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Structure and function of human skin

1.1 Introduction

Human skin is a uniquely engineered organ that permits terrestrial life by regulating heat and water loss from the body whilst preventing the ingress of noxious chemicals or microorganisms. It is also the largest organ of the human body, providing around 10% of the body mass of an average person, and it covers an average area of 1.7 m². Whilst such a large and easily accessible organ apparently offers ideal and multiple sites to administer therapeutic agents for both local and systemic actions, human skin is a highly efficient self-repairing barrier designed to keep 'the insides in and the outside out'.

Skin membranes can be examined at various levels of complexity. In some mathematical treatments of transdermal drug delivery (see Chapter 2), the membrane can be regarded as a simple physical barrier; more complexity can be introduced by viewing skin as various barriers in series. We can then introduce barriers in parallel by considering drug transport through pores in the tissue. Degrees of complexity also exist when examining basic structures and functions of the membrane. In some extreme cases it may be that transdermal drug delivery is limited by metabolic activity within the membrane. Alternatively, immunological responses may prevent the clinical use of a formulation that has proven to be optimal during in-vitro studies. A further complication is introduced in clinical situations where topical delivery is intended to treat diseased skin states; here, the barrier nature of the membrane may be compromised and so data extrapolated from in-vitro experiments using healthy tissue may be inappropriate.

This chapter provides an overview of the structure and function of healthy human skin, followed by a review of some physiological factors that can affect transdermal and topical drug delivery, such as skin disorders and age-related alterations to the membrane.

1.2 Healthy skin structure and function

Human skin is a highly complex organ though in many transdermal drug delivery studies it is often regarded somewhat simplistically as

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merely a physical barrier. *In vivo*, skin is in a process of continual regeneration, it has immunological and histological responses to assault (as would be the case if an exogenous chemical, such as a drug, were applied to the surface) and is metabolically active. Due to experimental and ethical difficulties, most transdermal drug delivery studies tend to utilise skin *ex vivo* (*in vitro*) which inherently reduces some of the above complexity – regeneration stops, immune responses cease and metabolic activity is usually lost in these studies. However, it should always be borne in mind that data obtained from excised skin may not translate directly to the in-vivo situation.

For the purpose of transdermal drug delivery, we can examine the structure and function of human skin categorised into four main layers (Figure 1.1):

- the innermost subcutaneous fat layer (hypodermis)
- the overlying dermis
- the viable epidermis
- the outermost layer of the tissue (a non-viable epidermal layer) the stratum corneum.

1.2.1 The subcutaneous fat layer

The subcutaneous fat layer, or hypodermis, bridges between the overlying dermis and the underlying body constituents. In most areas of the body this layer is relatively thick, typically in the order of several millimetres. However, there are areas of the body in which the subcutaneous fat layer is absent, such as the eyelids. This layer of adipose tissue principally serves to insulate the body and to provide mechanical protection against physical shock. The subcutaneous fatty layer can also provide a readily available supply of high-energy molecules, whilst the principal blood vessels and nerves are carried to the skin in this layer.

1.2.2 The dermis

The dermis (or corium) is typically 3–5 mm thick and is the major component of human skin. It is composed of a network of connective tissue, predominantly collagen fibrils providing support and elastic tissue providing flexibility, embedded in a mucopolysaccharide gel (Wilkes *et al.*, 1973). In terms of transdermal drug delivery, this layer is often viewed as essentially gelled water, and thus provides a minimal barrier to the delivery of most polar drugs, although the dermal barrier may be significant when delivering highly lipophilic molecules. The dermis has

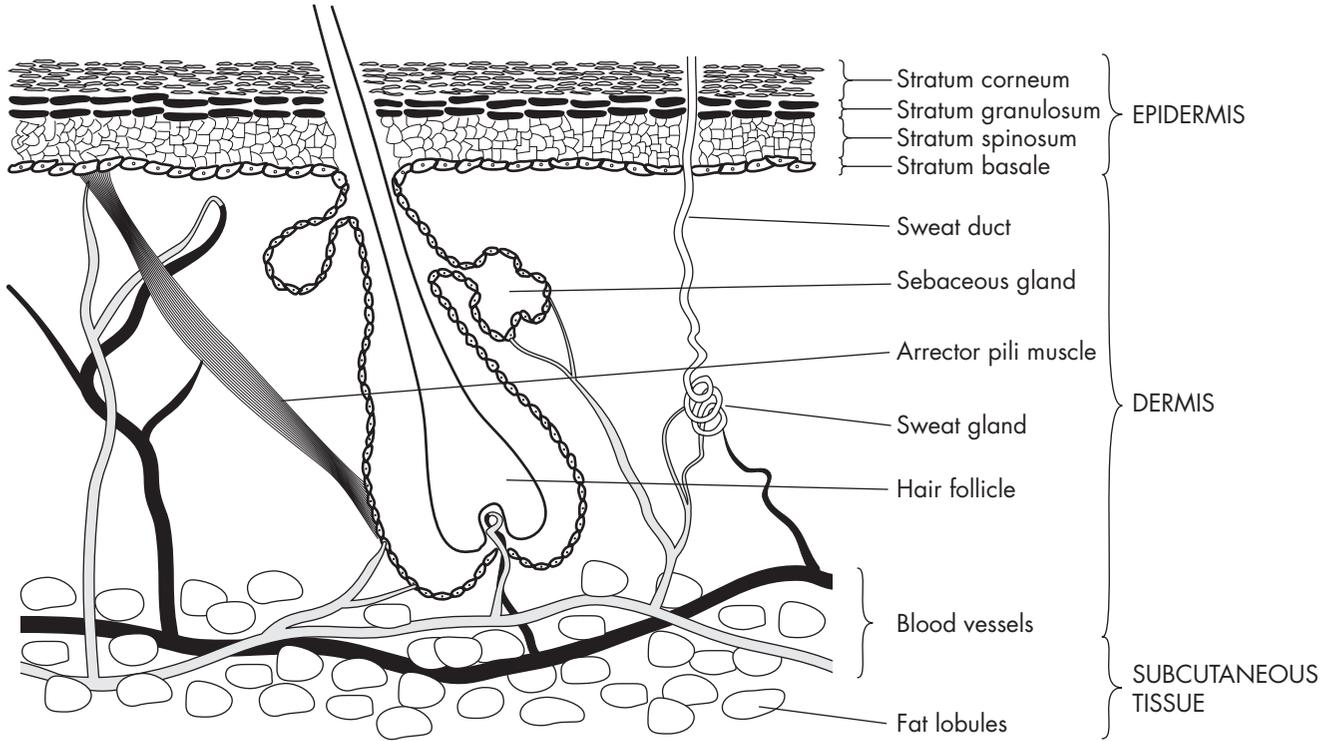


Figure 1.1 A diagrammatic cross-section through human skin.

