Exogenous Dermatology
Advances in Skin-Related Allergology, Bioengineering, Pharmacology, and Toxicology

In vitro Approaches to Cutaneous Safety
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A number of alternatives to animal models have been developed in recent years, mainly for the evaluation of non-aic (irritant) contact dermatitis. Unfortunately, due to the lack of relevant models and the complexity of the phenomena involved, several toxic effects such as comedogenicity, altered pigmented function and sensitization cannot be predicted in vitro. Most existing methods for assessing primary irritancy are based on cell culture models using epidermal cells. Several endpoints have been measured, including cytotoxicity and synthesis and release of inflammatory mediators. The cell culture models include conventional cultures of normal and transformed human keratinocytes (immersed monolayers) and three-dimensional cultures (partly immersed models). The latter have an architecture which resembles that in vivo, particularly with the presence of a stratum corneum that provides a partial barrier. In one of these models, EPISKIN, the cytotoxic action of chemical irritants (surfactants) and UV radiation has been tested. A satisfactory correlation was found between the amount of IL-1α released by keratinocytes and the primary irritancy indexes of a large panel of surfactants. Moreover, as described in vivo, solar radiation (UVB and UVA) induced the synthesis and release of IL-1α in this reconstructed epidermis model. Finally, in the case of UVA irradiation, IL-1α release was strongly increased following topical application of phototoxic compounds.

In conclusion, the use of cell culture methods, by a better understanding of the mechanisms of action, would allow us in the near future to predict the skin irritancy potential of topically applied compounds.

Contact Urticaria Today
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Immediate contact reactions of the skin, including contact urticaria, are a heterogenous group of inflammatory reactions that appear within minutes to an hour after contact with the eliciting substance and disappear within a few hours. Itching, tingling, burning and erythema is the weakest type of reaction. Local weal-and-flare is the prototypic reaction of contact urticaria. Sometimes eczema-like reactions are seen especially after repeated contact with the eliciting substance. Generalized urticaria and symptoms from organs other than skin, e.g. rhinitis, conjunctivitis, asthmatic attack and anaphylactic shock, are features of contact urticaria syndrome.
The contact urticaria syndrome is seen mostly in IgE-mediated allergic reactions (immunological immediate contact reactions) and dose-dependent local symptoms are features of immediate-type irritant reactions (nonimmunological immediate contact reactions). Over 200 substances have been reported to be able to cause immediate contact reactions.

Of the substances causing immunologic contact urticaria, the most important and widely studied is natural rubber and the protein allergens which it contains. About 15 allergenic proteins have been found in natural rubber latex sap or in marketed rubber products. Not only rubber proteins but even casein added to some rubber gloves during the manufacturing process may elicit allergic reactions in people allergic to milk.

Textile-Dye Contact Allergens
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The textile industry offers many beautiful fabrics for our use and enjoyment. Some of the chemicals employed, however, may cause dermatitis. The purpose of this presentation will be to present and discuss the chemistry of textile-dye allergens. The chemistry of 9 of the 49 established textile-dye allergens is not known. The remaining dye allergens were organized by application class and then chemical class within the application class. Their structures are provided.

Dye allergens belonged to 5 of 9 application classes with 70% being disperse, 14% basic, 11% acid, and 5% direct and vat. No allergens belonged to the reactive, naphthol, sulfur, or mordant classes. Therefore, dye contact allergens belonged to application classes with moderate to low fastness properties and not to classes with excellent fastness. This finding suggests that availability of dye for transfer from fabric to skin under moist conditions is particularly important.

Dye allergens belonged to each of the dye chemical classes, except diphenylmethane and the tri- and poly-azo groups of the azo class. About 48% of disperse dyes were monoazo, 26% anthraquinone, and 26% azothio-phene, quinophthalone, indigoide, nitrophenylamine, or diazo. Insufficient information was available about dye sensitization potential to allow examination of the chemical structure-sensitization relationship. Researchers are encouraged to perform quantitative structure activity relationships to refine the molecular basis of sensitization of textile dyes.

