Allergen penetration through the skin

The skin is directly in contact with environmental molecules which are present in the air or directly in contact with the epidermis. Despite the assumption that it has a barrier role which could prevent the penetration of molecules, the skin is permeable to all substances from the low molecular weight xenobiotics to the high molecular weight proteins. Only the degree of permeability varies depending on the physiological state of the skin and the chemical properties of molecules. Recent insights into the pathophysiology of allergic skin diseases have shown that allergen penetration is not the major factor in explaining why some patients become allergic while others maintain an immunological tolerance to the penetrating molecules. Indeed, the functional properties of some allergenic molecules able to induce activation of innate immunity appear to be far more important in the development of allergy than their ability to penetrate the skin easily.

Key words: xenobiotics, haptens, contact dermatitis, atopic dermatitis, tolerance

Cutaneous penetration - definitions

Percutaneous absorption corresponds to the transfer of a substance via the skin from the external environment as far as the blood. It can be defined as the sum of two phenomena: penetration of the molecules within the entire skin, followed by resorption in the blood or lymphatic systems from the papillary dermis and later, from the deep dermis. The penetration stage, in physical terms, is a passive diffusion through each structure of the tegument: horny layer, Malpighian layer, dermis and cutaneous annexes. It depends on a transfer, which occurs at the interface of the environment/horny layer, without which no diffusion is possible. This indispensable stage, starting from the external environment or the medium, corresponds with the release of the molecule which will be diffused and therefore be put at the disposal of the organism. Once absorbed, the substance is distributed in the organism then, after having been metabolised or not, it is eliminated. The stages leading to percutaneous absorption are similar to those found in any other route of administration.

The skin barrier function

Within the skin, the epidermis forms the main barrier to cutaneous penetration and within the epidermis it is the
harmy layer which is charged with this role [30]. The horn layer (i.e. the stratum corneum) presents practically the same resistance to absorption as the entire skin. This means that any alteration of this layer will be accompanied by an increase in the skin penetration of molecules and will allow the absorption of molecules which would not have been able to penetrate a healthy epidermis. The total elimination of the stratum corneum by delamination with adhesive tape (stripping) leads to an increase in penetration by chemical agents [5, 21]. The resistance of live epidermis to diffusion is very weak compared to that of the horn layer, but it must, however, be admitted that when the latter is removed by stripping, the epidermis can have a barrier function, notably with regard to very hydrophobic molecules. The dermis, in intact skin, only plays a very small part in the overall “barrier function”.

The resorption of absorbed molecules takes place through the blood (vascularisation of the dermal papillaries) and lymphatic systems. If the blood flow is insufficient to resorb the molecules as and when they arrive at the level of the deep dermis, local retention occurs. This phenomenon has been observed for numerous substances such as steroids (estradiol, progesterone, dexamethasone), toxic organophosphorous compounds (malathion, parathion, disopropyl fluorophosphate), non steroid anti-inflammatory drugs (indometacine, ifenamic acid, salicylic acid-based substances, ketoprofene, diclofenac) and enables us to better understand their mode of action at the level of structures situated below the application zone and where systemic diffusion and distribution by plasma does not seem to be necessary. The use of trace molecules like FITC (fluoresceine isothiocyanate) in murine models shows the rate of diffusion and distribution by plasma does not seem to be necessary. The use of trace molecules like FITC (fluoresceine isothiocyanate) in murine models shows the rate of diffusion and distribution by plasma does not seem to be necessary. The use of trace molecules like FITC (fluoresceine isothiocyanate) in murine models shows the rate of diffusion and distribution by plasma does not seem to be necessary. The use of trace molecules like FITC (fluoresceine isothiocyanate) in murine models shows the rate of diffusion and distribution by plasma does not seem to be necessary.

**Routes for skin penetration**

Two distinct pathways are available for penetration: 1) the transepidermal route where the substance will be diffused across the intercellular spaces of the horny layer or across the corneal cells themselves; 2) the cutaneous annex route which follows the pilo-sebaceous follicles and/or sweat glands. In the majority of cases penetration takes place via these routes together, both participating to the phenomenon and global penetration is the result of the conjunction of transepidermal and annex pathways.

