NECROTIZING ULCERATIVE GINGIVITIS

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PREFACE

Necrotizing ulcerative gingivitis is the most frequently encountered acute, specific gingival disease. It is a destructive pseudomembranous form of inflammation which is initially restricted to the interdental gingiva.

The initial lesions usually appear clinically as ulcerations involving the deep interdental tissues and the tips of the interdental papillae. Involvement of the marginal and even the attached gingiva may occur, although in most cases the ulcerative process remains confined within the papillary and marginal gingiva.

Wider extension of the lesions may occur in certain circumstances, with involvement of the alveolar or buccal mucosa, palate, tonsils or fauces. When the lesions are no longer contained within the gingiva the condition is more correctly described as necrotizing ulcerative stomatitis.

Tonsillar and pharyngeal lesions are stated to occur both in association with or in the absence of concurrent necrotizing ulcerative gingivitis. In the severely debilitated host, extension of the initial gingival lesions may lead to the development of the grossly destructive condition of cancrum oris.

The disease has been subject to numerous investigations over the last eighty years, since both Vincent and Plant described it as a condition distinct from diphtheritic angina, with which its more severe and disseminated manifestations were then frequently confused.

At that time, the common form of the disease was one of severe faucial infection and widespread ulcerative pharyngitis subsequent to tonsillar involvement. The severity of these lesions and their
distribution led to an accompanying sense of suffocation, i.e. angina. Hence the original descriptive term for the disease, Vincent's angina.

There is little complete agreement on any single aspect of the disease, with the exception of its amenability to palliative treatment by a legendary number of locally and systemically administered drugs, and its propensity for frequent recurrence.

Results of clinical and epidemiological observations, and of bacteriological, histopathological and lately ultrastructural and immunological investigations have proved inconclusive at best, and are frequently contradictory.

The relative lack of recent objective studies on necrotizing ulcerative gingivitis may be due to a decrease in the incidence of the disease, or difficulties in finding an acceptable animal model. Whatever the reason, the lack of such information makes necessary a detailed and critical evaluation of the existing studies and the conclusions drawn from them.

This review evaluates and correlates the available information on the aetiology, pathogenesis, incidence, diagnosis and treatment of necrotizing ulcerative gingivitis. Particular attention is paid to apparent fluctuations in the incidence of the disease, or variations in the severity of the presenting lesions. The rationale for some of the most frequently advocated forms of treatment is also discussed.

As a basis for understanding the disease process, a brief review of the structure, ultrastructure and function of the gingival tissues is presented in the introduction, together with an outline of the immunological mechanisms which may operate within these tissues.
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Part 1. INTRODUCTION.

The normal gingiva.
Gingival epithelium.
Gingival connective tissue.
Immunology.

THE NORMAL GINGIVA.

Morphology and Clinical Appearance.

Oral mucous membrane may be subdivided by its functional characteristics into three major types.  

Lining mucosa occurs in areas subjected to distension, such as the oral surfaces of the lips and cheeks, the floor of the mouth and the vestibules. It is covered by non-keratinizing stratified squamous epithelium.

Specialized mucosa is found in the dorsum of the tongue and in the vermillion or transitional zone of the lips.

Masticatory mucosa is present in those areas which may be subjected to the forces of mastication, i.e. the hard palate and the external surfaces of the gingiva. It is characterized by a keratinized or parakeratinized epithelium, overlying a dense lamina propria which is tightly bound to the underlying cementum or periosteum. Thus it occurs in relatively immobile regions.

The gingiva is that portion of the masticatory mucosa which covers the alveolar processes, and in health, the cervical areas of the teeth. Its coronal limit is the gingival margin, and its apical limit is the mucogingival junction externally and the uppermost dento-alveolar fibres of the periodontal ligament internally.

Epithelium lines the gingiva both on its external surface and on the surface adjacent to the teeth, and three different forms of gingival epithelium are described:

(a) Oral epithelium which extends from the gingival margin to the mucogingival junction and is of the orthokeratinizing or parakeratinizing variety.
(b) Sulcular epithelium which lines the gingival sulcus.

(c) Junctional epithelium which mediates the biological attachment of the gingiva to the tooth surface, i.e. it elaborates the epithelial attachment. (Figure 1).

Clinically and topographically the gingiva is subdivided into the free or marginal gingiva, the attached gingiva and the interdental or papillary gingiva.

Free gingiva is the coronal collar of smooth, movable unstippled gingival tissue which invests the necks of the teeth and forms the soft tissue wall of the gingival sulcus. It has been described as having a knife edged termination on the tooth surface, but a shallow furrow is more usually found between the gingival margin and the tooth surface at the orifice of the gingival sulcus. A rounded, rolled marginal gingival contour is found in the deciduous dentition and on erupting teeth.

In fully erupted teeth the gingival margin is located some 0.5 to two mms coronally to the cemento–enamel junction.

The gingival sulcus is the narrow space or potential space between the gingiva and the tooth surface, its apical extent being limited by the coronal or free surface of the junctional epithelium. Its depth in health is minimal in adults, and clinically it rarely exceeds two to three mms. Schroeder describes the histological sulcus depth in clinically close to normal tissue as being 0.4 ± 0.1 mms, but stresses that the clinical sulcus depth cannot be expected to reflect structural reality. He states that under conditions of strict normality there is no gingival sulcus present in mammals, including man.

This view is supported by experimental evidence obtained using dogs in which normal gingivae had been established by the rigid long term application of oral hygiene measures.

Listgarten states that clinical sulcus depth may not be the same as that of the histological gingival sulcus, which he considers to be a relatively shallow furrow 0.5 mms or less in depth. The greater reading
which is obtained clinically is attributed to mechanical disruption of the junctional epithelium by the measuring instrument.

Clinical sulcus depth is generally greater in the deciduous dentition, and may be up to six mm on erupting permanent incisor teeth. 5

In less than 50% of cases the free gingiva is demarcated from the attached gingiva by a shallow V-shaped free gingival groove. This runs parallel to the gingival margin at some 0.5 to 1.5 mm from it, and represents the approximate apical extent of the clinical gingival sulcus.

The presence or absence of the free gingival groove appears unaffected by mild to moderate gingival inflammation, or by the apical movement of the junctional epithelium from the enamel on to the cementum. 8 It cannot therefore be considered one of the criteria for normal gingiva.

When not present clinically, the groove may sometimes be demonstrated in histological section as an associated ridge and notch in the epithelium. Its presence appears related to a distinct fan shaped arrangement of the supra-alveolar collagen fibre bundles which run from the cementum into both free and attached gingiva, 6, 8, the dentogingival and dentoperiosteal fibres. (Figure 2).
Fig. 2 Clinical features and schematic section of labial/buccal gingival tissue. B = bone; C = cementum; D = dentine; DS = periodontal ligament; E = enamel; EA = epithelial attachment; GM = gingival margin; GS = gingival sulcus; IP = interdental papilla; JE = junctional epithelium; OE = oral epithelium; OSE = oral sulcular epithelium. 1 = cemento-gingival fibres; 2 = cemento-gingival fibres, horizontal portion; 3 = cemento-gingival fibres, alveolar portion; 4 = alveolo-gingival fibres; 5 = circular fibres; 6 = cemento-alveolar fibres; 7/8 = differently oriented periodontal fibres.


Attached gingiva is firm and resilient, and its surface is matt and stippled. It is continuous with the free gingiva, and extends from the level of the base of the gingival sulcus to the mucogingival junction. Here it is well demarcated from the red, movable, looser and non-keratinizing alveolar lining mucosa which covers the basal parts of the alveolar processes. In the palate, the attached gingiva blends into the similarly tightly adherent palatal masticatory mucosa.

The attached gingiva is firmly bound by its supra-alveolar connective tissue and lamina propria to the underlying cementum and periosteum. Its surface exhibits a variable degree of stippling which is due to the elevation of the epithelium by the insertion of bundles
of collagen fibres into the underlying connective tissue papillae. The stipple is due to epithelial depressions between these elevated areas. 4

Owings 9 investigated the relationship of stippling to the amount of gingival keratinization, and formed the opinions:

(a) ... that stipple represents areas of decreased metabolic activity at the intersections of epithelial ridges, and

(b) ... that dense, diffuse coarse stippling was equally associated with orthokeratinization and parakeratinization, but that sparse fine stippling was almost always associated with parakeratinization.

The rough stippled surface of the attached gingiva contrasts with the smoothness of both the marginal gingiva and the alveolar mucoea. Although the presence of stippling is one of the criteria for gingival health 3, 9, its distribution is variable within a range of normal. It may be totally absent in some areas such as the molar regions.

Stippling may be fine or coarse and is generally coarser in men. It is not very prominent in the deciduous dentition, and may not appear before the age of five or six. 4 It is more prominent on the vestibular than on the oral attached gingiva, usually coarsens with ageing, but tends to diminish again in old age particularly in women. 10

The width of the zone attached gingiva varies widely between individuals and in different areas of the mouth. Although the width is usually narrower in the deciduous dentition than in the corresponding permanent teeth, the pattern variation is similar in both, and the width does not appear to decrease with age in adults. 8

Recent evidence indicates that the width of the attached gingiva may increase with age in healthy periodontium with no gingival recession.
This is attributed to coronal movement of the cemento-enamel junction, accompanied by the gingiva and the alveolar bone, as a compensatory mechanism to maintain facial height in the presence of progressive wear of occlusal surfaces. ¹¹

The width of the attached gingiva varies from less than one mm to nine mms ¹¹, and a minimum width of one mm appears essential to preserve the health of the marginal gingiva. ¹²

It is usually wider in the maxilla than in the mandible, while in the palate it is continuous with the palatal masticatory mucosa. The widest areas are found vestibularly in the region of the anterior teeth, decreasing towards the cuspid and posterior segments, and being narrowest on the first tooth distal to the cuspid in both the deciduous and permanent dentitions. ⁸ In these areas there is an association with frenum and muscle attachments.

Lingually the pattern of variation is almost the reverse, the narrowest width being in the region of the anterior teeth and the widest adjacent to the premolar and molar teeth. ¹²

There are slight depressions in the attached gingiva between adjacent teeth, corresponding to depressions in the alveolar process between the eminences of the tooth sockets. These depressions may exhibit a vertical fold, the interdental groove.

**Interdental or papillary gingiva** is the tissue located at and within the interproximal spaces between adjacent teeth. Where a diastema exists the interdental gingiva is flattened or saddle shaped, and follows the contour of the alveolar bone to which it is tightly bound. ⁴ The free marginal gingivae of the adjacent teeth then form the mesial and distal margins of the interdental space.
When teeth are in contact, the free and attached gingival tissues which extend interdentally are formed into gingival papillae whose contour is dependent on the size and shape of the gingival embrasure. This is in turn determined by the contours of the approximating tooth crowns, and the size, shape and location of the contact area between them.  

In health, the gingival papillae represent the most coronal location of the gingivae. Buccal and lingual papillae may be present, with a concave depression or col between their tips, the shape of which depends on the contour and bucco-lingual dimension of the contact area. (Figure 3).

**Fig. 3**: Schematic lingual-buccal cross-section of interdental gingival tissue. B = bone; JE = col epithelium, comprised of junctional epithelium; CT = connective tissue; LP = lingual papilla; OE = oral epithelium; VP = vestibular papilla. 1 = circular fibres; 2 = transseptal fibres; 3 = alveolo-gingival fibres.

McHugh describes the presence of a col on all recently erupted teeth having proximal contacts. In the mature dentition it is more commonly present in posterior teeth, whilst in the anterior region the papillae may have a pyramidal shape. The base of the pyramid lies at the cemento–enamel junctions, while the mesial, distal, oral and vestibular surfaces form the steep sides which rise to join at the tip of a single papilla beneath the contact point.

The interdental region reflects earlier and more often than other gingival regions the signs of established gingival disease. It was originally thought that the surface of the col was lined by degenerating ameloblasts of the reduced enamel epithelium, and thus represented an area of morphological weakness.

Subsequent studies indicated that the epithelium of the col resembles the fused junctional epithelia of adjoining teeth. It is therefore non-keratinized and readily permeable, and the apparent vulnerability of the col area is attributed to this permeability, and to the morphological tooth to tooth relationship which favours the retention of bacterial dental plaque. Col epithelium may be replaced by less permeable oral stratified squamous epithelium at a later stage of development.

**Colour.** Oromucoosal pigmentation occurs most frequently in the mucosal surfaces of the lips, and in the gingiva and the palate.

Individuals, regardless of their race, or of widely differing degrees of pigmentation, have approximately the same number of pigment producing cells in any given region of skin or mucosa. Differences in pigmentation reflect differences in the activity of these cells, and in the distribution of the synthesized pigment.
There is a range of normal physiological pigmentation of the gingiva, due to differences in the intensity of melaninogenesis, the degree and depth of keratinization of the epithelium, and the arrangement of the gingival vasculature.\textsuperscript{4, 10, 15} Physiological gingival pigmentation is most frequent in, although not restricted to, the darker complexioned races\textsuperscript{16}, and may be diffuse or localized to certain areas of the gingiva.

Gingival colour is lighter in children than in adults. It apparently does not vary with age in adults, nor does it vary between the sexes. It is lighter in individuals with blonde rather than dark hair, and darker in those with darker eye colour.\textsuperscript{17}

Dummett\textsuperscript{15} describes the colour of healthy gingiva as variable between individuals, ranging from pale pink through to a deep bluish purple. Colour variations between these limits of normal may be uniform, unilateral, mottled, macular or blotched, and distributed to various or all parts of the gingiva.