Clinical Standardization of the TRUE Test™ Formaldehyde Patch
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Formaldehyde is a simple chemical, but a difficult allergen to standardize. The development of the formaldehyde patch of the TRUE test™ involved extensive formulation work to obtain optimal release, stability and clinical function. The best method proved to be the incorporation of the pro-allergen N-hydroxymethylsuccinimide (HMS) in a polyvinylpyrrolidone (PVP) vehicle. Dry HMS gave a stable patch. On contact with the humidity of the skin, HMS instantly cleaves to the carrier succinimide and formaldehyde which penetrates into the skin. This clinical study defines the optimal test dose, and indicates that the test has excellent ability to discriminate between patients sensitive or insensitive to formaldehyde.

Reflectance Spectroscopy of the Cutaneous Corticosteroid Vasoconstriction Assay and Other Cutaneous Pharmacological Tests

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The skin has several advantages as a model for compound testing. Some of the major benefits besides allowing an acceptable human experimental set-up are quick and reliable visual and objective readings. Using both noninvasive bioengineering techniques and visual estimations, the vascular reactions following topical corticosteroids, NSAIDs and anti-histamines were investigated using normal or pre-inflamed skin. Our reflectance spectroscopic analysis technique permits vascular reactions to be resolved into arterial and venous components. Using these refined techniques corticosteroid-induced blanching was predominantly venoconstriction and only the most potent corticosteroid generated a significant decrease in arterial hemoglobin and blood flow, explaining why the blood flow-dependent laser Doppler previously appeared less sensitive. Also UVB inflamed skin and post-occlusive hyperemia were included. Pre-inflamed skin appeared more discriminative and allowed ranking of less potent anti-inflammatory compounds. In conclusion, measured reflectance spectroscopy of the corticosteroid-induced blanching showed predominant venoconstriction and our results may explain why previous attempts to improve the cutaneous vasoconstriction assay using laser Doppler flowmetries have been unsuccessful. Pre-inflamed skin was more discriminative than normal skin but more specific types of skin inflammation mimicking diseased reactions are needed to accurately test the in vivo pharmacological potency.

Percutaneous Absorption: Physical Chemistry Meets the Skin

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The stratum corneum is a fascinating and impressive membrane structure. The manner in which this outermost and least permeable layer of the skin retards molecular transport is a remarkable feat of bioengineering, biochemistry, biophysics and physical chemistry. In particular, the composition and organization of the intercellular lipid domains of the stratum corneum have a profound effect upon its permeability properties to water, drugs and toxic contaminants.
In this paper, our current understanding of the relationship between a chemical’s physicochemical properties and its ability to permeate intact stratum corneum will be reviewed. The experimental evidence (including that from differential scanning calorimetry and infrared spectroscopy studies of stratum corneum), upon which our present comprehension of skin barrier function is based, will be discussed. The development of predictive algorithms for the calculation of percutaneous permeability coefficients, and the application of these models to transdermal drug delivery, and to the prediction of risk following dermal exposure to toxic chemicals, will be described.

Ability is determined by the arrangement of epidermal lipids. To study the skin barrier reactivity to different classes of irritants we used two models. Iritation induced by organic solvents and propanol. A clinical irritation was induced by applying xylene, toluene, propanol and toluene 20% in propanol. The baseline lipid composition and the lipid modifications at different times of observation of each subject was compared to clinical evaluation at 24 h, with transepidermal water loss (TEWL) and colorimetry. The modifications induced on skin by occlusion were used as control. Clinical scoring for toluene- and xylene-induced irritation showed a good inverse correlation with baseline epidermal ceramides. Propanol induced the same correlation between visual score and ceramides.

Irritation induced by surfactants. We induced a clinical irritation by applying sodium sulfate (SLS) 3 and 1% in comparison with distilled water. SLS characteristically induced an increase in TEWL. We correlate the irritation monitored by colorimetry and TEWL with the baseline ceramide composition and with the ceramide variations after irritation. Considering SLS 3%, we found a correlation between erythema and the quantity of ceramide 611 at 24 h and between the percentage of ceramide 1 and TEWL at 24 h.