**Transepidermal penetration**

The composite structure of the horny layer enables it to be presented schematically as a wall of ‘bricks’, made up of corneal cells (i.e. corneocytes) which are hydrophilic by nature of their protein content, surrounded by a lipophilic ‘cement’ made up of lipids which fill the extra-cellular spaces [14, 36]. In these conditions, diffusion through the horny layer can take place either directly by transepidermal passage, with successive transfer through the cells and the extra-cellular spaces, or by intercellular passage through the winding spaces left free between the corneocytes. Structural analysis of the horny layer shows that the lipids excreted in the intercellular spaces during the final stage of epidermic differentiation: free or esterified sterols, free fatty acids, triglycerides and sphingolipids, are organised in double layers which are arranged to separate the ‘hydrophilic’ and ‘lipophilic’ zones, thus creating an area of stratified diffusion with opposed physical/chemical properties [14, 31, 36]. The polar molecules can thus make their way towards the ‘hydrophilic’ regions of the intercellular lipidic layers while the ‘hydrophobic’ areas enable the nonpolar molecules to circulate.

The distance which a molecule travels is thus very different depending on whether it follows the transcellular pathway (the length of the diffusion pathway corresponding to the average thickness of the horny layer, i.e. from 10 to 40 µm) or the wounding path which leads through the intercellular spaces filled with lipids, whose length has been estimated at several hundred µm.

**Penetration via the cutaneous annexes**

Over most of the body, the horny layer is crossed by the pilo-sebaceous follicles and the sweat glands, these structures offering zones of less resistance (“shunts”) to the penetration of molecules. In man, these two types of annexes represent less than 0.1 to 1 % of the total skin surface. The anatomical structure of the sweat glands is complex and their resistance with respect to diffusion is difficult to evaluate. Because of this, their role as a penetration pathway is disputed, even if, despite the lack of recent studies, they seem indeed to participate in the penetration of iodine, histamine and adrenaline.

The pilo-sebaceous follicles are situated in epidermal invaginations and have always been considered as potential ‘shunts’, allowing a more rapid passage of molecules, in particular those which are diffused with difficulty across the horny layer [45]. Areas which are dense in follicles (the scalp, the arm pits) offer a higher permeability than those sites which are less hairy (forearms, palms).

**Cutaneous and molecular factors involved in skin penetration**

Diffusion through the skin depends on the heterogenous structural organisation of the different cutaneous layers, of their physical/chemical caracteristics (density, viscosity, solubility) and on the presence of proteins, as well as the potential for binding to these proteins [25]. The path of least resistance taken by the penetrating agent also depends on its relative affinity, or partition coefficient, for the different structures crossed and its volume.

**Cutaneous factors**

Several parameters govern the penetration stage:

– The metabolism of cutaneous enzymes is qualitatively similar to that found in the liver, but only represents a few percent of hepatic values. Metabolic activity can be a determining factor in absorption, in particular of non-protein chemicals, as is shown by the results of using potassium cyanide which greatly reduces trans-cutaneous diffusion.

– Anatomical variabilities in cutaneous penetration explain the difference in absorption of the same composition administered under identical conditions (vehicle, concentration, dose) on human skin, according to the anatomical area treated [60]. The skin of the scrotum offers the greatest permeability, the palmo-plantar zone is the least permeable. These anatomical variations have been observed for different molecules, among which are hydrocortisone, the organ-
The diffusion coefficient of the substance.

- The length of the pathway of the diffusion of the substance below the skin.

The difference between its concentration on the surface and the skin.

Several parameters govern penetration, among which the physical/chemical factors of the penetrating agent.

- The interaction of the substance with cutaneous molecules during transport can delay penetration.

The molecular size (molecular weight -MW-) of the substance which is being diffused is one very important parameter when considering penetration through healthy skin. Thus the diffusion constant for a large molecule is lower than that of a small molecule. The relation between the diffusion constant and the molecular size (measured by the molecular weight -MW-, or by the molecular volume -MV-) has been modeled in various ways. Kasting et al. proposed a relationship of an exponential type which can be used to predict the permeability coefficients [22, 52].

