of haemorrhage is of more immediate clinical importance in order that the systemic consequences of hypovolaemia may be avoided.

6.2.1.3 Trace elements and minerals.

Iron

Like vitamin C and oxygen, the presence of divalent iron is an essential requirement for lysine and proline hydroxylation, and thus for effective collagen synthesis (POLLACK 1979c; RILEY 1981). As was seen in Chapter 5, iron-deficiency is thought to be responsible for many of the observed changes associated with its corresponding anaemia. It is difficult however, to quantify the effect of iron-deficiency *per sé* in the repair processes (POLLACK 1979c), though some impairment of collagen synthesis is likely, with the production of less stable granulation tissue the result.

Copper

Copper is chiefly reported to be a component of lysyl oxidase, an enzyme which is responsible for the formation of covalent cross-linkages in collagen molecules (POLLACK 1979c; RILEY 1981). It is also reported to be essential for normal erythropoiesis, and thus has significance in wound oxygenation (CHVAPIL & KOOPMAN, 1982). However, copper deficiency is rare (POLLACK 1979c) and the effects of such deficiency on healing can only be conjectural until further investigation is performed.

Zinc

The biochemical functions in which zinc is thought to be necessary include: 1) enzymes and enzymatic function, 2) protein synthesis, and 3) carbohydrate metabolism. The role of zinc in the latter of these is somewhat controversial, though the histological findings in zinc-deficient and diabetic wounds are remarkably similar. However, there is strong evidence for zinc's involvement in at least 18 metalloenzymes (including alkaline phosphatase) and in protein

synthesis (DNA polymerase depends on zinc for normal RNA synthesis) (ZIELSDORF & WITT 1978; TENGRUP et al 1980a; ENGEL et al 1981; CHVAPIL & KOOPMAN 1982).

Thus, in wound repair, zinc deficiency leads to suppression of RNA and DNA synthesis with a concurrent decrease in cell proliferation and in polypeptide and protein synthesis (POLLACK 1979c). The wound is therefore characterised by delayed epithelialization and delayed gain in wound strength (NEWCOMBE 1972; SCHILLING 1976; HARRIS 1979b; TENGRUP et al 1980b). Zinc deficiency is also reported to alter intracellular bacteriocidal activity thus decreasing host resistance to infection (RILEY 1981; CHVAPIL & KOOPMAN 1982).

Whilst one study found inconclusive results (BUERK et al 1973), most researchers (on both animal and human subjects) agree that zinc deficiency does delay repair, and that the addition of zinc to the diet of such deficient individuals restores healing to normal (NORMAN et al 1975; WALLACE et al 1978; ZEILSDORF & WITT 1978; TENGRUP et al 1980a, 1981; BERLINGER 1982).

There is however no agreement on the effect of zinc supplementation on the healing of wounds where zinc is adequately supplied. Although some authors (HARRIS 1979b; TENGRUP et al 1980b) report enhanced early wound strength in normal rats from zinc supplementation, others (NORMAN et al 1975; ZEILSDORF & WITT 1978; CHVAPIL 1980; ENGEL et al 1981; BERLINGER 1982) report no effect from added zinc. Still others (WALLACE et al 1978; POLLACK 1979c; SCHUMANN 1982) report that excessive zinc is detrimental to healing.

The detrimental effects attributed to excessive zinc may relate to its effects on inflammatory cells, particularly macrophages, where

such levels are reported to cause inhibition of chemotaxis, phagocytosis and intracellular kill (CHVAPIL 1980). When in excess, zinc also inhibits lysyl oxidase (which functions to increase collagen cross-linkage) (POLLACK 1979c; SCHUMANN 1982).

It is important to note that although zinc deficiency is normally rare (POLLACK 1979c), surgical trauma, repetitive stress and some hormones produce sharp reductions in serum zinc levels (BUERK et al 1973; HALLBOOK & HEDELIN 1977; CHVAPIL & KOOPMANN 1982). Thus, transient zinc deficiencies may develop. Since albumin is the major carrier of zinc in the serum, liver disease (in which albumin production is decreased) may also lead to zinc deficiency (CHVAPIL & KOOPMANN 1982).

Summary.

In the case of deficiencies of copper, iron, zinc and perhaps other trace minerals, the major clinically apparent effect is that of delayed proliferation. While such a delay may again lead to increased oedema formation in theory, it is unlikely that this effect would be evident, except in extreme deficiency states. Zinc appears to be the most significant of these elements in modifying the early repair processes.

6.2.1.4 Oxygen.

Oxygen is essential for healing, just as it is for other energy requiring processes. Some is utilised in the numerous oxygenase and hydroxylase reactions which occur in healing tissue, including those of collagen synthesis. However, most of the oxygen consumed in wounds is used in the production of energy (HUNT & ZEDERFELDT 1969).