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numerous structures embedded within it; blood and lymphatic vessels, nerve endings, pilosebaceous units (hair follicles and sebaceous glands), and sweat glands (eccrine and apocrine).

The extensive vasculature of the skin is essential for regulation of body temperature whilst also delivering oxygen and nutrients to the tissue and removing toxins and waste products. The vasculature is also important in wound repair. The rich blood flow, around 0.05 mL/min per mg of skin, is very efficient for the removal of molecules that have traversed the outer skin layers. Capillaries reach to within 0.2 mm of the skin surface, and are found intertwined with the Malpighian layer of the viable epidermis. Molecules are thus removed, *in vivo*, from near the dermo-epidermal layer, ensuring that dermal concentrations of most permeants are very low. For transdermal delivery of most drugs, the blood supply thus maintains a concentration gradient between the applied formulation on the skin surface and the vasculature, across the skin membrane. It is this concentration gradient that provides the driving force for drug permeation. The lymphatic system also reaches to the dermo-epidermal layer and, whilst it is important in regulating interstitial pressure, facilitating immunological responses to microbial assault and for waste removal, the lymphatic vessels may also remove permeated molecules from the dermis – hence maintaining a driving force for permeation. Cross and Roberts (1993) showed that whilst dermal blood flow affected the clearance of relatively small solutes, such as lidocaine, lymphatic flow was a significant determinant for the clearance of larger molecules such as interferon.

There are three main appendages found on the surface of human skin that originate in the dermis, and these have been described in detail by Katz and Poulsen (1971). Hair follicles are found over the entire surface of the skin except for the load-bearing areas (soles of feet, palms of hands) and the lips. The sebaceous gland associated with the hair follicle secretes sebum; this is composed of free fatty acids, waxes and triglycerides which lubricate the skin surface and help to maintain the surface pH at around 5. Eccrine (or sweat) glands and apocrine glands also originate in the dermal tissue. Eccrine glands are found over most of the body surface, typically at a density of 100–200 per cm² of skin. Secreting sweat, a dilute salt solution at a pH of around 5, these glands are stimulated in response to heat and emotional stress. The apocrine glands are located near the dermo-epidermal layer but are limited to specific areas of the skin including the axillae, nipples and ano-genital regions. The lipoidal and ‘milk’ protein secretions are primarily responsible for imparting the odour of ‘sweat’.

In terms of transdermal drug delivery, the appendages (hair follicles, sweat ducts) may offer a potential route by which molecules could enter the lower layers of the skin without having to traverse the 'intact' barrier provided by the stratum corneum. These so-called 'shunt routes' may have a role to play in the early time course of the permeation process, and for large polar molecules and also in electrical enhancement of transdermal drug delivery. However, for most permeants, the fractional area offered by these shunt routes is so small that the predominant pathway for molecules to traverse the tissue remains across the bulk of the skin surface. The mechanisms by which molecules traverse human skin, including the influence of shunt route transport, are discussed in Chapter 2 (see Section 2.3).

1.2.3 The epidermis

The epidermis is itself a complex multiply layered membrane, yet varies in thickness from around 0.06 mm on the eyelids to around 0.8 mm on the load-bearing palms and soles of the feet. The epidermis contains no blood vessels and hence nutrients and waste products must diffuse across the dermo-epidermal layer in order to maintain tissue integrity. Likewise, molecules permeating across the epidermis must cross the dermo-epidermal layer in order to be cleared into the systemic circulation.

The epidermis contains four histologically distinct layers which, from the inside to the outside, are the stratum germinativum, stratum spinosum, stratum granulosum and the stratum corneum (Figure 1.2). A fifth layer, the stratum lucidum, is sometimes described but is more usually considered to be the lower layers of the stratum corneum. The stratum corneum, comprising anucleate (dead) cells, provides the main barrier to transdermal delivery of drugs and hence is often treated as a separate membrane by workers within the field. The term 'viable epidermis' is often used to describe the underlying layers, although the viability of cells within, for example, the stratum granulosum is questionable as the cell components degrade during differentiation.

1.2.3.1 *The stratum basale*

The stratum basale is also referred to as the stratum germinativum or, more commonly, the basal layer. The cells of the basal layer are similar to those of other tissues within the body; they contain the typical organelles such as mitochondria and ribosomes, and the cells are

