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Simon C. Wilkinson

Predictive Methods in Percutaneous Absorption

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*Dedicated to the pioneering research
in this field by:
Gordon Flynn
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Foreword

As a major function of the skin is to be a protective barrier and stop the ingress of exogenous chemicals, it may seem strange to dedicate decades of research effort into understanding of how, and to what extent, molecules pass through the skin! However, this topic, and the areas covered by this volume, is vitally important in understanding the potential beneficial or harmful effects of dermal penetration. Significant progress has been made through this research in understanding the structure, function and physiology of skin as well as how these factors influence the passage of molecules into and across it. This research area now goes beyond the physical measurement of the passage of molecules through the skin to include modelling and computational simulation technologies to assist our understanding of dermal penetration, as well as the chemistry behind formulation science. This knowledge has various applications in the pharmaceutical, personal product, biocide, fine chemical and many other manufacturing industries. This volume goes a long way to capture and define the state of the art in the experimental determination and computational calculation of percutaneous absorption.

However, despite some excellent research, we are still lacking the tools to consistently and reproducibly assess experimentally, let alone predict, the amount of a chemical that will reach the systemic circulation following topical application. There are many reasons for this, and this volume gives a thorough account of the problems, why they are important, and possible solutions. A substantial part of the problem is the quality, or otherwise, of the historical data with which we are developing models. Many of the data were not intended for the purpose for which they are now being used, rather being investigative studies of drug permeation or for risk assessment. As such, they more than adequately served their original purpose, but should be used with caution for modelling. A second problem is that of the formulated product which is applied to the skin. Modelling works best when the data used relate to the pure substance applied neat to the skin (or at most,

is applied as a saturated aqueous solution). We are only beginning to understand and model the effect that other chemicals—particularly formulation components—have on penetration, something that is highlighted in this book. Therefore, in order to take the science forward, the information provided and conclusions reached in this volume are vital to integrate into novel research programmes to answer questions such as “How can I reach a therapeutic dose of a drug when applied dermally?” or “What will be the risk of using this shampoo on a daily basis?”

This book has been written by experts in the field and will provide a valuable resource and starting point for all who wish to venture into this area or continue their study. This work especially fulfils the ambitions of Dr. Gary Moss who has been researching in this field for two decades, starting with his Ph.D. from Queen’s University Belfast. Following from his background in topical formulation development, he then combined this knowledge of experimental outcomes with a new-found interest in computational modelling methods—this was, after all, not long after the seminal publications of the Flynn compilation of skin permeability coefficients and the first Potts and Guy model. Gary has led and continues to lead the way in driving the process of data compilation and applying these approaches to other membranes (e.g. polydimethylsiloxane, Silastic®). Extending the concepts, through a variety of experiences garnered from positions in both industry and academia, he has worked to apply twenty-first-century modelling concepts to these problems, with careful reference to data quality, consideration of formulation and the adoption of novel machine learning methods. I have known Gary for over 20 years, and I have admired his scientific contributions to the field of transdermal research. There are few academic counterparts that can boast his knowledge of skin structure and function, transdermal drug delivery and toxicology applied to the permeation of exogenous chemicals into and across skin. This experience will become apparent to the reader of this textbook.

Simon Wilkinson is a toxicologist focusing on skin absorption and dermal metabolism, and has a strong interest in methodological approaches in percutaneous absorption. This expertise underpins one of the key themes in this book, which is the role of experimental data—usually derived for other purposes—applied to the modelling of percutaneous absorption and how this impacts on the model quality but also its relevance beyond theoretical or academic spheres.

An interesting finding, in research conducted by Dr. Moss and Dr. Darren Gullick, was the development of our understanding of skin permeability as a non-linear phenomenon, which led to the development of further investigations using the Gaussian process machine learning techniques and which has informed significantly this current volume. Perhaps one of the author’s key findings to date was the development of evidence for the nonlinear nature of the relationship between the physicochemical properties of a molecule and its percutaneous absorption, which

paved the way for further investigations using machine learning methods, which could be considered to be the future of predictive percutaneous absorption research.

The current edition is a timely addition to the literature, presenting and updating us with the state of the art of predictive percutaneous absorption.

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Chapter 1

Skin Structure and Physiology

Introduction

The skin is the most complex and diverse organ in the human body. It has a multitude of important roles, which include temperature regulation, mechanical resistance and various protective functions—for example, both from the ingress of exogenous materials, chemicals and biological species, and the egress of water from the body.