However, it is possible for molecules of a high molecular weight, in particular for proteins, to achieve penetration. The most interesting studies result from research into new ways of administering drugs percutaneously [19]. Using liposome formulations or transplanting a fatty acid onto the protein increases the diffusion of molecules with a high MW by rendering them more lipophilic and thus more easily available at the level of the inter-corneocyte lipids [15]. The preparations thus obtained use the intercellular pathways for penetration. Numerous molecules, defined as percutaneous absorption enhancers, are able to increase cutaneous penetration: ethanol, polyethylene glycol, lino- lenic acid, linomene etc... The utilisation alone or in association with penetrating agents has been able to increase the diffusion of proteins like LHRH [6]. Ultrasound increases skin penetration and its use has enabled the percutaneous penetration of large molecules like insulin, interferon gamma and erythropoietine [33]. The administration of an electric current of weak or strong voltage results in the same phenomenon of an increase in permeability. The association of an electric current with a detergent (sodium lauryl sulphate) induces conditions of highly increased cutaneous passage with the creation of intracutaneous penetration pathways capable of crossing the lipidic cellular membrane, the cornocyte envelope and the interior of the cornocytes [61]. It is thus possible to introduce molecules larger than 150 kD, like the immunoglobulins, at a rate which reaches therapeutic levels in the tissues (10 to 100 µg/hour/cm ). Iontophoresis also enables calcitonine to penetrate [11]. The association of iontophoresis and chemical enhancers induces important morphological modifications at the level of the horny layer, with dissociation of the cornocytes and rupture of keratin filaments, resulting in a further increase in the flow of macro-molecules crossing the epidermis, induced by the iontophoresis alone [6].

### Cutaneous penetration of non-protein chemicals

Non-protein allergens are chemicals of low molecular weight known as haptens, and they are responsible for contact dermatitis. Haptens are only immunogenic after covalent or non-covalent interaction with the amino acids of epidermal proteins. The great majority of haptens are electrophilic molecules which interact with the nucleophile residues of cutaneous proteins [29]. Metals do not bind in a covalent manner but establish weak interactions with the amino acids of cutaneous proteins.

Haptens are often derived from chemicals called pro-haptens, following skin metabolism stages which can, on the one hand, modify the diffusion and on the other, the allergenic capacity. Proof for the metabolism of pro-
haptens into haptens comes from the model of dimethylbenzanthracene (DMBA), a polyaromatic hydrocarbon (PAH). Allergic contact dermatitis to DMBA only occurs in mice which can metabolize it and inhibitors of PAH metabolism reduce the intensity of the eczema reaction [4]. One of the implications of these observations is that the individual cutaneous metabolism is at least as important as penetration in achieving sensitization and then an allergic reaction.

Allergic contact dermatitis is a delayed hypersensitivity reaction due to the activation in the skin of hapten-specific T cells. Two stages are necessary to its development: a clinically silent stage, sensitization, and the effector stage [10, 27]. Sensitization: the hapten, having crossed the horny layer, interacts with the proteins in the cutaneous cells. In particular it meets the network of epidermal dendritic cells (DC), the Langerhans cells. The interaction of the hapten/Langerhans cell induces: i) activation and maturation of DC which migrate from the epidermis to the dermis and then rejoin the lymph nodes draining the hapten penetration site; ii) processing of haptenized proteins and exposure at the membrane of the haptenized peptides in the peptide binding groove of MHC class II and class I molecules of DC. In the lymph nodes the DC present haptened peptides to naive T cells and activate those which express specific receptors. Thus the T cell clones CD4+ and CD8+ are generated and join the blood circulation and then the cutaneous tissue. It is interesting to note that CD8+ T cells are effectors in the allergic contact dermatitis reaction in murine models while CD4+ T cells are doted with regulatory properties [7, 23].

The effector stage: this occurs when the same hapten is applied on the skin. The hapten is diffused through the corneal layer and interacts with proteins of the epidermal cells. The hapten is metabolized by the cutaneous cells and is found in the cellular membrane in the form of haptenized peptides on MHC class I and class II molecules. The DC are thus capable of presenting the hapten to CD4+ and CD8+ T cells present in the dermis. The role of keratinocytes and other cutaneous cells only expressing MHC class I molecules appears to be essential for the activation of CD8+ effector T cells and has long been underestimated [2].