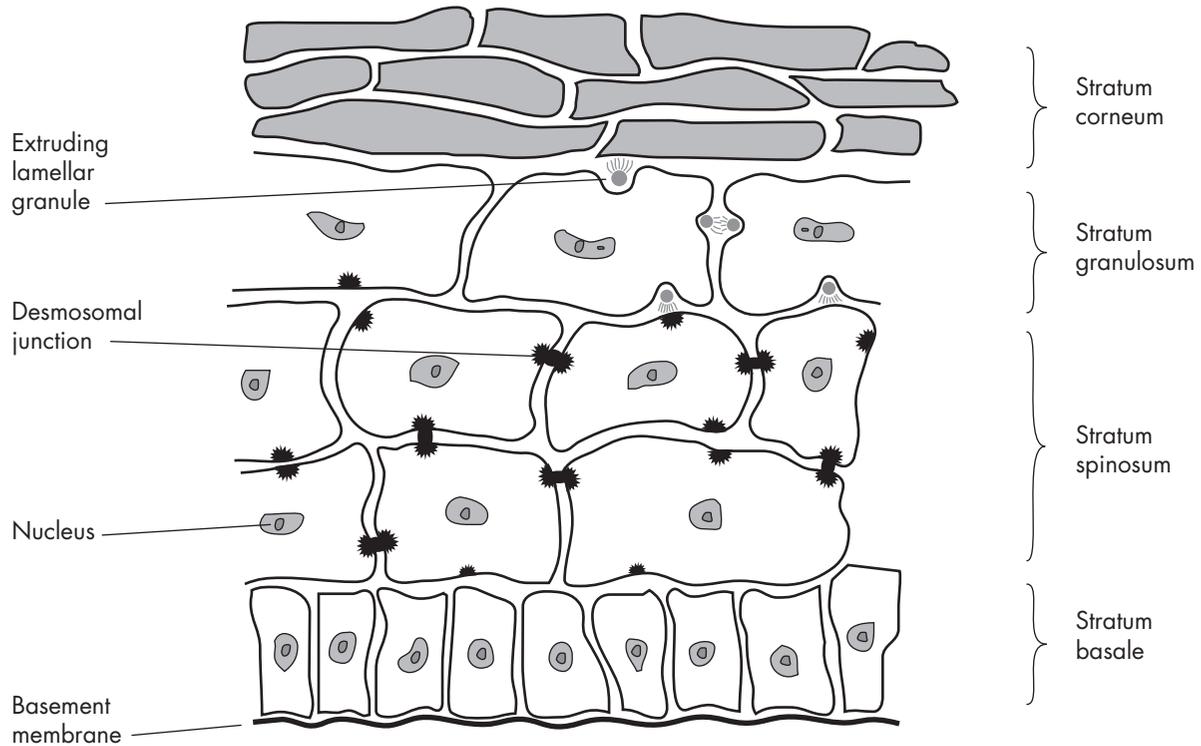


Figure 1.2 A representation of human epidermal cell differentiation.

metabolically active. This layer thus contains the only cells (keratinocytes) within the epidermis that undergo cell division (via mitosis). On average, dividing basal cells replicate once every 200 to 400 h. After replication, one daughter cell remains in the basal layer whilst the other migrates upwards through the epidermis towards the skin surface. The keratinocytes of the stratum basale are attached to the basement membrane (dermo-epidermal membrane) by hemidesmosomes, which act rather like proteinaceous anchors for these lowest layer cells. Loss of adhesion between the basal cells and the basement membrane results in shedding of the skin, as found in some blistering conditions. Within the stratum basale and the adjacent cell layer, the stratum spinosum, keratinocytes are connected through desmosomes, again highly specialised proteinaceous cellular bridges.

In addition to the keratinocytes, the stratum basale contains other specialised cell types. Melanocytes synthesise the pigment melanin from tyrosine. Two forms of melanin are found; eumelanin is the more common brown/black form, whereas the less common pheomelanin is red or yellow. Melanin granules formed in the melanocytes tend to be a mixture of these two forms. Melanocytes make surface contact with adjacent keratinocytes through dendritic connections, and this allows the pigment granules to pass from the melanocytes to the keratinocytes. On facial skin, there may be up to one melanocyte for every five basal layer keratinocytes, but on less exposed surface (such as the trunk) this ratio may be only one melanocyte to twenty keratinocytes. Melanocyte presence appears to be inducible with chronic exposure to light increasing the relative proportion of the pigment-forming cells within the basal layer. Melanins provide an energy sink within the skin; they absorb ultraviolet (UV) radiation and are free-radical scavengers. There are equal numbers of melanocytes in a given body site in darker and lighter skin types, but darker-skinned people have more active and efficient melanocytes.

Langerhans cells are also found within the stratum basale. Discovered and named after a medical student in 1860, it is only over the past 40 years that the role of these cells has been defined. They are themselves dendritic and through these processes connect to keratinocytes. Langerhans cells derive from bone marrow and are recognised as the major antigen-presenting cells of the skin. Antigens readily bind to the cell surfaces, and although Langerhans cells are not themselves efficiently phagocytic, they may present the antigens to lymphocytes in the lymph nodes. When compared to other membranes of the body, the skin comes into contact with many potential antigens and hence the

Langerhans cells play an important role in conditions such as allergic contact dermatitis.

One other specialised cell type is found within the basal layer, the Merkel cell. These cells are found in greatest numbers around the touch-sensitive sites of the body, such as the lips and fingertips. The cells are associated with nerve endings, found on the dermal side of the basement membrane, and it appears that they have a role in cutaneous sensation.

1.2.3.2 *The stratum spinosum*

The stratum spinosum (also known as the spinous layer or prickle cell layer) is found on top of the basal layer, and together these two layers are termed the Malpighian layer. This spinous layer consists of two to six rows of keratinocytes that change morphology from columnar to polygonal cells. Within this layer the keratinocytes begin to differentiate and synthesise keratins that aggregate to form tonofilaments. Desmosomes connecting the cell membranes of adjacent keratinocytes are formed from condensations of the tonofilaments, and it is these desmosomes that maintain a distance of approximately 20 nm between the cells.

1.2.3.3 *The stratum granulosum*

As they pass from the stratum spinosum to the stratum granulosum (or granular layer), the keratinocytes continue to differentiate, synthesise keratin and start to flatten. Only one to three cell layers thick, the stratum granulosum contains enzymes that begin degradation of the viable cell components such as the nuclei and organelles. The granular cells are so called because they acquire granular structures. Keratohyalin granules mature the keratins within the cell. Most importantly for topical and transdermal drug delivery, membrane-coating granules are also synthesised, probably in the endoplasmic reticulum and Golgi apparatus, and contain the precursors for the intercellular lipid lamellae seen in the stratum corneum. The lamellar granules are extruded from the cells into the intercellular spaces as the cells approach the upper layer of the stratum granulosum.

1.2.3.4 *The stratum lucidum*

The stratum lucidum is the layer in which the cell nucleus disintegrates and there is an increase in keratinisation of the cells concomitant with

further morphological changes such as cell flattening. Occasionally, droplets of an oily substance may be seen in this cell layer, possibly arising from the disintegration of lysosomes. The stratum lucidum tends to be seen most clearly in relatively thick skin specimens, such as from the load-bearing areas of the body (soles of feet and palms). Indeed, some dermatologists question whether this layer is functionally distinct from the other epidermal layers, or if it is an artefact of tissue preparation. Most researchers tend to view the stratum lucidum as the lower portion of the stratum corneum and hence bracket these two layers together.