The skin is the largest organ of the body. On average, it accounts for approximately 10 % of body mass, receives approximately one-third of the blood circulating throughout the body and has a surface area of approximately 2–3 m² (Woolfson and McCafferty 1993a, b). It provides a strong yet flexible self-repairing barrier to the external environment and protects internal body organs and fluids from external influences, harmful molecules and micro-organisms. The skin also forms an extensive sensory surface, transmitting sensations such as heat, cold, touch, pressure and pain to the central nervous system. The skin is a multilayered organ consisting of three main histological layers: the epidermis, the dermis and the subcutis. Mammalian skin is a stratified epithelium, and each layer will be considered individually, below, progressing from the deeper (innermost) tissues to the outermost tissues (those outermost tissues which are, ultimately, in contact with the external environment).

The Hypodermis (Subcutaneous Fatty Tissue Layer)

At the base of the skin, below the epidermis and dermis, lies the subcutaneous fatty tissue layer, often called the subcutis, or hypodermis. It provides support and cushioning for the overlying epidermal and dermal layers, a means of attachment to

deeper tissues below the skin. It acts as a depository for fat and an absorber of external forces, such as heat and physical shock, and contains the blood vessels that supply the skin. It is variable in thickness depending on the body site; it ranges from a few centimetres thick in some regions (i.e. the abdominal wall) to areas where there is little or no fat and where the hypodermal layer may be difficult to observe (i.e. the eyelid or the scrotum). As the dermis and hypodermis are both irregular connective tissues, it is often difficult to distinguish between them. However, the hypodermis is generally looser and contains a higher proportion of adipose cells compared with the dermis.

The Dermis

The dermis (or corium) lies immediately above the hypodermis. In terms of volume, it is the largest part of the skin, being commonly ten to twenty times thicker than the epidermis. It is usually 0.1–0.5 cm in thickness, depending on its location. It is a robust and durable tissue that provides flexibility and tensile strength to the skin. Its myriad functions include protecting the body from injury and infection and provision of nutrition for the epidermis. It also contains the main source of water within the skin. The dermis is comprised mostly of collagen, arranged in mechanically strong fibrous chains, which sit within a mucopolysaccharide gel-like structure (Wilkes et al. 1973). This matrix hosts a range of important structures, including nerve tissues, vascular and lymphatic systems, and the bases of various skin appendages. The lower part so the dermis consists of coarse fibrous tissues which provide the main supporting structural layer of the skin. It is also the locus of the blood vessels, which may extend to within 0.2 m of the skin surface (Woolfson and McCafferty 1993a, b). Towards the top of the dermis, the connective structures are more loosely formed and contain a finely structured papillary layer which encroaches into the epidermis. The transition between the predominately fibrous dermal tissues and the predominately cellular epidermal layers occurs at the dermo-epidermal junction.

The vasculature system of the skin is responsible for regulation of skin temperature, the supply of nutrients and oxygen to the skin, and the removal of toxins and waste products in assisting wound repair and healing. In the context of percutaneous absorption, it plays an important role in the removal of locally absorbed chemicals by carrying them into the systemic circulation. As the skin's blood supply can become relatively close to the skin's surface, penetrants are therefore removed from the skin at around the dermo-epidermal junction. This implies that the lower dermal and hypodermal layers of the skin play little role in the process of percutaneous absorption. It also implies that the blood supply to the skin provides the opportunity for penetrants to be removed from the local tissues and hence is important in the maintenance of a concentration gradient across the skin barrier.

Cross and Roberts (1993) also commented that the lymphatic system, which is located a comparable distance from the exterior of the body, may also play a significant role in the clearance of exogenous penetrants.

Skin Appendages

Human skin has associated with it several types of appendages, including hair follicles and their associated sebaceous glands (Fig. 1.1), and eccrine and apocrine sweat glands.

On average, human skin contains 40–70 hair follicles and 200–250 sweat ducts per square centimetre of skin. The skin appendages occupy approximately 0.1 % of the total skin surface, although this varies from region to region with, for example, the axillary, anogenital area and forehead having a larger than average concentration of hair follicles (Bronaugh and Maibach 1999). Hairs are formed from compacted plates of keratinocytes and reside in the hair follicles, which are invaginations in the epidermis. Sebaceous glands are associated with the hair follicles—usually formed as outgrowths of the follicle. They secrete an oily material, sebum, onto the skin surface. Sebum is a lipid-rich mixture which acts as a plasticiser for the *stratum corneum* and helps to maintain an acidic mantle of approximately pH 5 (Bronaugh and Maibach 1999). Eccrine glands are principally concerned with temperature control and are responsible for the secretion of sweat when stimulated by an increase in the external temperature or emotional factors. These glands commonly occupy approximately 10^{-4} % of the total skin area, and their structures ensure that they extend well into the dermis. Eccrine glands are found throughout the body, while apocrine glands are located in specific regions, including the axillae and anogenital regions.