Influence of Skin Lipid Composition on the Irritation due to Sodium Dodecyl Sulfate and Organic Solvents: An in vivo Study

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Epidermal main function is the elaboration of a stratum corneum envelope able to protect the skin from the dryness and from aggressive agents. The current concept of this skin barrier suggests that its perme-

Effect of Skin Vasodilation on the Components of Laser Doppler Flowmeter Signals
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The effect of histamine and methylxnicotinate on the components of the laser Doppler flowmeter signals was investigated. The vasodilators were applied topically to the arms of 11 healthy subjects. The three signal outputs of the laser Doppler flowmeter (flux, speed and concentration) were recorded and averaged for all the subjects. The percentage change over
baseline was evaluated for the three signal averages. Histamine consistently caused vasodilation as indicated by an increase in the flux signal of 255.6% (p = 0.012), in the concentration signal of 106.8% (p = 0.008) and in the speed signal of 51.5% (p = 0.069). Methylnicotinate caused vasodilation as indicated by an increase in the flux signal of 240.1% (p = 0.071) and in the speed sig-

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nal of 341.8% (p = 0.000), and a decrease in the concentration signal of -35.5% (p = 0.079). The results clearly show that not all vasodilators act on the cutaneous system with the same mechanism. The flux signal alone could not always reflect the variations in the physiology of the cutaneous system as this signal will not change if the speed signal increases and the concentration signal decreases, or vice versa, such that the net effect is to keep the flux signal unchanged. Hence the variations in the speed and concentration signals must be investigated rather than the flux signal alone.

Human Skin Blood Flow Response to Histamine
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To study inherent differences in skin function related to regional variation, we tested the hypothesis that different reactivities of small blood vessels via their direct and indirect activation by the released histamine play an important role in the observed regional variation of contact urticaria and perhaps other mediator-related diseases. Histamine was administered intradermally, thereby bypassing the spatially dependent penetration process. The induced response was quantified with laser Doppler cutaneous blood flow measurements. The extent of response and time parameters were compared in 20 volunteers at three anatomical sites. For comparison, topical administration was also performed. Significant differences in the measured responses at the three sites were observed: the increase in the cutaneous blood flow on the back was greater than the forearm (p < 0.01 prick test, p < 0.05 topical application), and that of both sites was greater than the ankle (p < 0.01 prick test, p < 0.05 topical application). There were no significant differences among the different sites in time parameters and no gender variations. As expected, the time required to reach maximum response was shorter for the intradermal method as compared to the topical application on the back (p < 0.001) and forearm (p < 0.05). On the other hand, the time required to decrease to 50% of the maximum response was not different for the intradermal and topical methods of histamine application. These blood vessel response observations may provide initial insight into inherent functional differences influencing cutaneous manifestations of endogenous and exogenous diseases.

Role of Skin in the Metabolism, Disposition and Toxicities of Topical and Systemic Drugs
Ademola, C. Scrofani, C. Cho, K. Kubota,
The contribution of systemic and topical metabolism to drug detoxification, delivery and toxicity were evaluated. Some of the drugs investigated include propranolol, theophylline, betamethasone 17-valerate, 13-cis retinoic acid and acetaminophen. The resistance of betamethasone 17-ester to skin enzymic hydrolysis (> 99% dose) and the retention of propranolol and its metabolites (between 6.3 and 20% dose) were observed, leading to more potential pronounced reservoir effects, irritation or toxicity following topical application of these drugs. Theophylline and betamethasone 21-valerate were rapidly metabolized by skin, both drugs and their metabolites concentrated rapidly in the epidermis, however, their metabolites diffused rapidly through the skin into receptor fluid (during in vitro studies) or blood (in vivo).

Studies also demonstrated that cutaneous drug-metabolizing activities dramatically increase the permeation of topical drugs; also topical and oral routes influence the metabolizing capacity of the skin and other tissues. In theophylline and betamethasone 17-valerate, topical administration of commercial cream (1%) result in 2-4 times greater total epidermal drug and metabolite concentrations than after oral administration. Although topical application of retinoic acid resulted in a dose-dependent increase in drug delivery; attenuated concentrations in blood after oral administration compared to the topical route were observed, suggesting potentially lower toxicity of topical compared with oral retinoic acid.

Binding studies showed that methoxypsoralen (8-MOP) and its main metabolite exhibited binding affinities higher than those of betamethasone 17-valerate but equivalent to retinoic acid and its metabolites. In 8-MOP the binding affinities were found to be higher in the epidermis (site of action) relative to the dermis (potential site of side effect).