1.2.3.5 *The stratum corneum*

The stratum corneum (or horny layer) is the final product of epidermal cell differentiation, and though it is an epidermal layer it is often viewed as a separate membrane in topical and transdermal drug delivery studies. Typically, the stratum corneum comprises only 10 to 15 cell layers and is around 10 μm thick when dry, although it may swell to several times this thickness when wet. As with the viable epidermis, the stratum corneum is thickest on the palms and soles and is thinnest on the lips. This thin membrane, consisting of dead, anucleate, keratinised cells embedded in a lipid matrix, allows for survival of terrestrial animals without desiccation. The stratum corneum serves to regulate water loss from the body whilst preventing the entry of harmful materials, including microorganisms. The stratum corneum has been represented as a 'brick and mortar' model (Michaels *et al.*, 1975; Elias, 1981) in which the keratinised cells are embedded in a mortar of lipid bilayers (Figure 1.3). However, it should be borne in mind that the keratinocytes are polygonal, elongated and relatively flat – approximately 0.2 to 1.5 μm thick with a diameter of 34 to 46 μm . Typically, it takes 14 days for a daughter cell from the stratum basale to differentiate into a stratum corneum cell, and the stratum corneum cells are typically retained for a further 14 days prior to shedding. Since the keratinocytes of the stratum corneum are cornified, they are also termed 'corneocytes'.

The barrier nature of the stratum corneum depends critically on its unique constituents; 75–80% is protein, 5–15% is lipid with 5–10% unidentified on a dry weight basis (Wilkes *et al.*, 1973). The protein is located primarily within the keratinocytes and is predominantly alpha-keratin (around 70%) with some beta-keratin (approximately 10%) and a proteinaceous cell envelope (around 5%). Enzymes and other proteins account for approximately 15% of the protein component. The cell envelope protein is highly insoluble and is very resistant to chemical

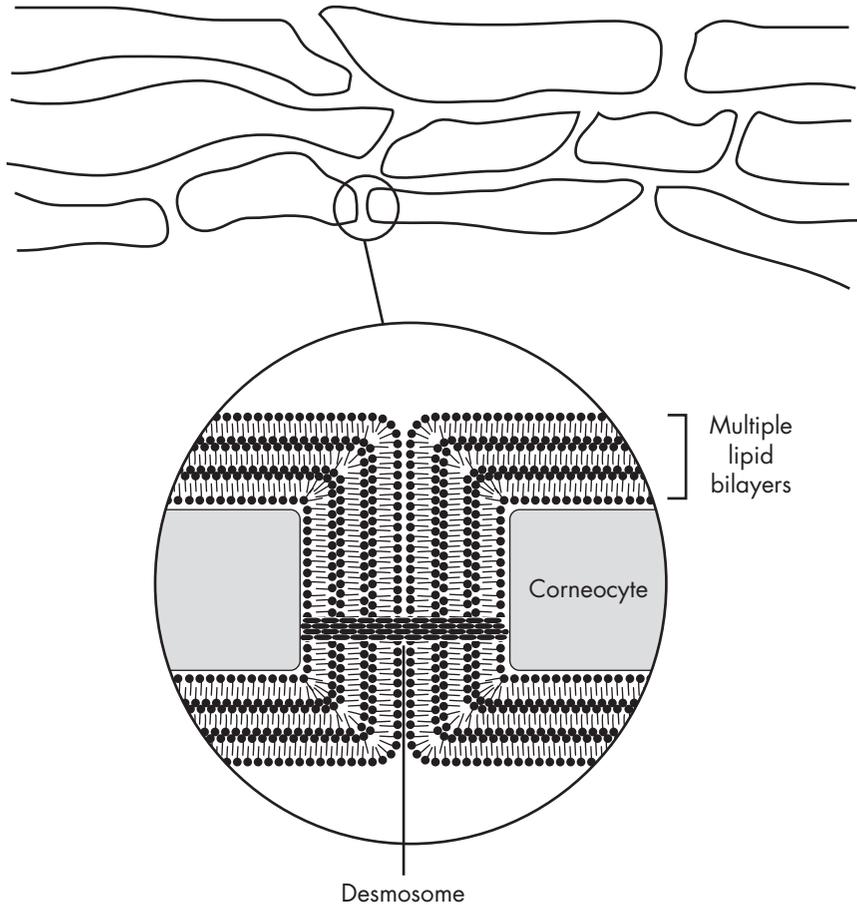


Figure 1.3 A representation of the 'brick and mortar' model of human stratum corneum.

attack. This outer keratinocyte protein has a key role in structuring and ordering the intercellular lipid lamellae of the stratum corneum; the keratinocyte is bound to a lipid envelope through glutamate moieties of the protein envelope. The lipid envelope thus provides an anchor to the keratinocyte and links the proteinaceous domains of the keratinocytes to the intercellular lipid domains.

Human stratum corneum contains a unique mixture of lipids and, for most permeants, the continuous multiply bilayered lipid component of the stratum corneum is key in regulating drug flux through the tissue (see Chapter 2). Thus, much effort has been devoted to probing the lipid composition of the intercellular domains, notably by Downing, Wertz,

Elias and co-workers (e.g. Elias, 1983; Wertz *et al.*, 1985). It is clear that the lipid content of the stratum corneum varies between individuals and with body site (Lampe *et al.*, 1983a), but major components of the domain include ceramides, fatty acids, cholesterol, cholesterol sulfate and sterol/wax esters (see Table 1.1). The stratum corneum lipids are arranged in multiple bilayers, but in contrast to all other lipid bilayers in the body, phospholipids are largely absent. However, eight classes of uniquely structured ceramides (designated ceramide 1 to 5, 6.1, 6.2 and 8) are present in the lipid matrix (see Figure 1.4) which, together with

Table 1.1 Lipid composition from human stratum corneum, expressed as a percentage of the total lipid content

<i>Lipid constituent</i>	<i>Skin site</i>		
	<i>Abdominal^a</i>	<i>Plantar^b</i>	<i>Unspecified^c</i>
Ceramides	18	35	41
<i>Ceramide 1</i>	14	–	3.2
<i>Ceramide 2</i>	4.3	–	8.9
<i>Ceramide 3</i>	–	–	4.9
<i>Ceramide 4</i>	–	–	6.1
<i>Ceramide 5</i>	–	–	5.7
<i>Ceramide 6</i>	–	–	12
Glucosylceramides	Trace	–	0.0
Fatty acids	19	19	9.1
<i>Stearic acid</i>	1.9	–	–
<i>Palmitic acid</i>	7.0	–	–
<i>Myristic acid</i>	0.7	–	–
<i>Oleic acid</i>	6.3	–	–
<i>Linoleic acid</i>	2.4	–	–
<i>Palmitoleic acid</i>	0.7	–	–
<i>Other acids</i>	<0.1	–	–
Cholesterol	14	29	27
Cholesteryl sulfate	1.5	1.8	1.9
Sterol/wax esters	5.4	6.5	10
Di- and triglycerides	25	3.5	0.0
Squalene	4.8	0.2	–
<i>n</i> -Alkanes	6.1	1.7	–
Phospholipids	4.9	3.2	–

^aLampe *et al.* (1983a,b); ^bMelnik *et al.* (1989); ^cWertz and Downing (1989).