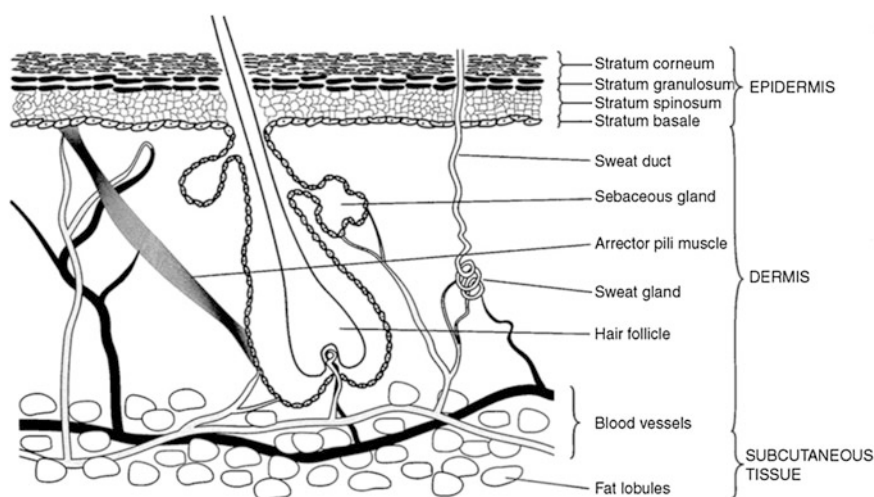


Fig. 1.1 Schematic diagram of the skin (© Williams (2003), used with permission)

The Subcutaneous Sensory Mechanism

The large size of the skin means that it acts as a major sensory organ for the body, particularly as it interfaces with the external environment. It provides information about the environment directly and indirectly, such as the effect of radiation on skin temperature. Fibres within the dermis form a plexus which lies parallel to the skin surface. The nerve plexus is comprised of unmyelinated and myelinated fibres. From the nerve plexus, individual fibres extend to supply particular locations in terminal branches which interconnect with and superimpose themselves upon each other in such a manner that every area in the skin is supplied by several different fibres, each of which ends in at least one particular receptor (Weddell 1941). Most of these receptors can be excited by different stimuli, but the different thresholds of stimuli required to provoke a particular receptor yield its specificity (Barlow and Mallon 1982).

The three main categories of cutaneous receptor, which are distinguished by their different sensitivities to stimuli, are the mechanoreceptors, thermoreceptors and nociceptors.

Mechanoreceptors are highly sensitive to pressure on the skin, or to movement of the hairs. Mechanoreceptors are usually described as rapidly adapting (RA) or slowly adapting (SA) types. RA mechanoreceptors include Pacinian corpuscles, which are found in both hairy and glabrous skin, and Meissner's corpuscles, which are located in the glabrous skin of primates. Pacinian corpuscles are small oval structures found in the deeper layers of the skin. They are 0.5–2 mm long and are composed of an "onion-like" lamellar structure which is formed from non-nervous tissue. Pacinian corpuscles are able to detect mechanical vibrations at high frequencies, which may be relayed at greater than one hundred hertz per second (Brodal 1981; Sinclair 1981). The Meissner corpuscle is an encapsulated myelinated receptor found in the dermis of human glabrous skin. It is surrounded by connective tissue which is attached to the basal projections of the epidermal cells by elastin fibrils. The Meissner corpuscle allows discrimination between highly localised sensations of touch, especially in the palmar regions where they are found in their highest density (Montagna 1964). Hair follicle receptors are myelinated fibres which are primarily associated with the tactile sensations (Elliott 1969). SA mechanoreceptors, including the Ruffini endings and the C-mechanoreceptors, respond during skin displacement, including the maintenance of a discharge of impulses when the skin is held in a new position (Barlow and Mallon 1982). The Ruffini endings are encapsulated receptors found in the dermis of hairy and glabrous skin. They provide a continuous indication of the intensity of the steady pressure or tension within the skin (Brodal 1981). C-mechanoreceptors are usually found in hairy skin and have small receptive fields (approximately 6 mm²). They emit a SA discharge when the skin is indented or when hairs are moved. However, repetitive stimulation produces a rapid fall in excitability and the receptors will fail to respond after 20–30 seconds as the receptor terminals become unexcitable after this time (Barlow and Mallon 1982).