The penetration of haptens via the epidermis is mainly due to their polarity as their molecular weight is generally less than 1kD. Cutaneous penetration is therefore rapid for lipophilic molecules and more difficult for hydrophilic molecules. In contrast to protein allergens, the penetration of haptens is not a problem and is therefore not a limiting factor in their allergenic capacity.

The fact that a hapten can induce allergic contact dermatitis in an individual, or not, does not then depend on its capacity for diffusion through the horny layer but rests above all on its ability to interact with proteins and to induce important modifications in their physical/chemical structure [29]. The quantity of haptens in contact with the integument is certainly the most important factor in immunological tolerance of haptens. Studies in murine models show that, at low doses, haptens do not in fact have allergizing capacities. Initially, this immunological non-response was interpreted as an incapacity of the immune system to recognise haptens present in only very small quantities. In fact, the non-response is an active phenomenon since animals treated in this way with cutaneous exposure at very low doses become tolerant of doses of stronger haptens which typically induce contact dermatitis in non-treated animals [49].

**Penetration of proteins through the skin**

It was long considered very difficult, if not impossible, for protein molecules (molecules of high molecular weight) to penetrate normal skin. Penetration is increased in certain pathologies, in particular in patients with atopic dermatitis (AD) who present a severe cutaneous xerosis. The proteins responsible for these allergic reactions are additionally endowed with enzymatic properties which favour penetration through the epithelium.

**Penetration of proteins through the skin**

Demonstration of the capacity of proteins to penetrate normal skin comes from clinical observations of urticaria and eczema at the site of skin contact with proteins [37, 54]. Urticaria on contact with latex in patients who have immediate hypersensitivity occurs in the minutes following putting on latex gloves. Skin contact alone can induce systemic manifestations (Quincke's oedema, anaphylaxis) in patients who are very sensitized. Protein contact dermatitis, which is observed in butchers, is reproduced with skin tests using bovine or porcine meats. AD patients frequently test positive to pneumallergens and/or trophallergens with a clinical and histological appearance typical of contact dermatitis [9, 41]. These tests are positive even when they are carried out on normal skin on the back at times when there are no AD eruptions.

The use of murine experimental models confirmed these clinical observations. Application of the protein ovalbumine on non-sensitized animal tegument results in the generation of specific IgE, which indicates that penetration and activation of B cells and type 2 ovalbumine-specific T cells have taken place [57]. If application of the protein is repeated, a contact dermatitis develops with all the histological characteristics of AD: predominance of T cells, infiltration of eosinophils, production of type 2 cytokines (IL-4, IL-5) and IFNγ. These studies go even further as the authors show that, once mice are sensitized cutaneously by ovalbumin, simple inhalation of the protein can trigger a bronchial hyper-reactivity in these animals [48]. Thus, ovalbumin can penetrate healthy skin, can induce a humoral immune (IgE) and specific T cell response, as well as inducing clinical signs of cutaneous allergy (AD) and respiratory allergy (asthma).

**Enzyme activity of protein allergens**

Little is currently known about the factors which determine the allergenicity of proteins found in the natural environment [8, 28]. One important step forward in this area was the discovery of the enzymatic properties (proteases, chitinases, lyases, amylases) of several respiratory and alimentary allergens [35, 47]. Thus, the current theory is that the enzymatic activity of allergens is necessary for their penetration through the epithelium, allowing direct access to the antigen-presenting cells.

The group of allergens which has been most studied is that of house dust, which is colonised by the acarian Der matothagoides pteronyssinus (Der p), whose allergens are concentrated in their faeces. The allergic responses are the result of the contact of faeces with the respiratory epithe-
Easing the penetration of allergens is not the only factor capable of explaining their allergenic capacities. Der pl polarizes the immune response towards the type 2 phenotype [12] while Der p9 induces the production of inflammatory cytokines by epithelial cells [24]. Taken altogether, these observations suggest that the presence in the air of these proteins with an enzymatic action capable of altering the epithelial barrier not only promotes penetration of these proteins, but also the penetration of all the proteins present in the environment, including those which do not themselves have allergenic or enzymatic capacities.

Alteration of the skin barrier in AD

The physiopathology of AD is complex, associating cutaneous and immunological anomalies [18, 26, 53]. The principal cutaneous alterations concern the epidermis and in particular the horny layer and are responsible for the dry skin (xerosis) which is characteristic of the condition. Xerosis is responsible for the typical clinical appearance of AD patients: dry, scaly, rough, dull, slightly wrinkled skin. It is responsible for the pruritus which increases the penetration of allergens.