Note: The presence of alkanes may be erroneous as there are no reported metabolic pathways for the production of alkanes within the skin, and it appears likely that their presence may arise from external contamination. In addition, Lampe and co-workers emphasised that the high triglyceride content of their stratum corneum sample was probably due to contamination by triglyceride-rich subcutaneous lipids.

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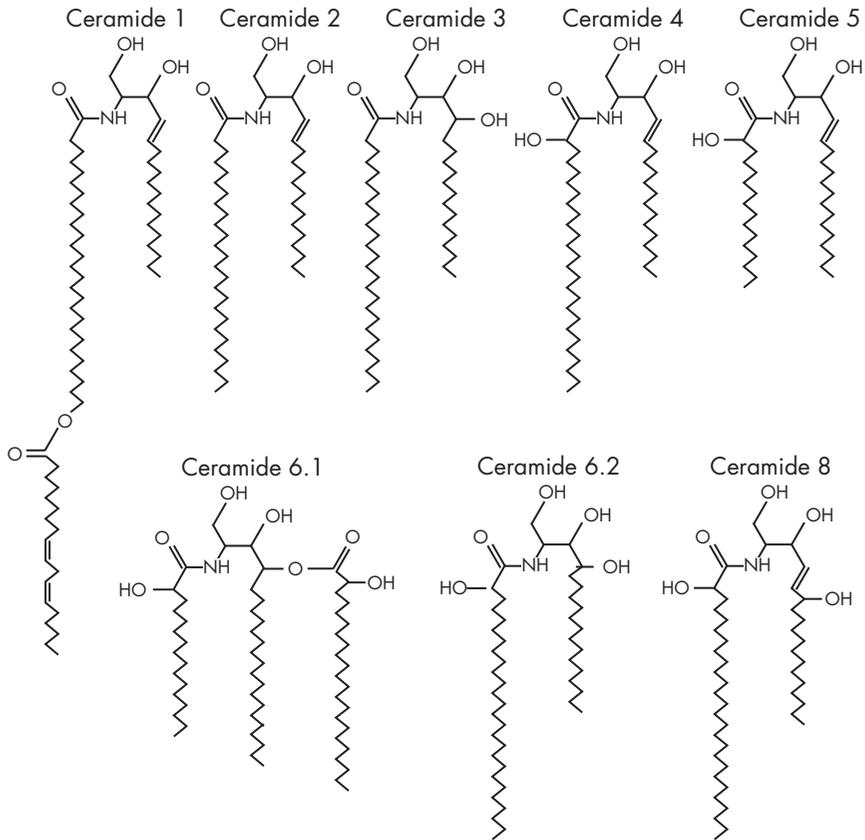


Figure 1.4 Structures of major ceramides found in human stratum corneum.

the fatty acids, cholesterol and cholesterol sulfate, provide the amphiphilic properties necessary to form lipid bilayers. Various functions in the stabilisation of stratum corneum lipid bilayers have been assigned to the different classes of ceramides – for example, ceramide 1 may stabilise the bilayer structure and act as a molecular ‘rivet’ between bilayers. However, it is clear that there is an array of micro-domains within the stratum corneum lipids; X-ray diffraction, spectroscopic and thermal investigations clearly show heterogeneous phase behaviour of the lipids illustrating that multiple states of organisation exist within the bilayers. In addition, the stratum corneum lipid bilayers may also include some intrinsic or extrinsic proteins, such as enzymes, which will affect the packing behaviour of the lamellae.

In addition to the keratinocytes and lipid lamellae, water plays a key role in maintaining stratum corneum barrier integrity. Water may mediate the activity of some hydrolytic enzymes within the stratum corneum since environmental humidity affects the activities of enzymes involved in the desquamation process. Additionally, keratinocyte water activity also regulates enzymes involved in the generation of natural moisturising factor (NMF). Water is also a plasticiser and thus prevents the stratum corneum from cracking due to mechanical assault.

NMF is a highly efficient humectant synthesised and hence is located within the stratum corneum. A proteolytic product from filaggrin, NMF is essentially a mixture of free amino acids, amino acid derivatives and salts; serine, glycine, pyrrolidone carboxylic acid, citrulline, alanine and histidine are the major components with lesser amounts of arginine, ornithine, urocanic acid and proline. This hygroscopic mixture retains moisture within the stratum corneum and helps to maintain suppleness.

1.2.4 Epidermal enzyme systems

As well as the cellular component of the epidermis, the tissue contains many drug-metabolising enzymes. Histochemical and immunohistochemical methodologies suggest that the majority of these are localised in the epidermis, sebaceous glands and hair follicles. Although present at relatively small quantities in comparison to the liver, they do allow metabolic activity that can effectively reduce the bioavailability of topically applied medicaments; a common misconception is that the skin is an 'inert' tissue. Indeed, most phase 1 (e.g. oxidation, reduction, hydrolysis) and phase 2 (e.g. methylation, glucuronidation) reactions can occur within the skin, though these tend to be at <10% of the specific activities found in the liver (Hotchkiss, 1998). However, esterases tend to have relatively high activities within skin and, considering that there is a large skin surface area, the metabolism of some drugs can be significant. For example, topically applied benzoyl peroxide is completely hydrolysed to benzoic acid by human skin (Nacht *et al.*, 1981). Such metabolic activity can also be of value; many prodrugs, notably the steroid esters such as betamethasone-17-valerate, are designed to have improved delivery characteristics (e.g. increased lipophilicity) and exploit esterase activity to liberate the free drug within the skin (see Section 4.5.1). Microorganisms present on the skin surface, such as *Staphylococcus epidermidis*, may also metabolise topically applied drugs.

For more detailed descriptions of the structure, function and differentiation of human skin, the reader is referred to the texts by Orland (1983), Marks *et al.* (1988), Bissett (1987) and Roberts and Walters (1998).

1.3 Physiological factors affecting transdermal and topical drug delivery

It is axiomatic that skin disorders will affect the nature of the skin barrier and hence will influence topical and transdermal drug delivery; a variety of skin conditions is thus described in Section 1.4. However, there are also physiological factors that can influence the rate of drug delivery to and through healthy skin, as described below.