Colour changes are regarded as important monitors of changes in gingival health\textsuperscript{6, 17}, but accurate observations of this type require an assessment of the norm for each individual's gingival complexion. (Plates 1 and 2).
PLATE 1. Lightly pigmented normal gingiva. Note stippling, scalloped gingival margins and well formed interdental papillae. Mucogingival junction is well defined.

PLATE 2. Gingiva more coarsely textured and more deeply pigmented than in Plate 1. Note marginal gingival enlargement, most pronounced in the lower anterior region.
Gingival Epithelium.

Three processes, mitosis, synthetic activity and later disintegration occur within epithelium, producing a constantly self-renewing multilayered cellular population in which the underlying cells form a cohesive tissue while those at the free surface are desquamated.

The stratified squamous epithelium of both the free and attached gingiva is either orthokeratinized, having surface cells which are clear structureless squames, or parakeratinized, in which case they retain flattened pyknotic nuclei. ¹

Both findings are normal, with parakeratinization present in about 75% of cases. ¹, ⁹

Epithelium of the lining mucosa, gingival sulcus and dento-gingival junction is usually described as non-keratinizing. ¹, ⁶ However, although the extremes of orthokeratinization and non-keratinization are evident in the oral epithelium, there is no absolute uniformity of cell populations, and a wide range of regional differentiation appears to exist. ¹, ¹⁸

Listgarten ² describes areas of the oral sulcular epithelium which exhibit a degree of keratinization, but states that portion of the sulcular epithelium which is derived from the junctional epithelium displays no tendency to keratinize. Attstrom ¹⁹ states that only a portion of the sulcular epithelium is non-keratinizing, while some of that portion which faces the tooth but does not make contact with it has characteristics similar to oral epithelium.

The interface between keratinizing oral epithelium and its lamina propria is uneven and tortuous. The underside of the epithelium has numerous ridges of varying depth and thickness which intersect in
a variety of patterns. Connective tissue papillae extend into the depressions enclosed by these intersecting epithelial ridges, which appear as pegs (rete pegs) in histological sections. They provide a greatly increased area of interface between the epithelium and the subjacent connective tissue, thus giving a much stronger junctional attachment and an increased area of exchange between them.

Normal junctional epithelium has no epithelial ridges, but in the absence of demonstrable inflammation they are considered by some to be a normal finding in sulcular epithelium.

**Intercellular junctions.**

Cohesion between epithelial cells is maintained by the cementing substance of the intercellular spaces, and by focal structural specializations of the cell plasmalemma to form various types of intercellular junctions. These mechanisms are assisted by irregularities in the contours of cell surfaces, and the close apposition of adjacent cell membranes. Spinous cells in particular have numerous peripheral cytoplasmic projections, resulting in narrow, irregular intercellular spaces.

Cell membranes between adjacent basal cells are fairly straight, and the intercellular spaces are not prominent. However, on the basal lamina aspect, the cell membrane is tortuous where it surrounds pedicles which project into the connective tissue. An irregular cell membrane is also found where the basal and spinous layers come into contact.
Three types of intercellular junction are described in the gingival epithelium. \( ^2, ^{14}, ^{20} \) (Figure 4).

\[ \begin{align*}
\cdots & (a) \text{ desmosomes} \\
\cdots & (b) \text{ gap junctions} \\
\cdots & (c) \text{ tight junctions}
\end{align*} \]

Fig. 4. Enlarged view of desmosome (D) and gap junction (GJ) of oral gingival epithelium. Tono fibrils (TF), viewed in an oblique plane of section, make contact with an electron dense attachment plaque (AP) adjacent to the inner leaflet of the cell membrane (CM). An intermediate contact layer (ICL) is situated between the outer leaflets of the cell membrane. At sites of gap junctions the cell membranes are closely juxtaposed but remain separated by approximately 20 Å; intercellular space (ICS); microvilli (MV).

The desmosome (Macula adherens) is the most common form of junction between gingival epithelial cells. Intracellular tonofilaments converge toward the cell periphery. Here they insert into the internal aspect of dense attachment plaques which are formed by thickening of the plasmalemma and condensation of adjacent intracellular cytoplasm. Most filaments appear not to terminate at the plaques, and probably loop through them so that their free ends remain in the cytoplasm.

A desmosome consists of the opposing parallel attachment plaques and the plasmalemmae of two adjacent cells, separated by light and dark lamellae which are thought to be oriented intercellular cement.

The central region of the gap between these plaques is the electron dense intercellular contact layer, and appears to be homologous with the peripheral density of hemidesmosomes.

Hemidesmosomes mediate adhesion between basal cells and their basement lamina, they are rarely found unattached on the surfaces of adjacent cell walls. They consist of a single attachment plaque and associated plasmalemma, beneath which a single thin peripheral density is located in the lamina lucida of the basement lamina. Tonofilaments which converge on the attachment plaque of a hemidesmosome may extend through the plasmalemma to terminate within the basement lamina.
The intermediate junction (Zonula adhaerens) consists of parallel adjacent cell membranes, with conspicuous bands of filamentous material in the adjacent cytoplasm, separated by an intercellular space about 200 Å wide which is filled with amorphous material. The zonula type junction is not localized, and extends in a continuous belt around the cell. The terms gap junction or nexus are also applied to the intermediate junction.

The tight junction may be of the macula, fascia or zonula ocludens type, and is formed by the fusion of adjoining plasmalemmea thus eliminating the intercellular space. The macula ocludens is a spot junction approximately the same size as a desmosome. If a more extensive area is involved the junction is described as a fascia ocludens, while the zonula ocludens is a belt like junction encircling the cell.

Maculae and fasciae oculudentes occur in oral epithelium. Zonulae oculudentes were described in gingival epithelium, but Listgarten suggests that these may actually have been fasciae.

Stratification of gingival epithelium:

Stratified squamous epithelial cells undergoing differentiation are known as keratinocytes. Pigment cells and other dendritic epithelial cell types and transients are collectively classified as non-keratinocytes. The two main features which differentiate keratinocytes from the cells of other tissues are their specialization for mutual cohesion, and for the synthesis of protein for retention within the cell.
Gingival epithelium is subdivided according to the morphology of its differentiating keratinocytes, into cell layers similar to those found in epidermis. These are the basal cell, prickle cell, granular and keratinized layers. A transitional layer or stratum lucidum occurs between the granular and keratinized layers in some thick glabrous regions of the epidermis, but is not usually found in gingival epithelium. 1

The basal cell layer (Stratum basale) contains the least differentiated keratinocytes. These small cuboidal or columnar cells have numerous fingerlike pedicles which appear to project into the subjacent connective tissue, but are separated from it by the basement lamina. 14, 20

Basal cell cytoplasm contains a high concentration of organelles in common with many other tissue types. However, basal cells cannot be considered functionally or structurally undifferentiated in the same way as undifferentiated mesenchymal cells or free stem cells of haemopoietic tissue, as they also contain tonofilaments which do not occur in cells of most other tissues. 1

Organelles and filaments identified in gingival basal cells include:

(a) ... a prominent nucleus 1, 25 which contains large proportions of the nucleic acids DNA and RNA. DNA, the genetic material of the cell, is localized in the karyoplasm and chromatin of the nucleus. It is concerned with cell growth and differentiation, and also with mitotic activity.

(b) ... one or more nucleoli 25, deep staining regions of the nucleus which have a high RNA content. There is a relationship between the number and size of the nucleoli and cell growth and active protein
synthesis. The nucleolus is regarded as an active participant in the cell's synthetic activities.

(c) ... a poorly developed Golgi complex. 20, 26 This membranous system of flattened sacs and vesicles in a curvilinear lamellated arrangement is most prominent in secretory cells. It is the region where newly synthesized proteins are segregated, concentrated and stored for redistribution. Synthesis of some polysaccharides may also occur within vacuoles of the Golgi complex.

(d) ... mitochondria 25, 26 are discrete organelles which occur as small spheres, ovoids or long slender threads. They are bounded by a double membrane, of which the inner one is folded into a series of flat membranous plates or cristae.

Mitochondria contain most of the enzymes required for the citric acid cycle, and play a vital part in the oxidative metabolism which makes energy in foodstuffs available to the cell. In general, the greater the cell's metabolic activity, the greater the number of mitochondria, usually preferentially located in regions of the cell where energy is utilized. Energy released by oxidative reactions is stored in the form of adenosine triphosphate (ATP).

(e) ... ribosomes 22, 27 are small nonmembranous organelles, granular bodies rich in RNA and associated protein, and the sites of cellular protein synthesis. They may be scattered as free ribosomes amongst the other organelles within the cytoplasm, or associated with the tubules and membranes (cisternae) of the rough or granular endoplasmic reticulum. They function singly or in small clusters known as polyribosomes or polysomes.

In general, free ribosomes synthesize proteins for the cell's own metabolic use, while the products of those attached to the outer surface
of the membranes of the endoplasmic reticulum are passed into cisternae. From here they pass to the Golgi complex for eventual export, i.e. secretion from the cell.

Basal epithelial cells contain abundant free ribosomes, but only sparse granular endoplasmic reticulum. In oral epithelium, both basal and parabasal cells show this continuing predominance of free ribosomes over granular-endoplasmic reticulum and over the Golgi complex.

(f) ... lysosomes are rounded cytoplasmic organelles, membrane bounded bodies filled with hydrolytic enzymes and responsible for intracellular digestion. The abundant small dense granules of polymorphonuclear neutrophils have the properties of lysosomes. Enzymes are released when lysosomes contact and fuse with autosomes containing ribosomes or mitochondria; breakdown of these organelles presumably provides material essential for cell metabolism.

Extracellular material is first ingested by phagosomes, and subsequently digested when these fuse with lysosomes forming phagolysosomes. This enclosure of material for digestion within the lysosomal membranes permits controlled degradation. Accidental release of lysosomal enzymes due to cell damage could result in autolysis.

The most characteristic feature of the cytoplasm of basal epithelial cells is the presence of fine strands of structural protein known as tonofilaments, which may become arranged in bundles or tonofibrils. Increasingly dense packing of these tonofilaments is one of the main features of cell differentiation in keratinizing epithelium.

The tonofilaments course towards the desmosomes and hemidesmosomes and it is believed that they function as an epithelial cytoskeleton.
maintaining tissue cohesiveness against applied forces.

Basal cells are primarily responsible for mitosis and the provision of new cells to replace those shed at the surface. Mitoses are most commonly encountered in these cells and in the lowest layers of the stratum spinosum. Together these may be regarded as the progenitor compartment or stratum germinativum \(^{10}\), although the actual proportion of suprabasal mitoses occurring in the oral epithelium is a matter of controversy. \(^1\)

**Basement lamina.**

Under light microscopy, the basal cells appear to be situated in contact with a basement membrane which lies between them and the underlying connective tissue papillae. This is however not a true membrane but a composite of connective tissue elements, including reticulin fibres and the basement lamina of the epithelium which is demonstrable with the electron microscope. \(^6, 23\)

The basement lamina is a structural entity of epithelial origin \(^21, 23\) having two identifiable layers, a lamina densa, and a lamina lucida which separates the amorphous, moderately dense lamina densa from the cell plasmalemma. \(^21\) The lamina densa consists of fine dense filaments embedded in an amorphous material, some of these fine filaments pass from the lamina densa through the lamina lucida to reach cell surface. \(^28\)

**Anchoring fibrils** are specialized striated fibrils which extend perpendicularly to the basal lamina on its connective tissue side, and form loops through which pass collagen fibrils of the connective tissue. \(^28\) This arrangement presumably contributes to the anchorage of the basal lamina, counteracting forces which might tend to separate the epithelium from the lamina propria. \(^14\)
Sprays of finer filaments fan out from the loops of the anchoring fibrils where these penetrate the lamina densa. It is suggested that they may continue through the lamina lucida to terminate either at the attachment plaques of the hemidesmosomes or by becoming continuous with the intracellular tonofilaments of the basal cells.¹ ²⁸ (Figure 5).

![Diagram of ultrastructure](image)

**Fig. 5** Diagram of the ultrastructure of the junction between epithelium and connective tissue.


The basement lamina is capable of filtering out molecules above a certain size, and can thus act as a protein filtration barrier.

The majority of metabolites entering or leaving the epithelium must traverse this region, which supplements the barrier function of the superficial layers of the epithelium. ¹⁴
Prickle cell layer (Stratum spinosum).

This is the largest of the strata of the oral epithelium and because of its relatively great width there are variations in the ultrastructural features of the cells within it. Those nearest the basal cell layer exhibit many of the features of basal cells, while the most superficial spinous cells resemble those of the stratum granulosum.

The major cells of the spinous layer are polygonal, and usually larger than basal cells. The term prickle cell refers to the microscopic appearance of apparently interconnecting cytoplasmic processes which were formerly regarded as intercellular bridges. Electron microscopy has shown that there is no actual cytoplasmic continuity between these projections, the intercellular junctions are provided by the desmosomes which are more numerous and somewhat larger than those in the basal layer.

Prickle cells contain relatively fewer organelles than basal cells. However, the ribosomes and the tonofilaments are more abundant and further organisation of the filaments into tonofibrils is also evident. In the more superficial layers the cells tend to become more flattened, with their larger diameter parallel to the basement lamina. The tonofilaments assume a similar orientation.

Membrane coating granules are organelles first encountered in the superficial cells of the stratum spinosum. They are found in groups in the superficial portion of the cell cytoplasm, and also occur in the higher strata of the epithelium. When they occur in keratinizing epithelium they have a striated appearance, due to the presence of a series of parallel lamellae. They are enclosed by a membrane and may have lysosomal functions.
Granular layer (Stratum granulosum).