Results from our studies and other investigations suggest differences in the levels of hepatic and cutaneous subcellular metabolism of theophylline, methoxypsoralen and acetaminophen. Correlations between drug metabolites by the human skin, cell culture and organotypic skin model suggest that these metabolites may potentially contribute, in part, to the observed cutaneous biologic effects of the parent drugs.

The principle working objective of Dr. Howard Maibach and myself has been to define percutaneous absorption in terms that are relevant to pharmacology and toxicology. In this endeavor, an in vivo study in human volunteers is the ultimate validation. In the absence of human studies, in vivo animal models such as the rhesus monkey provide data relevant to man for percutaneous absorption. An in vitro diffusion model with human skin can be used, provided that the diffusion system is not rate limiting (for example, a lack of chemical solubility in perfusion fluid). Percutaneous absorption is further defined by factors such as regional variation and multiple dosing, which bring laboratory data closer to the clinical
situation. The clinical situation can involve age, an infant who may be more susceptible to chemical bioavailability, or the elderly who command an ever-increasing need for medical aid. This latter situation often involves diseased skin, which lacks an effective model system. Surprisingly, in vivo studies in skin-diseased patients show there is not as great a loss of skin barrier property as assumed. Technology has entered the percutaneous absorption area with the likes of transdermal delivery (and the balance between skin drug delivery and skin irritation) and marvelous instruments to measure laser Doppler velocimetry and transepidermal water loss. All of these studies are filtered through regulatory agencies for final stamp of approval. This leads to our philosophy that the answers to questions are speculative until you get the data – just do it.

12 Assessment of Skin Delipidization as an Enhancing Factor in Percutaneous Penetration
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Polar solvent or active surfactant extraction of polar lipids from the stratum corneum (skin delipidization) produces perturbation of the cutaneous barrier. Through perturbed delipidized skins, the permeation of chemicals is enhanced, provided that the absorption induced is significant prior to barrier reactivation by the intrinsic biosynthetic processes of accelerated lipid production (skin lipogenesis). The resulting increased permeability coefficient was correlated schematically to the altered percutaneous penetration process. The effects of skin delipidization, specifically by organic solvents, were assessed both in vivo and in vitro. The pharmacodynamic measurements conducted on estrogen and lidocaine proved unequivocally accelerated absorption. In humans the pharmacokinetic evaluation of delipidized skin absorption of hydrocortisone formulated in a cream indicated that its slow rate of cutaneous permeation could not have been enhanced owing to the obstructive effect of the oily cream base and the ongoing restoration of the barrier function by the accelerated lipogenesis. The reviewed in vitro estimations depicted the appreciably enhanced percutaneous resorption of 5-fluorouracil, salicylic acid and salicylamide. These permeants exhibit low to moderate lipophilicities. The percutaneous penetration of the highly lipophilic and permeable dinitrochlorobenzene was not enhanced. Mild lipid depletion of the cutaneous barrier by incorporated ethanol effectively increased the absorption of β-estradiol.

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Patch testing for contact sensitization is still a primitive procedure – more art than science. Shibboleths flourish. Anyone can do a patch test – usually badly.

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Momentous, irksome problems remain. These include: (1) differentiation of allergic from irritant reaction; (2) clinical relevance of positive reactions; (3) influence of patch test devices and their sizes; (4) dosage-response relationships; (5) mythologies of the ‘angry back’ phenomenon, and (6) multicentered studies – a hoax?
The contact dermatitis societies need to match the quality of their investigations with their high-class recreational activities (read meetings).

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Contact Allergy: Animal Models
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Predictive contact allergenicity testing started nearly 50 years ago with the first publication of the Draize test [1]. Not much was known about the mechanism of induction and elicitation of allergic reactions. Further development had two different directions. Toxicologists tried to increase the sensitivity of the animal methods to improve the prediction of the contact allergenic potential of chemicals in animals and the risk evaluation for man. A large number of predictive tests had been developed [2, 3] and 7 methods were included in the guidelines of OECD and EEC. On the other hand, immunologists tried to get more information on the basic mechanisms of contact allergic reactions.

As a toxicologist one can just follow the guidelines to fulfill the requirements for registration or one tries to include some of the new knowledge in immunology for better risk assessment. Such testing will need more than one test and will need protocols adapted to the special requirement for the compound, e.g. for trans-dermal therapeutic systems. In the presentation, examples will be given on how standard protocols can be used for special purposes and what influences on induction and challenge must be considered. The changes of OECD and EEC regulations will also be reviewed.