1.3.1 Skin age

The most widely investigated physiological factor affecting transdermal drug delivery is that of skin ageing. There are clear structural and functional alterations that occur to the membrane as it ages (Fenske and Lober, 1986), though it is difficult to ascribe some of the age-related changes to inherent ageing processes or to cumulative environmental damages. For example, damage may result from repeated chemical assault (e.g. from soaps or cosmetics) or from a lifetime of exposure to UV radiation as sunlight. The literature contains some controversy over minor alterations to the viable epidermis with ageing, but it is generally recognised that the stratum corneum remains essentially invariant during a normal lifespan. This may be expected, since an intact stratum corneum barrier is necessary for terrestrial life. Potts *et al.* (1984) demonstrated that the moisture content of human skin decreases with age; since transdermal delivery is influenced by tissue hydration, this factor could alter drug permeation. However, other factors alter skin hydration and will probably have a greater influence than the age-related moisture decrease.

Beyond the skin membrane, there are some age-related alterations that theoretically can affect the amounts of a topically applied drug entering the systemic circulation. Blood flow (dermal clearance of molecules traversing the tissue) tends to decrease with age and this could, *in vivo*, reduce transdermal drug flux. However, for the majority of permeants dermal clearance tends not to be the rate-limiting factor in transdermal therapy.

There is good evidence in the literature for minimal or negligible differences in transdermal drug delivery on ageing of normal skin, though most of the studies reported have been performed *in vitro* and hence are essentially examining variations in the stratum corneum barrier, not in blood flow, etc. Ageing has no effect on transdermal permeation of water, estradiol, caffeine, methyl nicotinate or aspirin (Fenske and Lober, 1986; Roskos *et al.*, 1989, 1990) and thus would not be expected greatly to influence permeation of other molecules. However, it has been suggested that risk assessments on topical preparations for use in children (including cosmetics) should be separate from those of adults as children have a higher surface area to weight ratio and may have different metabolic activities (Plunkett *et al.*, 1992).

Whilst the ageing effects of normal skin on drug delivery are minimal, there are important morphological and hence permeability differences between normal (mature) skin and that of a neonate (pre-term infant). Skin development within the embryo begins approximately a week after conception with the formation of a single epithelial layer. This differentiates into epidermal and dermal tissue but at (normal mature) birth the dermis is only around 60% of its adult thickness; maturation of the dermis takes 3–5 months after birth. Within the embryo, a temporary outer skin layer – called the periderm – forms towards the end of the first month, which then thins towards the end of the second trimester concomitant with the appearance of a stratum corneum. However, the stratum corneum of the fetus is somewhat different to that of an adult, and it remains as a very thin membrane of only a few cell layers thickness until shortly before birth. Thus, although the immature fetus has an impaired skin barrier, advances in medical care allow neonates to survive from as early as 26 weeks.

There are concerns associated with the imperfect skin barrier of the neonate; the surface area to body weight ratio may be four times that in an adult, thereby causing difficulties with thermoregulation, transepidermal water loss, infection and absorption of exogenous chemicals. However, the reduction in the skin barrier properties can be advantageous for the delivery of drugs to the neonate; typically antibiotics, analgesics, cardiovascular and respiratory drugs need to be administered in a controlled manner. Oral therapy is problematic, and intravenous administration – though the norm – is difficult. Transdermal delivery has been used successfully to deliver clinical levels of caffeine and theophylline to neonates (Evans *et al.*, 1985; Amato *et al.*, 1991). However, it is not possible to provide a general transdermal formulation for drug delivery across neonatal skin; neonates vary in gestation time

(and hence in the degree of stratum corneum immaturity, and consequently in the permeability of the tissue) and the neonatal stratum corneum matures post delivery (Barrett and Rutter, 1994).

1.3.2 Body site

It is readily apparent that skin structure varies to some degree over the human body; the stratum corneum is thicker on the palms of the hands and soles of the feet (i.e. the load-bearing areas of the body) than on the lips or eyelids. However, the relative permeability of different skin sites is not simply a function of stratum corneum thickness as different permeants exhibit varied rank orders through different skin sites. Wester and Maibach (1989), in reviewing regional (site-to-site) variations in permeability comment that variations in drug absorption can be seen for sites with similar thickness of stratum corneum, and that some areas with different stratum corneum thickness provide similar levels of drug absorption.

Though site-to-site variation in permeability is complex, there are some general trends shown in the numerous literature reports on the subject. It is apparent that genital tissue usually provides the most permeable site for transdermal drug delivery. The skin of the head and neck is also relatively permeable compared to other sites of the body such as the arms and legs. Intermediate permeabilities for most drugs are found on the trunk of the body. Thus, a generalised rank order of site permeabilities is:

genitals > head and neck > trunk > arm > leg

Thus, there is a clear scientific rationale for selecting the application site based on permeability; scrotal tissue is the most permeable and hence offers the greatest prospect of drug delivery to clinical levels. Indeed, transdermal delivery of testosterone is highly effective through scrotal skin. However, for convenience, ease of patch removal and hence improved patient compliance, the trunk is often selected as a site of intermediate relative permeability for delivery of this steroid. Additionally, delivering drugs through scrotal tissue is restricted to around half of the population! Likewise, the relatively high permeability of skin on the head is used for scopolamine delivery; patches are applied to the postauricular (behind the ear) region where skin permeability is relatively high.

It is valuable to put the regional variations in transdermal drug absorption into context with respect to variation found for the same site

between different individuals (Southwell *et al.*, 1984). There is – as would be expected for a biological membrane – considerable variation in permeation across a given body site (say the trunk) of an individual (up to around 30%) and also considerable variation between the same body site on different individuals (up to around 40%). Such variability can thus exceed that resulting from regional differences if using tissue from, for example, the arm and the leg where the regional factor is small.

The influences of regional variations on transdermal drug delivery have recently been reviewed by Wester and Maibach (1999), who examine studies on numerous drugs and varied body sites of both humans and animals.

1.3.3 Race

There are surprisingly few literature reports examining racial similarities or differences in topical and transdermal drug delivery, yet many formulations and transdermal delivery devices are marketed for use around the world. The studies that have been reported show no differences in transepidermal water loss or in the delivery of benzoic acid, nicotine and aspirin between African, Asian and European skin (Berardesca and Maibach, 1990; Lotte *et al.*, 1993). However, there are significant differences in the stratum corneum water content between races (Berardesca *et al.*, 1991); it would be anticipated that these differences in hydration would be apparent through differences in drug absorption. With such limited data available the absence of racial variations in delivery of topically applied drugs cannot be assumed.