This layer is present in all orthokeratinized epithelium; in parakeratinized epithelium it is either poorly developed or altogether lacking. The deep cells of the granular layer still contain numerous organelles, but these decrease in number in the more superficial layers. The cells become flattened in a plane parallel to the epithelial surface, and the desmosomes adopt a similar alignment. Intracytoplasmic thickening occurs in the region of the desmosomal attachment plaques which may become obscured, and the nuclei exhibit signs of degeneration and may become pyknotic.

Membrane coating granules gather along the superficial plasma membrane with which they appear to fuse, while their lamellae are found to project into the narrow intercellular spaces where they may contribute to the intercellular substance, and thereby to the barrier function of the epithelium. The characteristic intracellular feature of the granular layer is the presence of round, electron dense, basophilic keratohyalin granules which are found in association with the tonofilaments. Parakeratinized epithelium may contain similar although fewer, smaller, more regular granules. The keratohyalin granules may be synthesized by the ribosomes, and have been associated with the formation of an embedding matrix for the fibrillar component of the epithelial keratin. The most superficial layers of the stratum granulosum lack virtually all organelles, including nuclei. At this level the keratohyalin granules begin to disappear, and the most prominent feature of the cell is its densely packed tonofilaments. The demarcation between the stratum granulosum and the stratum corneum is relatively abrupt.
Keratinized layer (Stratum corneum). (Figure 6)

This layer is absent in parakeratinized epithelium where the surface cells retain persistent condensed, pyknotic nuclei. In keratinized regions, the superficial layers consist of closely packed, markedly flattened cells lacking all organelles. They are filled with fibrils of eosinophilic keratin, and contain clear droplets which appear routinely within the gingival epithelium and which may be lipid droplets.

![Image of a cell membrane with labels IL, EL, D, and B, and a bacterium (B) attached to the surface cell by delicate strands of extracellular material.]

*Fig. 6.* Surface of the stratum corneum. The internal leaflet of the cell membrane (IL) is approximately three times thicker than the external leaflet of the cell membrane (EL). The cytoplasm is occupied entirely by closely packed keratin tonofilaments (TF). Modified desmosomes (D) are present between adjacent cells. Note the bacteria (B) attached to the surface cell by delicate strands of extracellular material, probably of bacterial origin.

The plasmalemma now exhibits both extracellular thickenings, and intracellular ones which are continuous with the attachment plaques of the desmosomes \(^2\) which persist in this layer although no longer connected with the tonofibrils. \(^1\) Membrane coating granules may contribute to the extracellular thickening.

Desquamation of the most superficial squames is due to intradesmosomal disruption \(^1\), the exact nature of which is uncertain. \(^2\)

Non-keratinized gingival (oral sulcular) epithelium, may be regarded as a form transitional between keratinized oral epithelium and junctional epithelium. It has basal cell and prickle cell layers with sparser, more randomly distributed tonofilaments, fewer ribosomes, fewer, smaller desmosomes and more irregular intercellular spaces with abundant cementing substance. \(^1\) The tonofilaments and ribosomes do not become as concentrated as in the keratinized epithelium, as the increase in number of organelles does not keep pace with the increase in cell volume.

There is no separately distinguishable granular layer \(^1\), and the keratohyalin granules are few or absent. Tonofilaments are less abundant and not arranged in bundles; only short tufts are associated with the attachment plaques of the desmosomes. \(^3\)

The surface cells are less flattened and retain apparently normal nuclei which still contain chromatin. \(^1\) The numbers of the other organelles become reduced, but some persist into the surface layers and the cells remain basophilic. Membrane coating granules are present, but they are of a circular, membranous vesicular form rather than laminated. Their function is apparently the same as they appear to contribute an amorphous material to the intercellular space. \(^1\)
Stratification in non-keratinized epithelium is indistinct and classification into histologically separate layers may be somewhat arbitrary. 18

**Non-keratinocytes:** Histological sections of oral epithelium reveal numbers of clear cells. These, with the exception of Merkel cells, lack desmosomal attachments to adjoining cells, and all have a common feature in that they do not produce tonofilaments. They undergo shrinkage of their cytoplasm during fixation, resulting in the appearance of characteristic clear perinuclear zones from which the descriptive term of clear cell is derived.

The classification of these cells and description of their function is still incomplete. 1, 24 Several major types are usually described in the oral epithelium, constituting an estimated 10% of the total cell population. 30

**Melanocytes** may be regarded as unicellular exocrine glands. They are pigment producing cells, predominantly located in the basal and only occasionally in the suprabasal layers, possessing dendritic processes which project into the overlying strata. 31 Although closely apposed to the basement lamina they do not form hemidesmosomes. Their function when they occur in the skin is light protective, but no function has been determined for the melanocytes within the oral mucosa.

Melanocytes are derived from melanoblasts which originate from neural crest cells and subsequently migrate to assume an intraepithelial location. They possess a well developed Golgi complex and large areas of granular endoplasmic reticulum, but do not contain tonofilaments.
The naturally occurring melanin precursor is the amino acid tyrosine. Tyrosinase is an enzyme essential for the early oxidative sequences in melanin formation. It is synthesized on the ribosomes and passed via the endoplasmic reticulum to the Golgi apparatus, where it accumulates in vesicles which develop into pre-melanosomes and later into melanosomes, the end organelles of melanin synthesis.

Tyrosinase catalyzes the oxidation of tyrosine into DOPA, dihydroxyphenylalanine, which then undergoes further changes through intermediate stages into the extremely dense, virtually insoluble pigment polymer, melanin. Progressive accumulation of the pigment within the melanosomes eventually obscures their internal structure, producing an homogenous opaque particle, the melanin pigment granule.

Since melanocytes transfer melanosomes to adjacent recipient keratinocytes via their dendritic processes, melanin acquisition may involve the keratinocytes in a phagocytic role. Some melanin is also deposited into connective tissue macrophages known as melanophages; it is believed that this melanin has been shed from within the epithelium and is undergoing degradation.

Differences in pigmentation between individuals are based on the functional activity of the melanocytes and subsequent pigment distribution, and not on numbers of melanocytes.
Langerhans cells are predominantly suprabasal dendritic cells, sometimes described as "high level" clear cells. They resemble melanocytes, but do not stain with DOPA reaction. Ultrastructurally they are distinguished from melanocytes because they contain characteristic rod shaped granules, Langerhans or Birbeck granules. There is no agreement on either their origin or their function.

Merkel cells appear as clear cells under light microscopy, but they do not have a dendritic form, they also possess some infrequent desmosomal attachments to adjoining keratinocytes. Although they are found in association with intraepithelial nerve endings there is no conclusive evidence that they are true sensory cells.

Other clear cells which are occasionally described are amelanotic melanocytes, and non-specific dendritic cells which may simply represent sections of Langerhans cells or melanocytes which do not include their characteristic organelles.

Small numbers of intraepithelial inflammatory cells, most commonly lymphocytes, are accepted as normal findings even in clinically normal gingival epithelium. They are regarded as short lived immigrants, said to be present in small numbers even when their numbers in the underlying lamina propria are low.
Junctional Epithelium:

The junctional epithelium is the thin collar of epithelium which surrounds the neck of the tooth. It maintains the biological attachment between the gingival connective tissue and the tooth surface, thus sealing the surface of the oral integument and maintaining the continuity of the epithelial covering. 14

In health it extends from the bottom of the gingival sulcus to the cemento-enamel junction, and is attached to the enamel and to small areas of coronal (afibrillar) cementum where these extend onto the enamel surface. However attachment can also occur to fibrillar cementum and dentine 2, and even to calculus which has been sterilized.

On erupted teeth, an amorphous electron dense dental cuticle of uncertain origin may be interposed between the basement lamina of the junctional epithelial cells and the tooth surface. Its presence is associated with the previous prolonged presence of epithelial attachment over that particular area 33, and it is generally regarded as a secretory product of the junctional epithelium. 14

The junctional epithelium is usually two to three mms wide 3, and varies in thickness from one to three cells at its apical limit 6 to 15 to 30 cells at its free surface at the base of the gingival sulcus.
All desquamating junctional epithelial cells are shed at this narrow free surface, and because they are arranged parallel to the surface of the tooth they are shed in a vertical axis, rather than on their flattened surface as is usual in other stratified epithelium.

Junctional epithelium differs from the oral epithelium in having only two strata, a stratum basale and a layer designated by Schroeder as the stratum suprabasale which resembles the lower levels of the stratum spinosum of oral epithelium. The suprabasal cells are flattened and arranged parallel to the long axis of the tooth surface; there is no stratum granulosum or stratum corneum, and the cells exhibit no tendency to keratinize.

All cells of the junctional epithelium display considerable ultrastructural similarities of their cytoplasm and organelles. They have a low nuclear-cytoplasmic ratio, a prominent Golgi complex and moderately abundant RNA, granular endoplasmic reticulum and mitochondria. Membrane bound surfaces are much more abundant than in the basal cells of oral epithelium, but tonofilaments are sparse, resulting in a lack of rigidity. The proportion of filaments and synthesizing organelles remains relatively constant regardless of the position of the cell within the epithelium.

The number of intercellular desmosomal attachments is small, and the intercellular spaces large, amounting to some 13% of the volume of the epithelium. There do not appear to be any membrane coating granules, and together these factors are presumed to account for the ready permeability of this epithelium.

Junctional epithelium cells in the region of the base of the gingival sulcus contain lysosomes, and may exhibit phagocytic activity towards bacteria.
Cell turnover within the junctional epithelium is very rapid, with a high rate of mitosis. Thus the rate of desquamation is high, as the shedding surface is relatively narrow when compared with the germinative surface. Figure 7. The turnover rate for junctional epithelium is estimated to be four to six days compared to 10–14 days for the oral epithelium.

Figure 7. Diagrammatic representation of gingival epithelium illustrating the relative ratio between the "germinative surface" and the corresponding desquamative surface, for oral epithelium \( A_o \) and junctional epithelium \( B_j \). Together with information on cellular turnover, these ratios suggest a much faster rate of desquamation at the free surface of junctional epithelium.

The high rate of desquamation is regarded as fulfilling the requirement for a high repair potential in an area of structural weakness which represents the only naturally occurring discontinuity of either skin or lining epithelium. 3

Two basement laminae are described for junctional epithelium. 2

(a) The external basement lamina unites the stratum basale by means of its hemidesmosomes to the underlying gingival connective tissue. Due to the absence of epithelial ridges this epithelium-connective tissue interface is usually straight. Anchoring fibrils are present in association with the external basement lamina. 14

(b) The internal basement lamina attaches the junctional epithelium to the tooth surface, also by hemidesmosomal attachment. It does not exhibit a clearly defined lamina densa and lamina lucida. Its width approximates that of the external basement lamina 2, with which it merges at the apical limit of the junctional epithelium.

It was proposed that the descriptive terms for these laminae be reversed, as the tooth can be considered external to the body. 2 The original classification persists and regards the tooth as the centre of the periodontal structural complex.

Junctional epithelium is initially composed of postsecretory (reduced or resorptive) ameloblasts of the reduced enamel epithelium. These form the primary epithelial attachment to the tooth surface by a basement lamina and hemidesmosomes. Following tooth eruption, these cells, which cannot replicate, gradually transform into cells resembling squamous cells, and are shed at the base of the gingival sulcus. They are replaced by more external cells of the reduced enamel epithelium.
whose structural features gradually come to resemble those characterizing junctional epithelium. The oral epithelium, which fused with the reduced enamel epithelium at eruption, may contribute to the formation of the coronal portion of the junctional epithelium.

Following several renewals of cell population it is no longer possible to determine the origin of particular cells within the junctional epithelium, and following surgical excision it can be reconstituted de novo by cells derived from the oral epithelium alone.  

Attstrom et al demonstrated the presence of small numbers of leucocytes within the junctional epithelium of apparently normal gingiva. Occasional mononuclear cells were found in both the basal and suprabasal layers, and a few PMNs were located between the cells adjacent to the enamel surface.

**Sulcular Epithelium.**

The coronal portion of the lateral wall of the gingival sulcus is lined by stratified epithelium which is histologically similar to that of the oral epithelium of marginal gingiva. This oral sulcular epithelium may achieve a degree of partial keratinization. It is continuous with but structurally distinct from the junctional epithelium, which forms the base of the gingival sulcus and a part of the apical portion of its lateral wall. In some areas individual junctional epithelial cells may persist for some distance coronally to the sulcus base, in which case the bottom of the sulcus is actually lined by epithelium on all sides.

The oral sulcular epithelium usually overlaps the internal surface
of the junctional epithelium for some distance in an apical direction. (figures 8a and 8b).

Diagrammatic view of the gingival sulcus. The sulcus is lined by the oral sulcular epithelium coronally, and by the free surface of the junctional epithelium inferiorly.

Figure 8a.

Histological section of gingival sulcus.
JE ... Junctional epithelium
CSE ... Oral sulcular epithelium
CT ... Connective Tissue
DC ... Dental cuticle

Figure 8b.
Gingival Connective Tissue.

The bulk of the gingiva is composed of dense connective tissue, whose structural components are:
(a) ... fibres
(b) ... blood and lymphatic vessels
(c) ... sensory receptors
(d) ... structural cells, and cells of the defence and repair systems

These are embedded in a gel-like colloidal matrix, the ground substance.

Collagen fibres are the predominant component of the gingiva quantitatively, and its most important functional component. They resist deformation in all directions, due to the interlacing of the fibre bundles into a three dimensional collagen network, the organization of which endows mechanical stability. 3, 30

Gingival connective tissue consists of supra-alveolar connective tissue attached to the cementum by Sharpey's fibres, and the lamina propria of the attached gingiva which is tightly bound to the underlying peristeum. It has a loose superficial papillary layer which makes intimate contact with the functioning surface epithelium, and has extensions which project into the spaces between the epithelial ridges. 14

A deeper zone of denser tissue, with a net like arrangement of fibre bundles is known as the reticular layer. 1, 4

The height and number of epithelial ridges and connective tissue papillae is much greater in the masticatory mucosa which must resist shearing stresses, than in lining mucosa where elongation and distension must occur simultaneously with that occurring in the underlying muscles.