Thermoreceptors are characterised by a continuous discharge of impulses at a given constant skin temperature which increases or decreases when temperature is raised or lowered. Thermoreceptors have small receptive fields (approximately 1 mm^2) and are classed as either “cold” or “warm” receptors, with the former located more towards the outer surface of the skin than the latter, at average depths of 0.15 and 0.6 mm below the skin surface, respectively (Barlow and Mallon 1982). While thermo- and mechanoreceptors contribute to the sensory quality of perceived pain, the nociceptors detect and signal high intensities of stimulation. Nociceptors generally reside at the dermo-epidermal junction and are either mechanical nociceptors (which respond to, for example, pinpricks or needles, or squeezing and crushing of the skin) or thermal, or mechanothermal, nociceptors (which respond to severe mechanical stimuli and to a wide range of skin temperatures) (Brodal 1981; Montagna 1964).

The Epidermis

The epidermis is the outermost layer of the skin. It is also the thinnest layer of the skin. Its thickness varies significantly around the body with, for example, the thickest skin being found on the weight-bearing planter surfaces (feet and hands, approximately 0.8 mm thick) and the thinnest skin being normally found on the eyelids and scrotum (0.06 mm) (Williams 2003). Despite the extensive vasculature present in deeper tissues such as the dermis, the epidermis has no blood supply and passage of materials into or out of it is usually by a process of diffusion across the dermo-epidermal junction and into the dermis. It is essentially a stratified epithelium, consisting of four, or often five, distinct layers (Fig. 1.2).

The Stratum Germinativum

The *stratum germinativum*, or basal layer, is the deepest layer of the epidermis. This metabolically active layer contains cells similar to those found in other tissues in the body and contains organelles such as mitochondria and ribosomes. It can be as thin as a single cell in depth and contains cuboid or columnar-to-oval-shaped cells which sit on the basal lamina. These cells are continually undergoing mitosis, as they provide replacement cells for the higher (outer) epidermis. Basal keratinocytes are connected to the dermo-epidermal membrane by hemidesmosomes and connect the basal cells to the basement membrane. The basal layer is also the location of other cells, including melanocytes, Langerhans cells and Merkel cells. The basal cells become flatter and more granular as they move up through the epidermis.

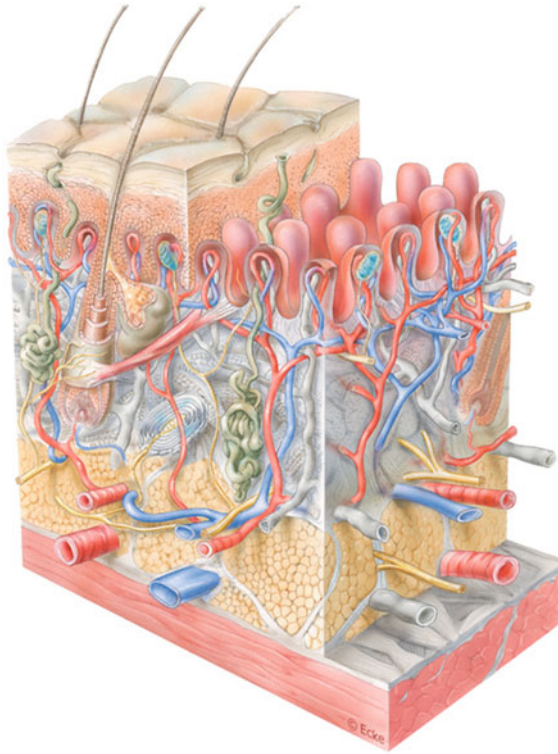


Fig. 1.2 Schematic representation of the epidermis (*source* BASF Personal Care and Nutrition GmbH; available at <http://www.skin-care-forum.basf.com>)

The Stratum Spinosum

The *stratum spinosum*, or prickle cell layer, sits immediately above the *stratum germinativum*. It is often described with the basal layer (where the basal layer is very thin) as a single layer—the Malpighian layer. Normally, however, it is several layers thick (usually 2–6 layers) and consists of morphologically irregular cells which may range from columnar to polyhedral in structure; such a progression in morphology is common as this layer progresses upwards. Each cell in this layer possesses tonofilamental desmosomes, often called “prickles” or “spines”, which give this layer its characteristic name and extend from the surface of the cell in all directions, helping to maintain a distance of approximately 20 nm between cells. The prickles of adjacent cells link via intercellular bridges and give three-dimensional structural rigidity and increase the resistance of the skin to abrasion throughout this layer. The prickle cell layer is metabolically active despite lacking in mitosis.