1.3.4 Other factors

Several other physiological factors may, to some degree, influence transdermal drug delivery. For example, keratinocytes tend to be slightly larger in females (37–46 μm) than in males (34–44 μm), but there are no reports of significant differences in drug delivery between equivalent sites in the two sexes.

The level of hydration of the stratum corneum can have a dramatic effect on drug permeation through the tissue, and increasing hydration is well known to increase transdermal delivery of most drugs. Indeed, occlusive dressings and patches are highly effective strategies to increase transdermal drug delivery since they create elevated hydration of the

stratum corneum. Controlling the level of tissue hydration is thus usually essential when designing experiments to study transdermal drug delivery (see Chapter 3).

Since diffusion through the stratum corneum is a passive process, then increasing the temperature clearly increases the permeant diffusion coefficient (at a fixed concentration gradient; see Chapter 2). The human body maintains a temperature gradient across the skin from around 37°C inside to around 32°C at the outer surface. Greatly elevating the skin temperature can induce structural alterations within the stratum corneum, and these modifications can also increase diffusion through the tissue. Again, appropriate temperature control is an important aspect of good experimental design, but for most situations in clinical application of topical medicaments slight variations in skin or environmental temperatures tend to have minimal effects on transdermal drug delivery.

Considerable variations are found in the structure and barrier functions of the skin from different animal species. Numerous animal models have been proposed for use in transdermal drug delivery studies, and these are examined in some detail in Section 3.3.1.

1.4 Pathological disorders

There are numerous dermatological textbooks that offer detailed descriptions of the clinical symptoms and pathology of skin disorders (e.g. Gawkrödger, 1997; Buxton, 1998). Some considerations relating to the formulation of preparations for treating skin disorders are described in Chapter 7. The intention here is to describe how some skin disorders alter the barrier properties of human skin, and hence describe the influence of these disorders on topical and transdermal drug delivery. Clearly, for many skin disorders where the barrier function is compromised, as treatment progresses the condition will improve; that is, the barrier function of the tissue may be restored to that approaching uninvolved tissue and consequently, as the disorder improves, drug delivery decreases. As will be seen from the following, in most diseased states, stratum corneum barrier resistance is decreased.

Dermatology, as with most specialities within medicine, has evolved from a somewhat empirical art to a scientific discipline. Currently, skin biopsies with associated microscopy and immunochemical techniques are used to assist diagnosis. However, much diagnosis is still based on expert examination of skin lesions together with knowledge of the distribution of the lesions and the time course of the disorder. Thus, classification of dermatological disorders can appear to be somewhat

Table 1.2 Some terminology commonly used in dermatology

<i>Term</i>	<i>Definition</i>
Atrophy	A thinning/loss of epidermis or dermis
Bulla	Large (>1 cm ²) elevated lesion that contains free fluid (a blister)
Crust	An accumulation of exudates (e.g. blood, serum)
Cyst	A sac containing liquid or a semi-solid usually found in the dermis
Erosion	A loss of the epidermis above the basal layer
Excoriations	Crusts and erosions due to scratching
Fissure	A slit through the whole thickness of the skin
Macule	Small areas (<1 cm ²) with a change in skin colour, but no alteration in skin level (no elevation or depression)
Nodule	A palpable solid lesion which usually extends from deeper skin layers to the outer surface
Papule	A solid elevated lesion usually <1 cm ²
Patch	As a macule, but larger (>1 cm ²)
Plaque	A solid elevated lesion, usually >1 cm ²
Pustule	An elevated lesion that contains pus, usually found in the dermis or subcutis (an abscess)
Scale	Excessive layering of the stratum corneum or keratinocytes on the skin surface
Scar	Fibrous tissue formed during wound healing
Sclerosis	A hardening of the skin
Ulcer	A loss of the epidermis and part or all of the dermis, leaving a moist depression
Vesicle	As a bulla, but smaller (<1 cm ²)

‘illogical’, especially when syndromes are linked with names that give little indication as to the nature of the condition. Terminology and the application of terminology can vary throughout the world, but more common terms used to describe skin lesions are given in Table 1.2.

1.4.1 Eruptions

Numerous disorders result in an eruption of the skin surface. In such cases the barrier properties of the stratum corneum are compromised, allowing easier passage of drugs (and potentially toxic materials) into and through the skin. Likewise, the erupted skin surface will allow increased water loss from the body.

Psoriasis is one of the most common of all skin diseases, though it manifests in various guises. It is a chronic recurring non-infectious scaling skin condition characterised by erythematous plaques covered with silvery scales. It affects typically 2% of Caucasians, but is less

prevalent in Africa and Japan. It may appear at any age and is equally prevalent in males and females. Plaques in psoriatic conditions arise from accelerated differentiation of keratinocytes through the epidermis and from increased mitosis of keratinocytes, not only from within the basal layer but also in the two or three cell layers above the basal layer. Numerous therapies exist, including those that inhibit mitotic activity [e.g. psoralen-UVA (PUVA) treatment]. Indeed, for topical therapy the loss of skin barrier integrity has been shown to be valuable for targeting drugs to the required site of action whilst minimising side effects (Anigbogu *et al.*, 1996).

Lichenoid eruptions are characterised by intensely itchy, flat-topped papules. In the most common of these conditions, lichen planus, the granular epidermal layer is thickened and lymphocytes are found in the dermis near the basement membrane, suggesting that the condition is associated with an autoimmune disease. As with psoriasis, many different lichenoid eruptions are seen, and lichen planus-like drug eruptions can appear following the ingestion of drugs such as chloroquine and chlorothiazide.