The papillary layer contains predominantly fine collagen fibres which form an open network enclosing the superficial elements of the microcirculation. 36 Reticulin fibres are found in association with these
fine collagen fibres in the region of the basement lamina. 4

Collagen fibres are coarser and much more densely packed in the reticular layer; there is an accompanying reduction in the amount of ground substance and in the cellular component. However, no distinct junction is evident between the two layers.

Gingiva contains no submucosa, although one is present in most areas of the hard palate where the gingiva and the palatal masticatory mucosa blend into one another. 1

Although the meshwork of collagen fibrils and fibre bundles is homogeneously distributed throughout normal gingival tissue 7, there is a preferential functional orientation of the bundles into the following major groupings 10: (Figure 2 and Figure 3).

Dentogingival fibres insert into cementum below the apical limit of the junctional epithelium around the whole circumference of the root. Some fan out coronally to terminate in the free gingiva, others extend in a more horizontal direction. Some dentogingival fibres may traverse the interdental gingiva, terminating in the buccal or lingual papillae of the adjacent teeth. 3

Dentoperiosteal fibres also arise from cementum, but extend apically over the alveolar crest to insert into the mucoperiosteum of the attached gingiva.

Alveologingival fibres are a small group arising from the alveolar crest radiating coronally into both the free and attached gingiva.

Circular fibres encircle the tooth within the free gingiva. 6 In the interdental region they may connect with similar fibres from the adjacent teeth, in the same way as the dentogingival fibres. 3

Transseptal fibres are a prominent horizontal group extending interproximally between the cementum of adjacent teeth.
Intergingival, transgingival and semicircular collagen fibre bundles have also been described in the gingiva of marmosets. 14

Connective tissue fibres.

Collagen fibrils are composed of fibrous protein which has high tensile strength and low elasticity; they aggregate into coarse fibres and become arranged in fibre bundles. 37

These fibrils are the most conspicuous structural component of the gingival connective tissues, and consist of a specialized high molecular weight protein, collagen, together with glycoprotein and hexose sugar components. 1

The basic unit of collagen is the tropocollagen molecule which is synthesized and secreted by the fibroblasts. These molecules become aligned into protofibrils 6, which undergo subsequent parallel alignment and both intra and extramolecular cross-linking into fibrils. These display a characteristic banding pattern, axial periodicity, when viewed by electron microscopy. 14

Two amino acids, hydroxyproline and hydroxylysine are unique to and characteristic of collagen protein. The presence of hydroxyproline is the basis for quantitative biochemical assays of collagen content. 37, 38 It is present in large quantities, 14% by weight, and constitutes an unvarying proportion of collagen. Hydroxylysine is involved in the process of cross-linking. 38

Increased cross-linking with maturation results in a reduction in the solubility of collagen in cold electrolyte solutions. 1 In gingival tissues, the insoluble collagen component remains extremely labile 37, and the newly synthesized non cross-linked salt soluble
content is also unusually large. 3

Gingival connective tissue may be unique in its high collagen turnover rate 37, which may explain its high potential for regeneration and repair. 14

Reticulin fibres 1, 4, 30 are chemically similar to collagen, and exhibit the same ultrastructural axial periodicity. However their structure is finer, and they exhibit argyrophilic staining properties. They may be an immature form of collagen, and are most apparent in developing connective tissue where they are the earliest visible fibrous structure.

Reticulin fibres are abundant in the subepithelial region subjacent to the basement lamina, where they may be responsible for maintaining continuity between it and the lamina propria. They are also described as forming branching networks which appear to bind bundles of collagen fibres into larger units.

Elastic fibres 1, 4, 39 are rarely found in gingiva, except where they are found in association with the walls of blood vessels. They are much more numerous in the lamina propria and submucosa of lining mucosa, and unlike collagen fibres they tend to run singly and to branch and anastomose.

Their function is to restore deformed collagen to its relaxed state, and the elastic fibre content of any region of the oral mucosa appears to be directly related to the degree of deformation which the region undergoes, and to its flexibility and distensibility.

Lozdan and Squier 39 demonstrated that the one consistent histological feature at the mucogingival junction is an abrupt and marked increase in the numbers of the elastic fibres.
Oxytalan fibres \(^1, 40, 41\) within the oral mucosa appear to be restricted to the lamina propria of the gingival tissues, where they greatly outnumber elastic fibres. Their function has yet to be determined. There are some histochemical similarities between oxytalan fibres and developing pre-elastic fibres, but they differ at the ultrastructural level.

They are described as a specially developed modified type of elastic tissue, distinct from elastic and pre-elastic fibres, which may partly contribute to the support of the vasculature. \(^40\)

**Blood supply.** Both the oral epithelium and the epidermis are avascular, and are dependent for their blood supply on capillaries within the underlying connective tissue. \(^1\) The gingiva is highly vascularized, well in excess of metabolic needs, which may represent potential for rapid repair in a vulnerable region. \(^30\)

The gingival blood supply is derived from three sources:

(a) ... Vessels on the periosteal side of the alveolar process, derived from terminal supraperiosteal branches of the labial, buccal, lingual, mental and palatine arteries. \(^6\) The larger vessels are found within the deeper layers of the lamina propria \(^1\), and are chiefly capillaries, arterioles and venules although small arteries are occasionally seen.

(b) ... Branches of the alveolar arterioles, derived from the posterior superior alveolar arteries, anterior superior branches of the infraorbital arteries, and inferior alveolar arteries in the mandible. These penetrate interdental septa through foraminae in the cortical plate and enter the gingiva. \(^14\)
... Some vessels of the periodontal ligament also enter the gingiva. 6

The gingival vascular bed, which is richer than that found in the skin, is formed by anastomoses between all these vessels. 4, 14

Vessels within the lamina propria course perpendicularly to the surface of the tissues, but on approaching the basement lamina of the epithelium they tend to run parallel to the contour of the gingival surface as a subpapillary network. They terminate in a rich distribution of terminal hairpin capillary loops, just beneath the epithelium-connective tissue interface in the peripheral parts of the connective tissue papillae beneath the epithelial rete pegs. 3, 42

Studies have demonstrated up to 50 capillary loops per square millimetre of buccal free and attached gingiva. 6 Some of these loops are the longest in the body, arising at the junction of the free and attached gingiva and extending to the free gingival margin. 1

Beneath the junctional epithelium the terminal blood vessels are arranged in a thin, delicate, flat anastomosing plexus 43, which extends under the epithelial surface from the gingival margin to the apical limit of the junctional epithelium. This crevicular plexus arises from a deeper zone which runs perpendicularly to the epithelial surface before branching into arterioles which then tend to run parallel to the gingival margin. 44 It contains a high proportion of post capillary venules which are prone to early damage in inflammatory reactions to injury. 3 Egelberg 43 comments on their greater disposition towards increased permeability than other vessels, their susceptibility to injury, and that they are a site of predilection for haemorrhage, thrombosis, endotoxin accumulation and allergic injury. These factors may be important in the production of necrotizing gingival lesions. 43
With the onset of gingival inflammation, the thin layer of vessels of the crevicular plexus is replaced by a vascular bed which is rich in loop formations. 43

Hock 45 used perfusion, vital microscopy and histological techniques to study the gingival vasculature in the erupting deciduous teeth of dogs and cats, and concluded that the loop formations in the gingival vessels may not develop until after the onset of chronic marginal gingivitis. She cautioned against accepting universality as normality in studies on human vascular patterns. The vasculature in clinically normal gingiva in which inflammation has been reduced, may have been influenced by the presence and the severity of pre-experimental gingivitis. 45

The col area has a blood supply provided by both capillary loops, and also vessels which resemble those of the marginal plexus. 42 This could be interpreted as evidence of inflammatory changes in this region, even under conditions of apparent clinical normality.

Gingival lamina propria contains all the elements of the microcirculation, with the probable exception of arterio-venous shunts. Precapillary sphincters are present, but are less responsive than those of skin, and the gingival capillaries remain open even when the gingival arterioles participate in induced vasoconstriction. 30

The oral vasculature plays no part in the thermoregulatory functions in man. 30

These observations are of importance when considering the pathogenesis of vasculonecrotic gingival lesions.

Lymphatic vessels 3, 4, 46 of the gingiva generally follow the course of the blood vessels, eventually draining to the regional lymph nodes. Major drainage is to the submandibular and submental groups,
and thence to the superior and deep cervical nodes. Palatal gingival lymphatics drain directly to the deep cervical nodes.

Vital retrograde lymphography has been used to study lymphatic drainage in the oral tissues of dogs. Few gingival lymphatics were demonstrated, least of all in the reticular layer of the connective tissue. Those found were predominantly of the perivascular type, and appeared to drain in an apical direction and towards the periosteal surface of the alveolar bone. 46

Nerves supplying the gingiva are peripheral extremities of afferent fibres. They arise from branches of the anterior palatal and the superior alveolar nerves in the maxilla, and the inferior alveolar, mental and lingual nerves in the mandible. 4

Both simple and organized nerve endings have been demonstrated, including terminal and ultraterminal intraepithelial fibres. Ultraterminal fibres are intraepithelial projections of nerve processes from organized endings in the subepithelial connective tissue. They may also be associated with Merkel cells, 1 which are regarded as pseudosensory cells.

Intraepithelial nerve endings are frequently found in the gingiva. Meissner tactile corpuscles and Krause end bulbs (which are temperature receptors) are also described. 14

Organized receptors are differentially sensitive to a particular sensory function, but there is no incontrovertible evidence to show that any particular type of organized receptor is exclusively responsible for any single form of stimulus. 30, 32

Numerous nerve endings are found in the interdental papillae. 32
Cells of the connective tissue.

The fibroblast is the most numerous, and the principal structural cell of the lamina propria. It is responsible for the biosynthesis, secretion and maintenance of glycoproteins, proteoglycans, collagen, reticulin, and elastin. It is therefore responsible for the major cellular functions of the connective tissue. 30

In addition, fibroblasts supply regionally differing determinants for the overlying epithelium, and possibly chalones (see Page 53) which regulate not only the mitotic rate of the fibroblasts but influence that of the epithelium as well. 30

Fibroblasts are large, highly differentiated cells capable of replication. 47 They are elongated, stellate branching cells which appear fusiform or spindle shaped in profile. The possession of long slender branching cytoplasmic processes results in a high ratio of surface area to volume, and the fibroblast is thus very favourably equipped for the uptake of nutrients.

Abundant organelles provide the basis for intense synthetic activity. The nucleus is elliptical and usually aligned parallel to the neighbouring collagen fibre bundles. In active cells it stains deeply, and the cell cytoplasm shows increased basophilia due to an increase in the amount of RNA associated with protein synthesis. There may be more than one prominent nucleolus, abundant granular endoplasmic reticulum, moderately numerous mitochondria and a well developed Golgi complex containing numerous membrane bound vesicles. 47

Newly synthesized monomeric tropocollagen protofibrils aggregate within the cisternae of the endoplasmic reticulum and probably the Golgi vesicles. Further lateral and longitudinal aggregation and
polymerization occurs extracellularly, as does the incorporation of protein-polysaccharide complexes between the aligning microfibrils. In normal connective tissue, the fibroblasts located in the area beneath and along the junctional epithelium are larger, and therefore presumably more active, than those found in more remote areas of the lamina propria. This finding is interpreted as evidence of the maintenance of a constant potential for rapid wound repair in this vulnerable region.

The reduction in collagen content which is observed during gingival inflammation may represent interference with the functional activity of the fibroblasts, resulting in deficiencies in collagen production and maintenance rather than the destruction of previously existent collagen.

However, the mechanisms responsible for fibrogenesis and for normal physiological turnover of collagen are not yet understood.

An increase in fibroblast numbers accompanies tissue promoted collagenolysis, and in these circumstances their role may be actively degenerative.

Fibrocytes are a feature of mature, stable connective tissue, and are regarded as fibroblasts in a quiescent phase, at a low level of functional activity. They have relatively little cytoplasm, but possess numerous long branching cell processes. The Golgi complex is scantly, the endoplasmic reticulum sparse, and the prominent nucleolus is lacking.

Undifferentiated mesenchymal cells may be found, but are difficult to distinguish from active fibroblasts. Classification is not morphological but functional, based on observations that the mesenchymal cells can differentiate into other cell types in response to certain stimuli. They are thought to be embryonic cells which persist in adults, and are located along the walls of blood vessels, particularly capillaries.
Macrophages are mobile cells having extensive phagocytic potential. They are scavenger cells and function to ingest and destroy invading microorganisms, foreign material and fragments of damaged tissue subsequent to injury. They are numerous and active in inflammatory foci. 49, 50 As a component of the monocyte-macrophage system they have an important immune function in recognising, ingesting and processing antigens for subsequent presentation to immunologically competent cells. 49

There is a wide range of morphological types of cells of this series, derived from bone marrow stem cells which differentiate into monocytes. These then migrate into the tissues as required and there subsequently complete their differentiation into macrophages. 49

Normal non-inflamed tissues contain fixed, stationary mononuclear cells, these are quiescent histiocytes which closely resemble fibroblasts. On suitable stimulation they can become phagocytic, and in inflammation they are indistinguishable from macrophages. The distinction between them is often arbitrary.