Surfactant-Induced Skin Dryness
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Human skin irritation tests of surfactant-based consumer products are usually evaluated by visual and tactile scores for, e.g., erythema, edema, and dryness. Recent advancements in the instrumental evaluation of skin physiology now permit an objective assessment of skin irritation. Subtle subclinical changes in the integrity of the skin, not accessible to the human eye, can now be quantified. The influence of surfactants on stratum corneum hydration has been controversially discussed in past reports. This is probably due to a complex bidirectional response of stratum corneum hydration following surfactant exposure. Thus we have demonstrated that surfactants may increase or decrease the water content of the stratum corneum, depending on the exposure condition and on the observation time. The boundary conditions of the influence of surfactants on stratum corneum hydration will be discussed.

Platelets and Chemokines
During the last years a new family of polypeptides has been detected which is referred to as chemokines due to their chemotactic activity on monocytes, memory T cells and eosinophils. Members of this family are among others interleukin-8 (IL-8), neutrophile-activating peptide (NAP-2), platelet factor-4 (PF-4), macrophage inflammatory protein (MIP-1α), β-thromboglobulin (β-TG) and RANTES (Regulated upon Activation, Normal T-cell Expressed and Presumably Secreted). We could show by means of immunoelectron microscopy that PF4, MIP1α, β-TG and RANTES are localized in the platelets within the same storage compartment, the α-granules (RANTES antibodies were kindly provided by J.-M. Schroder, PhD, Department of Dermatology, University of Kiel, Germany; immunoelectron microscopy was performed by M. Klinger, MD, Institute for Anatomy, University of Lübeck, Germany). After platelet activation by different stimuli such as thrombin or lipopolysaccharide, the contents of these granules are released at different rates and in different amounts. We could even demonstrate that interindividual variances in the released amount of PF4, β-TG and RANTES exist. Since these substances play an important role in chronic inflammation and allergic diseases, it is tempting to speculate about an involvement of platelets in these reactions. These findings also provide a new step towards the understanding of hypersensitivity reactions after platelet concentrate substitution in transfusion medicine.

Effects of Multilamellar Vesicles on the Disruption of Stratum Corneum Lipid Barrier in Hairless Mice

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Stratum corneum (SC) lipids play an important role in the permeability barrier and water content of the skin. Its water-holding function depends mainly on the lamellar structure composed of these lipids, but it is still not well known whether this structure is correlated with the permeability barrier. In order to investigate the effect of multilamellar vesicles having analogy to the lamellar structure of SC lipids on the disruption of the SC lipid barrier induced by acetone, the permeability barrier by transepidermal water loss (TEWL), and the water content by capacity and morphologic changes in SC lipids by electron microscopy using ruthenium tetroxide fixation were evaluated in the skin of hairless mice under various conditions including: distilled water-treated control (groups I and V); application of acetone (groups II and VI); application of acetone and multilamellar vesicles (groups III and VII), and application of acetone and vehicle (groups IV and VIII) for the air-exposed and paraffilm-occluded groups. The results were as follows. (1) Air exposure in group III had an accelerated effect on the recovery of increased TEWL and decreased capacity than in group II, and lamellar bilayers were not found in group II at 6 h after treatment. (2) Paraffilm occlusion in group VII had a significant effect in the recovery of increased TEWL and decreased capacity than in groups VI and VIII, and more lamellar bilayers were seen in the SC at 24 h after treatment than in group VI. (3) Occlusion significantly increased TEWL and had a tendency to slower and worsened formation of lipid bilayers.
Taken together, these results suggest that lamellar structure consisting of SC lipids may play a role in the permeability barrier as well as in the water content on the disruption of barrier.

Percutaneous Absorption of a Drug into Hair Follicles
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It is very important for drug delivery to analyze the percutaneous absorption of a drug into the target organ. There are only a few investigations about the percutaneous absorption of dermatological drugs into the target organs in skin.