Eczema, from the Greek 'to boil over', is a further non-infectious eruptive condition, in which blistering occurs. The term eczema has no clear universally accepted definition, and is often included within dermatitis simply meaning inflammation of the skin. Such inflammations can arise from various stimuli. Atopic dermatitis is a chronic inflammation of the epidermis usually with a strong genetic predisposition from parents who may have, for example, asthma or allergic rhinitis. Atopic dermatitis may affect up to 15% of infants, with the onset usually occurring before the child's first birthday. Various studies have clearly demonstrated that the stratum corneum barrier is highly compromised for patients with atopic dermatitis, with transepidermal water loss from the body increasing by up to 10-fold (Ogawa and Yoshiike, 1992; Aalto-Korte and Turpeinen, 1993). Contact dermatitis can result from a direct irritant action of a substance on the skin (irritant contact dermatitis) or further exposure, following previous sensitisation of the skin, from a contact allergen (allergic contact dermatitis). Irritant dermatitis is the more common of the two manifestations, and can be caused by many chemicals, solvents and detergents. Irritant dermatitis tends to have a rapid onset of action (4–12 h after exposure) and is seen at the site exposed to the irritant. Sodium lauryl sulfate was used to induce irritant dermatitis before in-vivo percutaneous absorption of several drugs was assessed (Wilhelm *et al.*, 1991). Clearly having implications for therapy, hydrocortisone absorption was shown to increase nearly

three-fold through the affected site, with a two-fold increase seen for indomethacin. Allergic contact dermatitis affects 1–2% of the population and is often seen at body sites such as the ear lobes/neck, wrists and feet. Typical allergens include nickel and chromates (used in jewellery), and dyes or leather-tanning chemicals. As with other eruptive conditions, impairment of the skin barrier has been reported with contact hypersensitivity reactions.

1.4.2 Infections

Intact skin is a highly effective barrier against the ingress of microorganisms. However, the tissue also carries microbial flora, including bacteria and yeasts and if breached, then infection can result. Commonly found skin bacteria include staphylococcal species (e.g. *Staphylococcus epidermidis*), micrococci, corynebacteria and propionibacteria. These organisms tend to aggregate around hair follicles where they can be numerous. For example, micrococci may number around 60 per cm² on the forearm, but up to 500 000 per cm² in the axillae. With such potentially high numbers of organisms, it is also conceivable that some topically applied drugs may be metabolised prior to penetrating the tissue.

Many diseases of the skin are caused by staphylococcal (often *Staphylococcus aureus*) and streptococcal (e.g. *Streptococcus pyogenes*) organisms. One example is impetigo, presenting as thin-walled easily ruptured blisters, and commonly spread by contact between children, though treatment is usually with oral antibiotics. Most seriously, *Streptococcus pyogenes* is the causative organism of necrotising fasciitis, an acute condition usually resulting from a minor trauma. An ill-defined erythema will rapidly become necrotic and can be fatal unless rapid surgical debridement of the tissue and systemic antibiotics are employed.

Other bacterial infections, less common than those caused by the Gram-positive cocci, include infections due to mycobacteria; tuberculosis is caused by *Mycobacterium tuberculosis* which generates the condition lupus vulgaris, characterised by red/brown plaques often on the face and neck. Other opportunistic pathogens can infect susceptible skin sites, such as psoriatic lesions causing secondary infections.

Other than bacteria, there are several viral conditions that can erupt from the skin surface. Warts (verrucae) are common benign cutaneous tumours caused by the human papillomavirus. They are usually self-limiting, though are transmitted by direct contact. Over 90 subtypes of the human papillomavirus have been identified, and some of

these subtypes are associated with specific clinical lesions; type 2 with common hand warts, types 6 and 11 with genital warts. Generally, the epidermis becomes thickened and is hyperkeratotic causing the eruption, and the keratinocytes of the stratum granulosum are vacuolated because of the viral infection. Many treatments for hand warts are topically applied, though cryotherapy is usually indicated for most genital warts. Targeting of, for example, salicylic acid to the viral particles within the wart has been demonstrated from topical applications (Lawson *et al.*, 1998). Herpes simplex ('cold sores') infections are also treated topically, and again are very common self-limiting vesicular eruptions. Herpes zoster (shingles) is similarly self-limiting, though secondary bacterial infections can be problematic.

Fungal skin infections are also common and range in severity. Two types of causative organisms are common: *Candida* species which are yeasts; and the dermatophytes that are multicellular. *Candida albicans* is an opportunistic pathogen causing thrush, especially where the normal bacterial flora is disturbed (e.g. following antibiotic therapy). Dermatophyte infections target the keratinised tissues of the body – hair, nails and stratum corneum and are common as athlete's foot (*tinea pedis*), ringworm (*tinea corporis*) and 'jock itch' (*tinea cruris*).

It is clear that many of the above infections vary in their severity. Consequently, the damage caused to the skin barrier integrity will vary with severity of the infection. In cases such as necrotising fasciitis it is obvious that the barrier is seriously impaired. With the presence of a hand wart, only a very small fraction of the skin surface is involved, and the effect of hyperkeratinisation on drug flux through the wart site is probably marginal. What is apparent, however, is that in all the cases of infection outlined above, the effect on barrier integrity will be to always diminish its effectiveness. This may be advantageous where topical therapy for the infection is desired, but the barrier is dynamic and will be restored as the condition improves; hence, drug flux across the repairing tissue will be expected to slow.

1.4.3 Ichthyoses

Ichthyoses are defined as disorders of keratinisation and epidermal differentiation. They are usually genetically determined and are characterised by excessively dry and scaly skin. In the UK, the most common ichthyotic condition, ichthyosis vulgaris, is found in 1 in 250 to 300 people but is often so mild that no clinical treatment is necessary. Sex-linked recessive ichthyosis (*ichthyosis nigricans*) is found in approx-

imately 1 in 2000 males; though the fully developed disease is only found in males, females may have mild symptoms of the condition. Considerable thickening of the stratum granulosum with an increase in stratum corneum thickness is seen with ichthyotic conditions. However, even with a thickened stratum corneum, Lavrijsen *et al.* (1993) have shown that the barrier integrity is compromised in ichthyotic patients.

1.4.4 Tumours

Skin tumours are common, and numbers of cases are rising rapidly in Western countries. The majority of skin tumours are benign, and those from viral warts have been described above. Benign tumours can result from a proliferation of basal layer keratinocytes or melanocytes. Some benign tumours (such as actinic keratoses) manifest as scaly hyperpigmented crusting lesions, are premalignant and usually result from an outdoor occupation or from considerable recreational sun exposure. Malignant tumours can derive from keratinocytes (such as squamous cell carcinoma), from melanocytes (malignant melanoma), or from other basal cells in the epidermis (basal cell carcinoma). Most malignant tumours are excised and may be followed by radiotherapy or chemotherapy. However, some tumours may be controlled rather than cured (such as slowly evolving T-cell lymphomas), in which case topical preparations including steroids may help to improve symptoms. Again, the stratum corneum around an eruptive tumour will be compromised.

1.5 References

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