Macrophages contain large numbers of phagosomes, and are capable of synthesizing lysosomal enzymes. 47, 50

Melanophages are macrophages of the lamina propria which have ingested melanin granules shed from within the epithelium. They do not exhibit tyrosinase activity and thus cannot themselves synthesize the melanin which they contain. 1

Mast cells occur in large numbers in normal gingival connective tissue, frequently in a perivascular location. Their primary role appears to be defence and repair. They synthesize, store and secrete important vasoactive and anticoagulant mediators of the inflammatory response 47, including histamine, heparin, and in some animals, but apparently not in man, serotonin.
Mast cells are large spherical or elliptical cells, capable of slow motility. They have a small centrally placed nucleus, sparse endoplasmic reticulum and a well developed Golgi complex. They contain large numbers of highly distinctive metachromatic granules within their cytoplasm, the metachromasia is chiefly attributed to the presence of heparin. Mast cells are rarely observed actually within an inflammatory focus. Their total numbers may increase in moderate inflammation, but they degranulate close to the centre of the focus and their numbers tend to decrease with an increase in the severity of inflammation.

Robinson and De Maro noted a large mast cell population in normal gingiva, and an inverse relationship between mast cell population and the severity of experimentally induced gingivitis. The col area had the least mast cells of any experimental region, which was interpreted as a probable response to the heavy concentration of irritants in this area.

Gingival connective tissue of apparently normal clinical status may contain small numbers of leucocytes, usually located close to the blood vessels or in a narrow zone immediately beneath the junctional epithelium in close relationship to the base of the gingival sulcus. There is little or no infiltration below the sulcular epithelium, it occurs from the bottom of the gingival sulcus to the apical limit of the junctional epithelium. Leucocytes are only present in significant numbers as part of a host defence reaction to inflammation.

Lymphocytes are most commonly found at the coronal end of the infiltrated region, in the deeper regions plasma cells occur in increasing numbers, and they predominate in the apical region. Lymphocytes are classified as small, medium and large, all having
similar overall morphology although the larger ones have a relatively
greater amount of cytoplasm. No clearly defined functional differences
are yet known to exist between the three types.

Small lymphocytes and their progeny are the fundamentally
immunocompetent cells of the body. They are simple round cells having
a large dense nucleus and a minimal rim of surrounding cytoplasm, with
few mitochondria and little granular endoplasmic reticulum. Two
populations are described, one with a life span of possibly hundreds
of days, and a shorter lived variety with a life span of only several
days. Cells of both life spans are classified into two major groups
based on their immunological functions.  

T-cells develop various properties when stimulated by immunogen,
some enlarge and divide, forming a population responsible for
immunological memory function, the others develop into the effector
cells of cell mediated immunity. 

B-cells also develop a memory cell population following immunogenic
stimulation, others mature into plasma cells (see Page 70).

Plasma cells are derived from the short lived B-cell population
via an intermediate precursor, the plasmablast. They have a round
eccentrically placed nucleus, a well developed Golgi complex and a high
concentration of granular endoplasmic reticulum. The nucleus is
characterized by its chromatin granules which are arranged in a
cartwheel or clock face fashion. The function of the plasma cell is
immunoglobulin secretion.

Ground substance: The cellular and fibrillar elements of the
gingival connective tissues are embedded in an amorphous matrix, the
ground substance, which appears structureless at the microscopic and
ultrastructural levels, but nevertheless possesses a high degree of
molecular organization. Its structural components are mucopolysaccharides, proteoglycans, acid mucopolysaccharides, glycoproteins and tissue fluids which contain plasma proteins, soluble fibre precursors and breakdown products, metabolites, enzymes, hormones, vitamins and electrolytes. 53

Metabolites reaching the cells from the circulation, and also catabolites—moving in the opposite direction—must pass through this ground substance. It is therefore involved in the growth, organization, differentiation and regeneration of the tissues, and as a result it is chemically heterogeneous and its composition varies. 53

The major, covalently linked carbohydrate–protein complexes contained in the ground substance are:

(a) ... Glycoproteins, whose carbohydrate content is of low molecular weight. 1, 14

(b) ... Proteoglycans which contain uronic acids and amino sugars, and are often described as acid mucopolysaccharides.

Their mucopolysaccharide carbohydrate fractions may be either sulphated, e.g. chondroitin sulphates A, B, and C, of which B (dermatan sulphate) is found in skin and mucosa, or free, e.g. hyaluronic acid which exists in polymeric form.

Hyaluronic acid has well developed water holding capacity and is able to exclude other large molecules from its molecular domain 1, 53 while still being involved in the transport and diffusion of metabolites within the tissues. Bacterial invasion may occur as a result of the hydrolytic action of bacterial hyaluronidase on the polymeric integrity of hyaluronic acid.

Glycoprotein and proteoglycan levels are susceptible to change due to hormonal influences or pathological states such as inflammation.
Immunology.

Immune mechanisms evolved to protect the healthy individual from foreign, i.e. non-self substances such as invading microorganisms or their toxic metabolic products. 52

When an acute inflammatory response is first induced in host tissues, noxious agents are localized, inactivated or destroyed by the activation of a variety of host effector systems such as the complement and kinin systems. However, if the acute phase of the inflammatory reaction is unsuccessful in overcoming the challenge, chronic inflammation may supervene.

Permanent tissue damage may then occur, possibly as a result of cellular self injury due to extended triggering of the immune response. 54

Resistance to pathogens is based on:

(a) ... Non-specific factors, described as innate immunity or resistance, which are capable of acting independently of the immune system 55, and

(b) ... Specific or acquired immunity 56, a state of altered reactivity which results from antigenic stimulation of the host. The three fundamental characteristics of this immune response are specificity, memory and self-recognition. 52, 57

It is frequently difficult to maintain the precise distinction between these two forms of response, which may act concurrently or synergistically. 50 It has been suggested that the major function of immunological mechanisms may simply be the generation and augmentation of non-specific effector mechanisms. 55 (Table 1).
**Immunogens** are substances of various chemical types, usually complex high molecular weight macromolecules, which have the capacity to induce an immunological response de novo. Thus the immune system is stimulated to produce antibodies or sensitized effector cells which are directed specifically at the inducing substance, but not at other unrelated substances.

**Antigens** are substances which are capable of specific reactivity with preformed antibodies or effector cells. They are capable of initiating antibody production in previously sensitized animals.

Specificity is governed by a portion of the chemical structure on the antigen molecule known as the antigenic determinant. Different antigens may have some of their antigenic determinants in common, they are then described as heterophile antigens.

Some substances such as bacterial endotoxin possess a non-specific form of adjuvanic平, capable of enhancing the antigenicity of an otherwise weak antigen.

Proteins, polypeptides and lipopolysaccharides are capable of acting as antigens as are the lipoteichoic acids of most Gram-positive bacteria.

**Haptens** are small molecules, often simple chemical substances, incapable alone of eliciting an immune response. When a hapten binds to a larger "carrier" molecule, usually a serum or tissue protein, it then acts as the determinant of antigenic specificity for the resultant protein hapten conjugate which is immunogenic. The hapten alone, without the carrier molecule, is capable of serological reactivity with specific antibodies.
**Immunopathology** is the term used to describe all immune phenomena and reactions which are associated with disease, regardless of whether they are helpful, harmful or inconsequential to the host.

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<td>Phagocytosis—lysozyme—interferon</td>
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<tr>
<td>Specifically acquired immunity</td>
<td>Maternally derived Ig in baby</td>
</tr>
<tr>
<td>Specifically acquired immunity</td>
<td>Protection by preformed heterologous antibody or homologous γ-globulin</td>
</tr>
<tr>
<td>Active</td>
<td>Exposure to infection</td>
</tr>
<tr>
<td>Induced</td>
<td>Immunization with toxoid, or killed or attenuated organisms</td>
</tr>
</tbody>
</table>


**Innate immunity.**

*(Non-specific Immunity, Primary Defence Mechanisms, Resistance).*

Many effective but non-specific antimicrobial mechanisms are present at birth, and do not depend on previous exposure of the individual to any particular infective microorganism. Although these mechanisms are important, the later development of acquired specific immune responsiveness is indispensable to the survival of the individual.
Host determinants of innate immunity.

Species and strain. 52, 63 Different species vary markedly in their susceptibility to pathogenic microorganisms. Susceptibility does not necessarily imply lack of resistance. A species may be readily infected and yet recover rapidly from such infection, as occurs in humans and the common cold.

Individual genetic factors. 55, 63 Heredity plays a part in determining individual susceptibility, but this influence is difficult to distinguish from acquired immunity developed early in life due to environmental differences between communities.

Age. 63, 64 Infectious diseases are usually more severe in the young, possibly due to immaturity of immunological defence mechanisms. Cell loss and organ system deterioration are important factors in determining the response in senescent individuals.

Hormonal, nutritional and stress induced factors 55, 63 are implicated in determining individual susceptibility to infectious agents. Inadequate diet and disturbed homeostasis may be correlated with increased susceptibility to some bacterial diseases. Possible mechanisms are leucopaenia associated with dietary deficiency, or decreased phagocytic activity due to malnutrition or excess of glucocorticosteroids. 63

Stress conditions resulting in raised adrenalin and corticosteroid output can depress epithelial cell turnover by increasing the activity of locally produced tissue hormones known as chalones, which inhibit mitosis. 1
Physical determinants of innate immunity. 63

Mechanical barriers resist penetration by potential pathogens. Such barriers are provided by the physical resistance and desquamation of the stratified epithelium of the integument, the trapping effect of moist mucous membrane surfaces, and the action of ciliated epithelium as in the respiratory tract.

Active antimicrobial determinants. 55, 63, 65

Bacterial interactions at mucous surfaces may have a protective effect; the commensal bacteria can compete with the invading pathogens. Fatty acids in sweat and sebaceous gland secretions generate a low pH which inhibits bacterial colonization and replication. Mucous secretions can inhibit the penetration of cells by viruses through competition with cell surface receptors for viral neuraminidase. 66 (Table 2).
<table>
<thead>
<tr>
<th>Specific Host Determinants</th>
<th>Physical Determinants</th>
<th>Active antimicrobial Determinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species and strain</td>
<td>Mechanical barriers</td>
<td>Antibacterial and antifungal skin secretions.</td>
</tr>
<tr>
<td></td>
<td>(Skin, mucous membrane)</td>
<td></td>
</tr>
<tr>
<td>Individual genetic factors</td>
<td>Moist surfaces</td>
<td>Antibacterial and antifungal mucous membrane secretions.</td>
</tr>
<tr>
<td></td>
<td>Anatomical traps</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(e.g. nasal cavity)</td>
<td></td>
</tr>
<tr>
<td>Hormonal, nutritional and stress related factors</td>
<td>Mechanical cleansing (e.g. cilia)</td>
<td>Antibacterial and antiviral substances in tissue fluids. (e.g. lysozyme, interferon).</td>
</tr>
<tr>
<td>Age</td>
<td>Flushing and dilution (e.g. saliva, urine)</td>
<td>The acute inflammatory response. Phagocytosis.</td>
</tr>
</tbody>
</table>
Non-specific humoral factors.

Lysozyme \(^{55, 67}\) is a low molecular weight bactericidal enzyme with a limited antibacterial spectrum, present in most secretions except cerebro-spinal fluid, urine and sweat. It is found in high concentration in the granules of polymorphonuclear leucocytes and in macrophages \(^{50, 67}\), and causes hydrolysis of the cell wall of many Gram-negative bacteria. It may be an essential factor in the destruction and lysis of some Gram-negative bacteria in combination with antibody and complement. \(^{65}\)

Human sera and tissues contain other basic polypeptides of limited bactericidal activity. \(^{63}\) These include spermine, protamine, histone, properdin and C-reactive protein which is not present in health but appears in the serum during acute inflammation. \(^{68}\)

Interferon is a non-specific antiviral agent produced by host cells subsequent to viral infections. It assists in the recovery from rather than the prevention of viral infections, by inhibiting intracellular viral replication. \(^{67}\)

Non-specific cellular mechanisms.

Phagocytosis is a non-specific defence mechanism whereby bacteria are recognized, engulfed and intracellularly destroyed by macrophages and polymorphonuclear leucocytes (PMNs). \(^ {67}\)

Its efficiency is enhanced by a mechanism of the immune response known as opsonization. Opsonins are immunoglobulins, which together with complement coat the surfaces of invading bacteria and render them more susceptible to phagocytosis. \(^{55}\) Activation of complement by antigen–antibody interaction also attracts larger numbers of PMNs by chemotaxis.

Although this aspect of the defence mechanisms is basically protective to the host, it has the potential to cause tissue damage
due to the release of lysosomal enzymes from disintegrating phagocytes. 50

**COMPLEMENT:**

Complement activity is the collective term for the operation of a system of factors, present in low concentration in normal serum, which is activated by antigen-antibody interaction resulting in the release of biologically active by-products which mediate aspects of the inflammatory response. 55, 68

The capacity of complement to lyse antibody coated erythrocytes is well documented, but the dramatic haemolytic potential of the system is now regarded as less important in vivo than its potential for biological amplification by its ability to promote inflammation, facilitate phagocytosis and promote chemotaxis. 69 (Figure 9).

**Figure 9.** Immunoglobulin and complement coats greatly increase the adherence of bacteria (and other antigens) to macrophages. Uncoated or IgM (I) coated bacteria adhere relatively weakly to non-specific sites but there are specific receptors for IgG (Fc) (●) and C3 (●) on the macrophage surface which considerably enhance the strength of binding. The augmenting effect of complement is due to the fact that two adjacent IgG molecules can fix many C3 molecules. Although IgM does not bind specifically to the macrophage, it promotes adherence through complement fixation.