The stratum corneum in AD patients is thinner than in normal subjects and contains fewer intercellular lipids [59]. The lamellar organisation of the corneocytes is altered [40]. The hydrolipidic film on the surface is altered and washing aggravates this deficit [59]. The lipidic abnormalities mainly concern the ceramides, whose numbers in the corneal layer are very reduced, probably by a functional deficit in acylase sphingomyeline [34]. The alteration of the skin barrier in AD patients is shown by the reduction in the water-content of the stratum corneum and by the increase in transepidermal water loss. These anomalies are observed in the inflammatory zones but also in the skin areas with a clinically normal appearance [46, 58].

It is still not known whether the diminution of the barrier function of the stratum corneum is innate and pre-exists the development of the disease or if it is acquired and is the consequence of chronic cutaneous inflammation. The skin barrier defects observed in AD are considered by some as a primitive, genetically determined abnormality [39, 51]. The murine model of spontaneous AD which develops in NC/Nga Tnd mice argues in favour of this hypothesis, as the animal presents water-retention anomalies as well as altered composition of the epidermal ceramides [1]. However, observation of a normalisation of the transepidermal water loss and the water content of the stratum corneum in AD patients who have been ‘cured’ for several years goes against the idea of an intrinsic anomaly of the cutaneous barrier in AD [32].

Alteration of the barrier function could also be the consequence of cutaneous inflammation linked to the penetration of pneumallergens. One clinical study compared the alteration of the cutaneous barrier at the sites of atopy patch tests with the sites of patch tests to contact hapten, in AD patients who presented with nickel contact dermatitis [17]. This study compared the transepidermal water loss for each patient, on the sites of the two types of tests, with that of normal skin and showed that the barrier function of the epidermis is only decreased on the sites which are positive to pneumallergens. These studies show that the cutaneous inflammation induced by chemical contact allergens (type I T cells) is not responsible for a reduction in the barrier function, even in AD skin, while inflammation induced by contact allergen proteins (type 2 T cells) is able to severely alter the barrier function. Thus one can imagine that the alteration in the skin barrier induced by the pneumallergens increases the quantity of allergens which are able to penetrate, resulting in a vicious circle and perpetuating the eczema lesions.

Conclusions

The penetration of molecules which come into contact with the skin is a phenomenon which depends on numerous factors, but which is possible for small, non-protein chemical molecules as well as for proteins of a high molecular weight. Altogether, recent studies show that the barrier function of the epidermis only plays a small part in sensitization via cutaneous pathways in the main allergic conditions with cutaneous manifestations. This is explained by the very superficial subcorneal localisation of epidermal Langerhans dendritic cells, the principal antigen presenting cell responsible for the uptake of molecules which have penetrated. In allergic contact dermatitis it is accepted that sensitization occurs by epicutaneous pathways resulting in the induction in the lymph nodes of specific T cells capable of returning to the skin and inducing cutaneous lesions typical of eczema at the site of re-exposition to the hapten. In atopic dermatitis, the physiopathological processes are less clear. There is no doubt that eczema lesions can be triggered by cutaneous exposition to pneumallergens as patients develop eczema on contact with pneumallergens applied as patch tests. The possibility that patients can be directly sensitized by cutaneous exposition to pneumallergens is more controversial. Nevertheless, it is possible to immunize an animal with only cutaneous exposure to high molecular weight proteins with the induction of specific IgE and the development of the inflammatory lesions of atopic dermatitis [48]. Studies are in progress to determine if these mechanisms play a role in the development of atopic dermatitis in man and if the cutaneous penetration of allergens is not only responsible for the expression but also the induction of allergic immune responses [18, 53].

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**Announcement**

**Board Certification in Dermatopathology**

The International Board of Dermatopathology will organize under the auspices of the International Committee for Dermatopathology the first Certifying Examination in Dermatopathology (Diploma in Dermatopathology) in Frankfurt/Main, Germany, on December 6, 2003.

Participating Societies: International Society of Dermatopathology
European Society for Dermatopathology
Ibero-Latin American Society of Dermatopathology

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