The Stratum Granulosum

The *stratum granulosum*, or granular layer, lies immediately above the *stratum spinosum* and is usually one to three cells deep. It consists of flattened, granular cells whose cytoplasm contains characteristic granules of keratohyalin, which is responsible for their characteristic “granular” appearance. In the *stratum granulosum*, degradation of cell components becomes significant; visually, this is seen in the flattening of cells compared to the layers immediately below the granular layer, and also in a substantial decrease in metabolic activity which eventually ceases towards the top of this layer due to the degeneration of cell nuclei, which leaves them unable to carry out important metabolic reactions.

The Stratum Lucidum

The *stratum lucidum* sits immediately above the *stratum granulosum*. It is easily observed on thick skin, but may be missing from thinner skin, which is why the epidermis is often described as having either four or five layers. The *stratum lucidum* is often considered to be functionally indistinct from the *stratum corneum* and that it may be an artefact of tissue preparation and cell differentiation, rather than a morphologically distinct layer. The cells of the *stratum lucidum* are elongated, translucent, and mostly lack either nuclei or cytoplasmic organelles. This layer is significantly more keratinised, and contains significantly flatter cells, than the underlying layers of the epidermis.

The Stratum Corneum

The outermost layer of the skin is the *stratum corneum*, or horny layer. It is the final result of cell differentiation and compaction prior to desquamation and removal from the body. It is a compacted, keratinised multilayer which is dehydrated in comparison with the adjacent layers of the skin. It is, on average, 15–20 cells thick—around 10 μm in thickness when dry, although it can swell to many times its thickness when wet. The formation of keratin and the resultant cell death are part of the process of keratinisation or cornification that produces what is, in effect, the *stratum corneum*, the outer envelope of the body. In areas of the skin where the *stratum lucidum* is clearly present, the *stratum corneum* is usually much thicker, and this also mirrors the thickness of the viable epidermis around the body. Thus, the epidermis in those regions, such as the palms and soles, can be up to 800 μm in thickness, compared to 75–150 μm in other areas. Cells of the *stratum corneum* are physiologically inactive, continually undergoing a process of shedding while themselves being constantly replenished from the upward migration of cells from the underlying epidermal layers (Woolfson and McCafferty 1993a, b).

The *stratum corneum* is the major rate-limiting membrane of the skin and is responsible for the regulation of water loss from the body as well as limiting the ingress of harmful materials from the external environment (Scheuplein and Blank 1971). It is currently believed to consist of two alternating amorphous lipophilic and hydrophilic layers and is comparatively more lipophilic than the other epidermal layers. While generally having lower water content than other layers of the skin, the *stratum corneum* water content is highly variable and depends on both moisture content of the external environment of the body and the location on the body. The exceedingly dense *stratum corneum* may also swell to many times its own thickness in the presence of water. The water content of the *stratum corneum* generally decreases as the external interface is approached. The *stratum corneum* possesses approximately 40 % water by weight (in a relative humidity of 33–50 %). By weight, the *stratum corneum* is composed of approximately 40 % protein (mostly keratin) and 15–20 % lipid (triglycerides, cholesterol, fatty acids and phospholipids) although it should be noted that the exact composition will vary around the body. The *stratum corneum* lipids originate from a number of sources, including the discharged lamellae of membrane-coated granules, intercellular cement and the keratinocyte cell envelope (Anderson and Cassidy 1973).

The cells of the *stratum corneum* are flattened and elongated and are approximately 1 μm in thickness. They occupy an area of 700–1200 μm^2 ; thus, there are approximately 10^5 cells per cm^2 . They form a closely packed array of interdigitated cells (which facilitates the formation of cohesive laminae) which are the cells stacked in vertical columns (MacKensie and Linder 1973). Each cell is contained within a mainly proteinaceous envelope rather than the conventional lipid bilayer cell membrane. This envelope provides the *stratum corneum* with the majority of its mechanical strength, in particular through the disulphide bonds of the intracellular keratin and by linking cells that are embedded in an intercellular lipid matrix (Matolsty 1976).