Opsonic adherence of C3 to PMNs and macrophages is probably not significantly different from immune adherence, i.e. the ability of antigen-antibody complexes to adhere to cell bound C3 on nonsensitized particles such as erythrocytes. Immune adherence is thought to assist phagocytosis by binding microorganisms to a relatively rigid object thus facilitating ingestion. 68

There are nine serum protein components 68 in the effector system (C1 - C9), the first of which comprises three major subfractions, Clq,Clr and C1s. 52 Although activation of the system is usually by a specific immune (Ag-Ab) complex, complement antedates the appearance of acquired immunity in the phylogeny 66 and can be activated by processes, alternate pathway factors, other than the reaction of C1 with antigen-antibody complex. However when activated in this way the system is poorly lytic.

Bacterial endotoxins can activate C3 and subsequent stages, bypassing C1, C4 and C2. 55 Properdin is thought to be a necessary component in this bypass activation. 68

Proteolytic enzymes from damaged tissue can initiate acute inflammatory reactions by releasing anaphylatoxin from either C3 or C5. 66 This relatively low molecular weight substance is capable of causing release of histamine from mast cells in a reaction distinct from that mediated by reaginic antibody.

The Complement Sequence. (Figure 10)

Activation commences when Clq combines with a receptor on an antibody molecule following its combination with the appropriate
antigen. The activation of this first component enables it to activate the next one in the sequence, initiating what is described as a cascade effect with sequential amplification. C4 follows C1 in the numbering sequence as the components were numbered before the sequence was established. 55, 68

Complement is believed to be the principal immunologically relevant effector system present in the serum. Some of the biological effects of its activation are listed in Table 3.

**Figure 10.** Sequence of complement activation showing formation of fragments chemotactic for polymorphs and with anaphylatoxin activity causing histamine release. Activated C567 complex is also chemotactic. Immune adherence through C3 to macrophages, platelets or red cells facilitates phagocytosis. Fixation of C8 and 9 generates cytolytic activity. Cells bearing AC1423567 are susceptible to cytotoxic attack by lymphocytes.

<table>
<thead>
<tr>
<th>Complexes and Compounds Involved</th>
<th>Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1, 4.</td>
<td>Together with IgM, neutralization of Herpes simplex virus.</td>
</tr>
<tr>
<td>C1, 4, 2.</td>
<td>Possible generation of kinins, increased vascular permeability.</td>
</tr>
<tr>
<td>C3b.</td>
<td>Enhanced leucocyte phagocytosis. Opsonic adherence to PMNs and to macrophages. Immune adherence.</td>
</tr>
<tr>
<td>C3a.</td>
<td>PMN chemotaxis. Anaphylatoxin, histamine release—contraction of smooth muscle, increased vascular permeability.</td>
</tr>
<tr>
<td>C1, 4, 2, 3.</td>
<td>Immune adherence.</td>
</tr>
<tr>
<td>C5.</td>
<td>Probably the principal Anaphylatoxin, also effective chemotaxis.</td>
</tr>
<tr>
<td>C5, 6, 7.</td>
<td>Chemotaxis.</td>
</tr>
<tr>
<td>C8, 9.</td>
<td>Membrane damage. Cytotoxic and cytolytic effects on erythrocytes, nucleated cells and microorganisms.</td>
</tr>
</tbody>
</table>
The Acute Inflammatory Response.

The normal initial reaction of tissue to sub-lethal injury is a non-specific inflammatory response directed at the dilution and also the localization of the irritant. 70

Infectious lesions are inflammatory lesions, and the fundamental principles controlling host reaction to injury are strictly applicable to them. However bacteria may afford a prolonged continuous stimulus for the inflammatory reaction due to their ability to proliferate, and they are capable of elaborating substances which modify the normal host response.

Thus disease represents the composite effect of an interplay between microorganisms, their metabolic products and the host defence mechanisms.

The acute inflammatory response is initially a vascular one, followed by cellular phenomena. An immediate vasodilatation occurs, accompanied by increased vascular permeability which allows the entry of a protein rich exudate into the tissue spaces. The exudate contains permeability increasing polypeptides (kinins), and plasma derived substances which when activated by enzymes, also produce kinin like substances. Capillary permeability is further increased by chemical substances released in the area of injury, e.g. histamine is released from injured tissue and degranulating mast cells, and serotonin from damaged endothelial cells and platelets. 70

Loss of fluid elements concentrates the cellular elements of the blood, leading to a slowing of the rate of blood flow and possibly to eventual stasis. Margination of polymorphonuclear leucocytes occurs, these then pass through the vessel wall by diapedesis.
and become concentrated in the area of injury by the chemical attraction of substances liberated from injured cells, a process known as chemotaxis.\textsuperscript{50}

In the later stages macrophages make their appearance and ingest bacteria, cell debris and dead polymorphonuclear leucocytes.

Phagocytosis by macrophages destroys microorganisms and inactivates toxins.\textsuperscript{50} It also provides a further close link between the non-specific and the acquired immune responses, by ensuring that the lymphoid cells are not presented with excess antigen which could result in the induction of tolerance (immune paralysis) rather than the development of responsiveness.\textsuperscript{49, 63} In addition, not all ingested antigen undergoes intracellular degradation. Some persists in a highly immunogenic state at the macrophage cell surfaces and is presented to immunocompetent lymphocytes, amplifying rather than initiating the immune response.\textsuperscript{49}

Conversely, the phagocytic activity of the macrophages is enhanced and given specificity by the preferential binding of cytophilic antibodies to their cell surface membranes.\textsuperscript{50, 52}
Macrophage Arming Factor is a type of cytophilic antibody which bestows immunological specificity on macrophages, enabling them to destroy target cells and possibly microorganisms. 50 (Figure 9 and Figure 11).

**Figure 11.** Cytophilic and opsonizing antibody provide alternative mechanisms for promoting adherence of bacteria to macrophages. The receptor site binds more strongly to complexed than free IgG and it is likely that the opsonizing route is the most significant.

The Shwartzman Reaction.

This reaction is produced by an initial intradermal injection (the preparatory injection) of bacterial endotoxin, followed some 12-24 hours later by an intravenous eliciting injection. This induces the response \(^7^n\), which is regarded as a form of non-specific hypersusceptibility. \(^7^2\)

The response is not however dependent on Ag-Ab reaction, and the eliciting reaction may be brought about by endotoxin from different bacterial species to those of the preparatory injection, or by washed Ag-Ab precipitates, agar or kaolin. \(^7^n\) Thus it is not strictly an allergic phenomenon, although its clinical manifestation is somewhat similar to the Arthus type reaction of immediate hypersensitivity.

Weinberg and Swartz \(^7^3\) state that convincing evidence that the reaction occurs in disease in man is still lacking, and there is no agreement on the extent of any possible involvement in human disease. The clinical description of the phenomenon is based on experiments with rabbits.

The basic lesion is a haemorrhagic necrotic reaction. PMN "cuffing" occurs about the small veins at the site of the preparatory injection. PMNs appear to be essential to the production of the phenomenon \(^3^7\) which does not occur subsequent to introduced leucopaenia. \(^7^4\) The eliciting injection produces a peripheral vasoconstriction at the prepared site, and obstructive leucocyte-platelet thrombi are formed. Complement is fixed on endotoxin particles; immune adherence to platelets (in the rabbit) and to erythrocytes (humans) occurs. The coagulation system is activated and intravascular coagulation results leading to necrosis of the vessel wall.
Lysosomal enzymes are stated to be of importance in causing the inflammatory changes associated with the lesion.

A generalized form of reaction is also described when both the preparatory and eliciting injections are given intravenously 24 hours apart. 50

Specific (Acquired) Immunity.

Acquired immunity may be classified as follows 66:

(a) ... **Passively Acquired Immunity**, which is the immunity derived by a non-immune individual from an actively immunized one. It may be natural, as in the case of the infant which is protected by maternally derived IgG, or induced by the therapeutic or prophylactic administration of immune serum or cells.

(b) ... **Actively Acquired Immunity**, which involves the stimulation of the immune system of the individual on whom the immunity is conferred. Again, this immunity may be natural, i.e. developed as a result of exposure to infection, or induced after deliberate artificial immunization with toxoid, or killed or attenuated organisms. (Table 1).
When an individual is challenged for the first time by an immunogen, several days elapse before antibodies can be demonstrated in the host's serum. This primary response results in a relatively low level of low affinity (IgM) antibody production which then declines after reaching a peak level, although some remains in the circulation. 52

On subsequent exposure to the same antigen, even many years later, any circulating antibody is rapidly depleted. However, there is a second anamnestic response by the primed individual resulting in more rapid, more abundant and more sustained production of antibody. 52

Thus a memory has been imparted to the immune system, and coupled with this memory is specificity of the response which is directed at the original antigen and not at other unrelated ones. 57 (Figure 12).

![Figure 12: Kinetics of appearance of antibody after first and second doses of antigen four weeks apart](image)

The immune function is described as having two aspects:

(a) ... the Afferent side is responsible for the recognition of antigen, its processing, and in general the induction of the immune response.

(b) ... the Efferent side deals with the specific humoral or cellular executive effector mechanisms which give practical effect to the immune response. Thus the efferent aspect is responsible for the manifestations of changed reactivity. 49, 75

These dual functions are performed by cells, macrophages, small lymphocytes and their derivatives, originating in the lymphoid tissues contained in the bone marrow, spleen, lymph nodes, thymus, gut tissues such as the appendix, and also in the foetal liver. 52

The Peyer's patches of the human gut are presumed to be the equivalent of the Bursa of Fabricius in chickens, responsible for the development of the immunocompetent cells which synthesize humoral antibodies. 52

**Small Lymphocytes.**

The small lymphocyte 47, 52, 76 is the cell of major importance in the immune response. Two distinct populations are described, thymus dependent (T-cell) lymphocytes and bursa dependent (B-cell) lymphocytes. These populations are responsible for the two separate functional limbs of the immune system, the cell mediated and the humoral immune responses.

T-lymphocytes are derived from haemopoietic stem cells arising from the bone marrow. These enter the circulation and come under the influence of the thymus gland where they mature and proliferate, developing into long lived T-cells, also known as thymus processed or thymus dependent lymphocytes.
Tritiated thymidine incorporation studies have shown that there is a high rate of mitosis of lymphoid cells in the thymus. Its essential function appears to be the proliferation and differentiation of primitive bone marrow derived lymphocyte precursors and the production of a factor which induces immunological competence in the matured lymphocytes. 77

Mature T-cells enter the pool of recirculating lymphocytes and are found in the blood, lymph and spleen. They are presumed to account for approximately 90% of human peripheral blood lymphocytes. 78 However some become established as long lived cells in the peripheral lymphoid tissues. The thymus dependent areas of the lymphoid tissues are adjacent to the central arterioles in the white pulp of the spleen, and the post-capillary venules in the medulla of the lymph nodes. 52

CELL MEDIATED IMMUNITY. (CMI)

On appropriate immunogenic stimulation the T-cells again proliferate. Some then remain in the circulation as sensitized or primed memory cells specific to that particular antigen, the others differentiate into killer cells, lymphoblasts, which are the effector cells of CMI and react directly with the antigenic target, with cytotoxic effect. 52

Spontaneously arising neoplastic cells may be eliminated by cell mediated immune responses, giving rise to the concept of another function of the immune system, the immunological surveillance of the host. Thus the immune system can activate destructive inflammatory responses not only against invading foreign substances and microorganisms, but also against host cells which by mutation, damage or viral action have developed aberrant (non-self) antigenic determinants. 75, 79
When sensitized cells come into contact with antigen, non-antibody effector substances known as lymphokines are released.\textsuperscript{66, 79} These are regarded as being responsible for the non-specific component of the cell mediated immune response, and \textit{in vitro} they have powerful pharmacological properties including\textsuperscript{52}:

(a) \ldots The promotion of vascular permeability.

(b) \ldots Release of a mitogenic factor which causes the proliferation and stimulation of yet unsensitized lymphocytes.

(c) \ldots Cytotoxicity, which may affect host tissues such as fibroblasts as well as target cells against which the response is directed.

(d) \ldots Chemotaxis and activation of macrophages.\textsuperscript{50}

(e) \ldots Localization of macrophages preventing their normal migration due to release of a macrophage migration inhibitory factor (MIF).

(f) \ldots The stimulation of osteoclasts.

(g) \ldots Cohen and Winkler\textsuperscript{80} describe an eosinophil chemotactic factor, a lymphokine which can only function after interacting with immune complexes and may thus link between CMI and humoral immunity.

CMI appears to be adapted to deal with peripherally immobilized antigens which do not readily reach the lymphoid tissues, and is encountered in the defence against neoplasms and against some viral, bacterial, mycotic and parasitic infections.\textsuperscript{52} Macrophages play a major efferent role in these situations.\textsuperscript{49}
HUMORAL IMMUNITY.

The B-cells are thymus independent, although they arise from the same bone marrow stem cell populations as do the T-cells. Following processing in "bursal equivalent" areas, probably the Peyer's patches, they emerge as immunocompetent cells which constitute about 10% of the peripheral circulating lymphocyte population, although the majority remain sessile in the lymph nodes.

On initial contact with immunogen they also proliferate. Some then survive as memory cells while the others differentiate through a series of stages such as plasmacyte and plasmablast into mature plasma cells.

The cell mechanisms leading to immunoglobulin production appear to rely on transport of antigen to the lymphoid tissues, and its possible processing by macrophages. The function of the plasma cell is the synthesis, secretion and release into the blood and tissue fluids of effector substances. These are binding proteins, antibodies which are specific to the particular immunogen invoking the response. Plasma cells are short lived end cells which do not undergo mitosis. Their cytoplasm is packed with granular endoplasmic reticulum carrying polyribosomes responsible for the synthesis of immunoglobulin for extracellular use. (Figure 13).
Fig. 13. Gingival plasma cell. Note the typical "clockface" arrangement of the condensed heterochromatin (HC) within the nucleus and the closely packed parallel cisternal of the granular endoplasmic reticulum (ER). Mitochondria (M).

In many situations the host response to foreign substances is by a combination of both humoral and cell mediated immune responses. 78

Cellular co-operation between T- and B- cells is also of importance in the secretion of some antibodies, particularly to weak antigens, the T-cells being regarded as helper cells which in some way increase the antigenic stimulation of the B-cells. 52, 60

The mechanisms of this co-operation are not understood, but animal experiments have established that antibody secretion by B-cells alone may in many cases be poor or modest. However the addition of T-cells results in a considerable increase in antibody synthesis. 52 It is now widely believed that this co-operation between lymphocytes is important for humoral immune responses to such antigens as serum proteins, heterologous erythrocytes and hapten-protein conjugates. (Figure 14).

It is also regarded as essential for the change from IgM to IgG synthesis which occurs during secondary immune responses. 52

Recent evidence suggests that B-cells also have the capacity to respond mitogenically, to produce lymphokines, and possibly to participate in cytotoxic reactions. 81
Cellular basis of the immune response. Precursor bone marrow stem cells are processed by the thymus gland and the "Bursa Equivalent" to become immunocompetent T and B cells, respectively. Blastogenic transformation and division of the cells to become memory cells, activated cells, or plasma cells occur upon interaction with antigen in the presence of macrophages. B cells may also be activated to release lymphokines.

Cellular cooperation in the humoral immune response. Ab = antibody.

Figure 14: From: Baer, P.N. and Morris, M.L. Textbook of Periodontics. Philadelphia. Lippincott. 1977
Antibodies.

The antibodies produced by plasma cells are a class of heterogeneous but related proteins known collectively as immunoglobulins. Five major types are distinguished. These, listed in order of decreasing concentration in the serum are Immunoglobulin (Ig)G, IgA, IgM, IgD, and IgE. Further sub-classes are described, four for IgG and two each for IgM and IgA.

Antibodies against a particular antigen may be found in only one, or in any permutation of the five immunoglobulin types.

The immunoglobulins have similarities in their basic structure. All are composed of a symmetrical basic Ig unit or multiples thereof, containing two identical light and two identical heavy chains bound by disulphide bonds. The alphabetical notation distinguishes them structurally according to differences in the antigenic determinants on their heavy chains.

IgG: This is the major Ig in the human constituting some 80% of the total. It is the most abundant of the serum globulins, but as it diffuses more readily than the others it is also the one found in greatest quantity extravascularly, the distribution in health being equally between the blood and the tissue fluids. IgG is the predominant Ig produced in the gingiva.

It is the major Ig synthesized during secondary responses. As the predominant species it has the greatest role in the neutralization of toxins and in enhancing phagocytosis either as a cytphilic or as an opsonizing antibody.

In man, only IgG among the immunoglobulins passes the placenta as a result of active selective transportation, and reaches the foetal circulation where it becomes a major defence mechanism against infection in the newborn, an example of passively acquired immunity.
Papain digestion of the IgG molecule splits it into three fractions, two specific antigen binding sites known as the fragment antigen binding (Fab) portions, and a single fraction crystallizable (Fc) portion. The latter makes no contribution to antigen binding specificity, but is believed to determine biological specificity such as distribution within the tissues, selective transport across the placenta, mast cell affinity and the ability to fix complement. Thus the Fc portion is responsible for the antibody effector functions of the immune response. (Figure 15).

Fig 15 |Diagram of immunoglobulin (IgG) structure.

The Fc fragment contains sites for complement fixation, reactivity with rheumatoid factors, membrane transmission, skin fixation, macrophage fixation, and regulation of catabolism. (From Turner, M. W. and Hulme, E., The Plasma Proteins: an Introduction, London: Sir Isaac Pitman and Sons. 1971 with permission.)

Macrophages can adhere to immune complexes formed by bacteria and IgG antibody as they have specialized cell surface receptors for sites on the Fc fragment. 49

**IgM**: This is the first Ig to appear in vertebrates. It appears to be a more primitive type of immunoglobulin preceding IgG in the phylogeny of the immune response, and it is the major Ig synthesized by the foetus. 52, 60

IgM comprises about 6% of the total immunoglobulins, and because it is a macroglobulin of high molecular weight it is largely confined to the bloodstream. 52 It is a pentamer, with each subunit consisting of two heavy and two light chains and resembling an IgG molecule except that it possesses more carbohydrate residues.

IgM appears early in the response to infection, it is frequently the first Ig detectable after exposure to antigen. It is an efficient opsonin for particulate antigens, has high complement fixing ability and well developed cytolytic and agglutinating properties as well. 54

(Table 4).

| Table 4: Biological properties of major immunoglobulin Classes in the human |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **IgG** | **IgA** | **IgM** | **IgD** | **IgE** |
| Major characteristics | Most abundant Ig of internal body fluids particularly extra-vascular where it defends micro-organisms and their toxins | Major Ig in sero-mucous secretions where it defends external body surfaces | Very effective agglutinator; produced early in immune response—effective first line defence vs. bacteraemia | ? | Raised in parasitic infections, Responsible for symptoms of atopic allergy |
| Complement fixation | + | - | + | ? | - |
| Cross placenta | + | - | - | - | - |
| Fix to mast cells (in homologous skin) and basophils | - | - | - | - | + |
| Fix to macrophages | + | ? | - | ? | ? |

IgA: As well as occurring in the serum, and accounting for about 13% of total immunoglobulin in health, IgA is the principal immunoglobulin of human exocrine secretions such as saliva, tears and gastrointestinal secretions. As it occurs in these locations in much higher concentration than IgG, it is accordingly regarded as a possible important defence mechanism for exposed external body surfaces.  

Externally secreted IgA differs from the monomeric form found in serum in that it is locally produced within the lamina propria as a dimer. It also carries an additional glycoprotein, the secretory component or transport piece which is synthesized by the local epithelial cells and is believed to be responsible for increasing molecular resistance to degradation by proteolytic enzymes.

IgA is an effective agglutinating antibody, which could be interpreted as a factor facilitating plaque formation. However, it has been shown to possess the capacity to block bacterial cell receptor sites and inhibit bacterial colonization of epithelial cells. This property is strain specific and correlates with agglutinating activity.

The efficiency of IgA in bacteriolytic reactions is doubtful. However due to the availability of two Fc portions, the dimer form gains a low ability to fix complement, possibly by activation of the C3 alternate pathway. Following attachment to Gram-negative organisms this activation of the complement system facilitates the digestion of the bacterial cell wall by the enzyme lysozyme.  

Secretory IgA appears to be responsible for the first line of defence against invading microorganisms in the oral cavity.
may explain the low level of IgA in the serum. 81, 82
(Figure 17). Fixing to cells is evident irrespective of the presence of antigen, and
Like IgG its physiological role is uncertain, but it is suggested that
IgG constitutes about 1% of total immunoglobulins, but as yet nothing is known of its physiological functions. 52
IgG is a reactive or homocytotropic antibody. It possesses
a marked ability to attach to surface receptors on mast cells, basophils
and possibly other tissue cells, and to remain fixed. This property of
IgG is a reaginic or homocytotropic antibody. It possesses
81, 83
Hibberd, 84 suggests that the evolutionary emergence and
survival of IgE and IgM indicate the possession of a physiological
advantage which is as yet undetermined.

FIG. 16.

From: Brudtsø, P. Immunology of Inflammatory Periodontal Lesions.

Oral cavity
Buccal
mucosa
IgA
IgM
IgG
IgA and SC
Gland-associated synapses
Primary IgA and IgM
Free SC
IgA
IgM
IgG
IgA and IgM
Pocket epithelium
Staple and space
Thus IgE is for the most part fixed on the surfaces of the cells which will effect or be affected by the biological consequences of its specific reaction as an antibody.

Combination of antigen with cell bound IgE results in degranulation of mast cells and the release of vasoactive amines such as histamine and heparin. IgE has recently been identified in normal human gingiva.
HYPERSENSITIVITY:

The ability to develop a specific protective immunity to certain infectious agents is only one aspect of the development of the capacity to elaborate immune responses. The term hypersensitivity is applied when an immunologically primed or sensitized individual, on subsequent exposure to the same antigen, develops reactions which are physiologically disruptive or damaging to tissue, rather than a protective secondary response. This may happen when hereditary or other factors unduly influence selection of the immune mechanism, as in atopic individuals, or when antigen is presented to the tissues of the hypersensitive individual in large doses or repeatedly.

Regardless of the clinical outcome, the fundamental nature of the response remains the same, altered reactivity developed by the individual after an initial exposure to an immunogen. In its original usage, the term allergy referred to altered reactivity of either kind. It is now usually restricted in use to describe hypersensitivity reactions to foreign substances, sometimes described as allergens.

"The notion of immunological surveillance recognises, perhaps more clearly than the concept of immunity, that hypersensitivity is a normal sequel to contact with antigen, not a disease per se. To overcome the challenge presented by microorganisms seeking lodgement in the tissues, or check the proliferation of a clone of mutant cells, the destructive manifestations of specific hypersensitivity need not necessarily exceed the microscopical level. But, there are circumstances where the degree, extent, nature or effect of the tissue response to an immune reaction may be such as to constitute a disability in itself." 75

Four major types of hypersensitivity are described in the classification devised by Cell and Coombs. 86
Types 1, 11, and 111 are dependent on the release of pharmacologically active mediator substances following antigen–antibody interaction. These humoral responses are derived from B-cell activity and are referred to as immediate hypersensitivity reactions. Type IV, or delayed hypersensitivity depends on cell mediated immune activity provided by T-cells, and the release of lymphokines following reaction between antigen and sensitized lymphocytes.

**IMMEDIATE HYPERSENSITIVITY REACTIONS:**

**Type 1. Anaphylactic or reagin dependent reactions.**

These occur when IgE homocytotropic antibodies, bound to mast cells and possibly to tissue cells by their Fe fragments, react with the appropriate antibody. The resultant explosive degranulation of mast cells releases, powerful pharmacological mediators including:

(a) Histamine, which is present in mast cell granules as the precursor histidine. It has a muscle constricting effect on the walls of small vessels, and also causes increased vascular permeability and capillary dilatation by direct action on the endothelial cells of the venules resulting in the creation of gaps at the intercellular junctions.

(b) 5-hydroxytryptamine, serotonin, which causes increased capillary permeability and smooth muscle contraction in animals, but has yet to be demonstrated in human anaphylactic reactions.

(c) Slow reacting substance of anaphylaxis (SRS-A) which is capable of producing prolonged contraction in certain smooth muscles and has pronounced bronchial constricting capacity in man.

(d) Bradykinins, which have histamine like effects. They are
exceptionally potent in producing arteriolar and venular dilatation.\textsuperscript{52}

The anaphylactic reaction is attributed to the action of these mediators.\textsuperscript{79} It may occur either as a systemic form, usually following parenteral administration of the antigen to a sensitized individual, or as a localized form. The systemic reaction may be induced by an insect bite, or by the administration of heterologous serum or certain drugs such as penicillin.\textsuperscript{52}

A broad description of the syndrome is one of profound generalized shock, with an associated pulmonary oedema due to prolonged smooth muscle contraction accompanied by capillary dilatation. The onset of symptoms may be extremely rapid and the outcome fatal, although this severe form of response is uncommon. In less severe cases a histamine mediated generalized urticaria may result.

Eosinophils are found in large numbers in the blood and the tissues in anaphylactic reactions. Their role is uncertain, but may be the detoxification of histamine\textsuperscript{87}, or inhibition of its release by the production of prostaglandins.\textsuperscript{31}

Localized anaphylactic reactions occur in sensitized individuals due to local access and action of the allergen on certain tissues such as mucosal surfaces. The action of the pharmacological mediators is restricted to the tissue or organ in which the reaction takes place.\textsuperscript{52}

Bronchial asthmas, hay fever and allergic conjunctivitis are examples of localized anaphylactic reactions. The level of IgE in patients with allergic asthmas has been shown to be much higher than in normal individuals\textsuperscript{87}, the antibody level correlating well with susceptibility though not necessarily with the severity of symptoms.\textsuperscript{83}
It is suggested that reagin dependent allergy may be implicated as an aetiologic factor in periodontal disease, and the presence of IgE in healthy gingiva has been reported.

Type II. Cytotoxic Hypersensitivity Reaction.

Whereas Type I reactions are dependent on cell bound antibody, the Type II reaction is initiated by serum antibody reacting with an antigenic component which is either part of a tissue cell or intimately associated with its surface, such as a cell fixed drug which is acting as a hapten.

Antibodies directed against such cell associated antigens are usually of the IgG or IgM class, and have a cytolytic or cytotoxic effects by various mechanisms such as the opsonic adherence of phagocytes or the activation of the complement system. Cells coated with antibody are also rendered susceptible to non-phagocytic destruction by cytotoxic cells of the monocyte class which may be atypical leucocytes.

An example of complement dependent antibody toxicity is the lysis of foreign erythrocytes which occurs in incompatible blood transfusion. The isohaemagglutinin involved is usually IgM.

Some haemolytic anaemias may be caused by an immune reaction against bacterial endotoxin, which becomes coated on host erythrocytes as a cell fixed antigen.

Type III. Toxic-complex syndrome, complex mediated hypersensitivity.