The upward movement of cellular material through the epidermis ends in the *stratum corneum*, which constantly sheds its outermost layers in a process called desquamation. This process involves the cleavage of intercellular bridges and may suggest a certain amount of metabolic activity and regulatory control in a layer often considered to be, to all intents and purposes, inert (Michaelis et al. 1975). Typically, the daily desquamatory loss from the *stratum corneum* is typically not more than 1 g, although as the rate of *stratum corneum* shedding is, in healthy skin, equal to the rate of epidermal cell regeneration, the *stratum corneum* remains approximately the same thickness.

The Stratum Corneum Barrier

The *stratum corneum* skin barrier has traditionally been described as a “bricks and mortar” structure (Michaelis et al. 1975; Elias et al. 1981). The “bricks” represent the tightly packed corneocytes, and they are embedded in a “mortar” of lipid bilayers. These flattened, highly proteinaceous cells are the final point of

keratinocyte differentiation and are interconnected by structures called corneodesmosomes (Fig. 1.3). The “bricks” are enclosed within a continuous and highly ordered lamellar lipid bilayer. Structurally, ceramides are the most important components of this lamellar phase; they are polar lipids which contain hydroxylated alkyl side chains that, under normal conditions, are packed both hexagonally and orthorhombically. As discussed above, the keratinocyte “bricks” of the skin barrier may hydrate extensively, resulting in significant changes to the packing, structure and permeability of the *stratum corneum* (Michel et al. 1988; Norlen 2006, 2007; Rawlings 2003, 2010). The *stratum corneum* corneocytes change in their morphological and biochemical functions as they progress from the lower to higher levels of the *stratum corneum*. Such transitions are associated with increases in transglutaminase-mediated protein crosslinking and increased levels of intercorneocyte ceramides and fatty acids, resulting in a progression from fragile to rigid structures, described by Rawlings (2010) as the transition from “*stratum compactum*” to “*stratum disjunctum*”. This transition occurs alongside an increase in the occurrence of the protein (pro)filaggrin, which is thought to play a key role in the aggregation of keratin filaments within corneocytes (Rawlings 2010).

Despite the fundamental correctness of the “bricks and mortar” model of this *stratum corneum*, advances have been made in recent years, which have elaborated our understanding of the *stratum corneum* structure and its barrier function. New species of ceramides and the synthetic pathways that generate them are still being identified (Rawlings 2010). Cryoelectron microscopy studies have proposed the existence of a

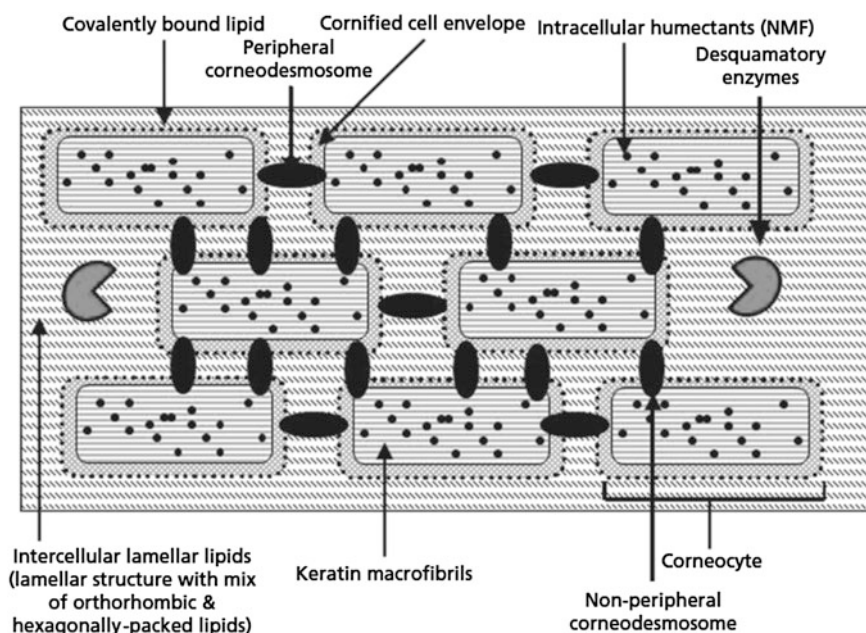


Fig. 1.3 Schematic structure of the *stratum corneum* (Rawlings 2010, used with permission)