Immune complexes formed by combination of soluble antigen with circulating humoral antibody form microprecipitates in and around small blood vessels. An acute perivascular inflammatory response is produced, in which many different forms of mediators may be involved. Complement fixation results in the release of anaphylatoxins with subsequent
histamine release and increased vascular permeability. Chemotaxis of PMNs leads to phagocytosis and this may result in local tissue damage due to the release of proteolytic enzymes. Further mediators of inflammation may also be produced if cell lysis occurs as a result of complete complement activation when C8 and C9 are fixed. Ag-Ab complexes may activate the Hageman factor and in turn the plasma kinin system, and platelet aggregation may lead to further release of vasoactive amines and the production of local ischaemia due to the formation of microthrombi. (Figure 18).

**Figure 18.** Type III—Complex-mediated hypersensitivity.

The outcome of the reaction in vivo depends not only on the absolute amounts of antigen and antibody, but also on their relative proportions which determine the nature of the complexes and their subsequent distribution, and also on the ability of the antibody to activate complement.

In gross excess of circulating antigen, soluble complexes are formed which may cause systemic reactions. In gross antibody excess the complexes are rapidly precipitated and also readily phagocytosed. They are potentially irritant when they occur in high local concentration. 55

Soluble complexes which persist in the tissues can form in moderate excess of either agent.

Serum sickness is the systemic form of reaction brought about by antigen excess, as for example following prophylactic parenteral administration of heterologous serum. 52 Foreign serum protein requires several weeks for total elimination from the circulation. If for example 10-20 ml of horse anti-diptheria serum are administered there will be a considerable amount still remaining in the circulation by the time the immune response develops and antibodies are synthesized against the foreign protein, approximately eight days after the initial injection. 87

Ag-Ab complexes are formed and deposited in various parts of the body, leading to a variety of symptoms which may include pyrexia, lymphadenopathy, generalized urticaria, arthritis, glomerulonephritis and coronary artery vasculitis. Increased vascular permeability caused by vasoactive amine release assists deposition of complexes in the vascular bed. 66
**Arthus type reactivity** is the Type 111 reaction caused by localized high extravascular concentration of soluble antigen, and the presence of large amounts of IgG antibody. Antigen diffuses towards the walls of the small blood vessels where immune complexes are formed in the subendothelial layer and a localized vasculitis develops. 52, 55 The reaction is characteristically erythematous and oedematous due to the action of the vasoactive mediators, and there is dense infiltration by polymorphonuclear leucocytes due to the chemotactic effect generated by complement fixation. Ischaemic necrosis may follow vessel blockage in the later stages. (Table 5).

Attempts have been made to explain the pathogenesis of periodontal disease on the local formation within the gingiva of Ag-Ab complexes and the production of an Arthus type reaction. 61, 90, 91 However, conclusive evidence has yet to be demonstrated. 92

**DELAYED HYPERSENSITIVITY REACTION:**

**Type IV. Cell mediated hypersensitivity.**

These are slowly evolving (24-48 hours) mixed cellular reactions, characterized by a mononuclear inflammatory infiltrate, contrasting with the PMN response seen in Type 111 reactions. This form of reactivity may be transferred to nonsensitive individuals by the injection of sensitized lymphoid cells but not by serum. 75 (Table 5).

There is no participation by free antibody in the reaction, the response is initiated by lymphoid effector cells derived from thymus dependent T-cell lymphocytes. 50 These respond to exogenous antigenic material by the release of lymphokines and the development of cytotoxic activity against specific target cells. 86 (Table 6).
Table 6. Recognition and effector systems in the host response.


Macrophages are active in an efferent role in lymphokine mediated delayed hypersensitivity responses. Such "armed" or activated macrophages have increased bactericidal activity as a result of stimulation by lymphokines.

The Tuberculin (Mantoux) reaction is a classical example of delayed hypersensitivity. The sensitized individual is given an intradermal injection of purified protein derivative from tubercle bacilli, which results after some 24 hours in the appearance of an erythematous, indurated inflammatory reaction in the skin which persists for some time.
Histologically the picture is one of essentially mononuclear perivascular infiltration, composed of monocytes and large numbers of macrophages. Some PMNs are present initially but soon migrate from the area. The cellular infiltration may persist for some days after resolution of the inflammation. 87

Cell mediated hypersensitivity is encountered in allergic responses to intercellular infective agents such as viruses and streptococci, in contact dermatitis resulting from sensitization to certain metals or simple chemicals, in the rejection of homologous grafts, in drug sensitization and in autoallergic disease. 87

There is considerable speculation over the possible role of delayed hypersensitivity reactions in chronic periodontal disease. 61, 90, 92, 93

Unlike immediate anaphylactic hypersensitivity reactions which are hapten specific and independent of the carrier protein, delayed hypersensitivity has the characteristic feature that the specificity for the eliciting antigen resides as much in the carrier protein as in the hapten carried. 75
### Summary of different types of hypersensitivity

<table>
<thead>
<tr>
<th>Type</th>
<th>I: Anaphylactic</th>
<th>II: Cytotoxic</th>
<th>III: Complex-mediated</th>
<th>IV: Cell-mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody mediating reaction</td>
<td>Homocytotropic Ab, Mast-cell binding</td>
<td>Humoral Ab, ± CI*</td>
<td>Humoral Ab, ± CF</td>
<td>'Ab' bound to T-lymphocyte</td>
</tr>
<tr>
<td>Antigen</td>
<td>Usually exogenous (e.g. grass pollen)</td>
<td>Cell surface</td>
<td>Extracellular</td>
<td>Extracellular or cell surface</td>
</tr>
<tr>
<td>Response to intradermal antigen:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. reaction</td>
<td>30 min.</td>
<td></td>
<td>3-8 hr.</td>
<td>24-48 hr.</td>
</tr>
<tr>
<td>Appearance</td>
<td>Wheal and flare</td>
<td></td>
<td>Erythema and oedema</td>
<td>Erythema and induration</td>
</tr>
<tr>
<td>Histology</td>
<td>Degranulated mast cells; oedema; eosinophils</td>
<td></td>
<td>Acute inflammatory reaction; predominant polymorphs</td>
<td>Perivascular inflammation: polymorphs migrate out leaving predominantly mononuclear cells</td>
</tr>
<tr>
<td>Transfer sensitivity to normal subject</td>
<td>Serum antibody</td>
<td></td>
<td></td>
<td>Lymphoid cells</td>
</tr>
<tr>
<td>Exemples</td>
<td>Atopic allergy, e.g. hay fever</td>
<td>Haemolytic disease of newborn (Rh)</td>
<td>Complex glomerulonephritis</td>
<td>Mantoux reaction to TB</td>
</tr>
</tbody>
</table>

AUTOIMMUNITY:

One of the fundamental principles of the development of immunological competence is self recognition. Thus in normal circumstances there will be no reaction against the organism's own tissue antigens. Holborow states that in nature the best example of immunological tolerance is the unresponsive state which occurs in animals and man to their own tissue components, even though these are immunogenic to allogeneic and xenogeneic animals.

Ehrlich (1901) proposed the concept of "horror autotoxicus" which suggested that disease resulting from antibody formation against antigens in the host's tissue constituents could not occur. In fact, it is now known that the occurrence of autoimmune reactions is not uncommon, and with regard to some autoantigens not necessarily abnormal.

There are various human diseases in which serum antibodies or sensitized cells may be demonstrated which are specific with host cell antigens. For example autoantibody serum factor directed against host cell nuclei (anti-nuclear factor, ANF) is demonstrable in the sera of patients with systemic lupus erythematosus (SLE). Anti-tissue antibodies of the IgM type may be readily induced, arising secondarily to various forms of tissue damage. They have chemotactic effects and may be responsible for initiating a PMN response to deal with tissue breakdown products associated with normal cell turnover, thus lending support to the concept that not all autoantibodies are necessarily autoaggressive.

THEORIES OF ANTIBODY SYNTHESIS:

(a) ... In the INSTRUCTIVE theory, antibody production may be envisaged in terms of the antigen acting as an instructive template, which can modify the globulin
molecules of any Ig producing cell to a specific spatial configuration complementary to the determinants of that antigen.

This directive ability of the antigen would either bring about a reconfiguration of already formed protein chains, or produce chemical rearrangement of the forming immunoglobulin molecule. This hypothesis does not explain the immunological inertness of the body's own antigenic components.

(b) ... The alternative SELECTIVE theory holds that the genetic apparatus contains the information required for the synthesis of various antibodies. Burnet's clonal selection theory of acquired immunity has as its basic concepts:

(i) That each small family or clone of mesenchymal cells has the genetic information available to make one, and only one, particular antibody, and

(ii) That the gene of antibody specificity is present in the cell before antigenic stimulation takes place, and that molecules of the antibody are built into the cell surface as receptors, the antibody having no part in directing the pattern of the antibody to be formed, only in stimulating its synthesis by the cell.

A clone of virgin antigen sensitive cells may receive either a positive antigenic signal leading to clonal expansion, or a negative signal which results in clonal suppression or elimination. Tolerance to self antigens would result from the inhibition during foetal development of the clones of immature cells which have the ability to react with the antigenic determinants of the individual's own tissues. Since the lymphoid tissues tend to differentiate relatively late during development
most such potentially antigenic substances are already formed and freely available. Thus the suppression of those clones capable of reacting with them would result in a specific unresponsiveness to autoantigens, the development of an acquired immunological self tolerance.

In the terms of this clonal selection theory, autoimmune responses could be manifested as a result of the termination, circumvention or failure of development of such acquired tolerance.

AETIOLOGY of the AUTOIMMUNE RESPONSE:

The following potential mechanisms exist whereby humoral or cell mediated autoimmune responses might be invoked:

Evasion of Normal Tolerance to Autoantigens.

(a) ... The release of normally inaccessible "sequestered" antigens into the circulation results in their coming into contact with immunologically competent cells. Spermatozoa and eye lens protein are in normal circumstances inaccessible to the lymphoreticular system, but if a contact is established as a result of trauma or disease then they are not recognised as self and an autoimmune reaction may result. An antigen which is usually sequestered intracellularly may produce a reaction if it becomes available as a consequence of cell death.
(b) ... Alteration of the autoantigen by drugs, chemicals or microorganisms may result in the formation of protein hapten conjugates with the tissue protein acting as the carrier molecule. Tissue protein may undergo change or denaturation with age, and viruses have been suggested as playing a part in altering cell surface antigenicity. 94

(c) ... Heterophile antigenicity. Microorganisms may have at least some antigenic determinants in common with some tissue components. 94 Group A haemolytic streptococci possess at least one antigen which cross reacts with human heart tissue, about 50% of patients recovering from such streptococcal infections have heart reactive antibodies present in their serum. 84

_E. Coli_ is thought to cross react with human colon, and colon autoantibodies are present in ulcerative colitis. 95

The possibility that bacterial endotoxin may through its adjuvanicity play a role in inducing the formation of autoantibodies has yet to be evaluated _in vivo_. 97

Breakdown of acquired tolerance mechanisms.

An alteration in the cellular processes which are essential to the maintenance of normal tolerance could be brought about by the action of chemicals, drugs or microorganisms on the lymphoid tissues, or by a mutation leading to an inherited deficiency in the lymphoid system.

The resultant immunological deficiency would be manifested in the proliferation of normally suppressed autoreactive lymphocytes, described by Burnet as "forbidden clones", and the development of autoimmune responses. There is speculation that the presence of some
viruses may interfere with normal lymphocyte reactivity to antigens, resulting in a response to antigenic stimuli to which the lymphocyte would normally remain tolerant.  

Weir suggests that a further possible mechanism for the development of autoimmune responses may be:

**Stimulation of pre-existing potentially autoaggressive B-cells.**

Some recent evidence suggests that B-cells lose tolerance more readily than T-cells, and that self-tolerant T-cells may be bypassed by presenting autoantigens to the B-cells in a highly immunogenic form, possibly through some form of adjuvanicity.

**CLASSIFICATION OF AUTOIMMUNE DISEASES:**

Roitt describes a spectrum of autoimmune diseases ranging from organ specific in which the autoantibodies have specificity for a single organ, through an intermediate range which is characterized by lesions tending to be localized to a single organ although the antibodies are non-organ specific. At the other end of the spectrum lie the truly non-organ specific diseases in which neither the lesions nor the autoantibodies are confined to one organ.

(a) **Organ Specific Disease.** In a disease such as Hashimoto's thyroiditis, circulating antibodies with absolute specificity for thyroid constituents are produced. The lesion is limited to the thyroid gland which becomes heavily infiltrated by lymphocytes, histiocytes and plasma cells and finally becomes replaced by fibrous tissue.

(b) In the intermediate range, primary biliary cirrhosis is characterized by an inflammatory cell infiltration of the small bile ductules. However the serum antibodies present are not liver specific.

(c) **Non-organ Specific Disease** is said to occur when neither
the lesions nor the autoantibodies are confined to any one organ. In the systemic lupus erythematosus the pathological changes are widespread and mainly effect the connective tissue; skin, joints, serous membranes, blood vessels and particularly kidney glomeruli may be affected.

The autoantibodies produced, ANF are directed against the DNA and possibly other components of the cell nuclei of many different body cells. 87

More than one autoimmune manifestation may occur in the one patient, in such cases the diseases are often within the same region of the autoimmune spectrum. 95

A familial tendency to the development of the autoimmune diseases is recognized. 95 It is thought that this may be due to genetic predisposition 97 which leads to increased susceptibility to environmental influences, rather than direct inheritance.

The pathogenesis of autoimmune diseases may, by definition, be considered as immunopathological hypersensitivity reactions. Cytotoxic (Type I), Immune Complex (Type II) and Cell Mediated (Type IV) reactions, either singly or in combination, have been suggested as the mechanisms involved in autoimmune reactions. 87, 95

Some authors have implicated localized autoimmune processes in the pathogenesis of chronic destructive periodontal disease, provoking a self-perpetuating immune reaction. Much of this work is necessarily on animals, and the conclusions are guarded. 90, 98