With regard to the investigation on the absorption of a drug into hair follicles, the usual separation methods by simple plucking possibly bring the hair follicles into contact with the epidermis. It contains a much larger amount of the drug thus the hair follicles become contaminated with the drug. The contamination can be avoided by our method in which the hair follicles were plucked using a hot (more than 100°C) depilatory wax. This peels the epidermis together with the wax.

Percutaneous absorption of glyceryl monopentadecanoate (PDG, hair-growing agent) into hair follicles in vitro and in vivo was investigated using this method. (1) The quantity of PDG in the hair follicles (Qf) increased dose dependently. (2) The increasing rate was higher in vitro than that in vivo. (3) In vitro the Qf linearly increased until 48 h. (4) In vivo the Qf did not increase after 5 h. (5) In vivo the Qf decreased exponentially after removal of the unabsorbed drug from the skin surface.

The lower quantity and the rapid decrease in PDG in vivo may result from the active elimination by blood flow or metabolism in viable hair follicles. These findings will help to develop an effective drug preparation.

Scabies 1994
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The scabies pandemic continues. Scabies presents itself in very special (atypical) forms including: scabies in infancy; scabies in the elderly; animal-transmitted scabies; urticarial and vasculitic scabies; crusted (Norwegian) scabies; dyshidrosiform sebidiid; bullous scabies, and scabies of the scalp.

The subject of scabies in HIV/AIDS will be presented in some detail. HIV/AIDS will be the most common disease triggering crusted (Norwegian) scabies. Scabies becomes more atypical as the patient is progressively immunosuppressed. The diagnosis of scabies may not be made for months to several years. Patients with HIV/AIDS, when hospitalized, are not uncommonly the source of hospital epidemics of ordinary scabies. Laboratory diagnosis (including some newer techniques), immunology (cell-mediated immune reactions dominate), and epidemiology will be presented. Discussion of differential diagnosis will be emphasized.
The next segment will be on therapeutic agents, including suggestions for their use. These agents include: (1) permethrin; (2) lindane 1% lotion; (3) precipitated sulfur (6%), and (4) oral ivermectin (undergoing study at this time).

Therapeutic failure will be separated from tolerance (resistance) and reinfection.

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Correlation of in vivo and in vitro Percutaneous Absorption with a Mathematical Model
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A database was established using in vivo and in vitro dermal penetration data obtained from phenols, steroids and fragrance chemicals for human skin. The data include flux measurements ($J_{avg}$, $J_{max}$), derived permeation coefficients, and those estimated from a modified Potts-Guy relation. These values were subjected to cross comparison and to individual correlations with mechanistic and clinical descriptors. Descriptors include: MR (polarizable volume); log $P(0/W)$; H-bond acceptor count (HBA); H-bond donor count (HBD), and the degree of occlusion for the in vivo studies. Multiple regression studies were used to correlate all sets of data.

$K_{p_{max}}$ (human in vivo) derived from $J_{max}$ correlates strongly with in vitro log $K_p$ for semi-occluded experiments ($r = 0.832$) and not at all for occluded experiments ($r = 0.114$). Log $J_{max}$ (in vivo) correlates better with estimated log $K_p$ (in vitro; $r = 0.778$) than log $J_{avg}$ ($r = 0.574$). This is expected as $J_{max}$ is a better approximation of steady-state flux than $J_{avg}$.

In QSAR studies, log $J_{max}$ correlates with MR, log $P(0/W)$ and the degree of occlusion ($n = 34$, $r = 0.857$, $F = 27.57$). Occlusion has a strong positive effect on log $J_{max}$.

One of the key conclusions from this study is the need to rank the degree of occlusion in order to compare in vivo and in vitro data effectively. Percutaneous absorption, as measured by the finite dose technique of Feldman and Maibach, is the result of complex factors; data do not directly correlate with simple measurements or calculations of log $K_p$.

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Four Decades of Topical Corticosteroid Assessment
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There is no doubt that the clinical introduction of hydrocortisone in 1952 revolutionised the clinical management of dermatological conditions. Several classes and potencies of topical corticosteroids were researched and developed in the ensuing two decades. Together with the development of the drug entities, assessment methods to test the potency and clinical efficacy of the drug congeners had to be improved. Early in vivo assay systems employed were often invasive or traumatic and would probably not be acceptable by the code of ethical standards universally accepted today. These assessments included non-immunological and
immunologically based inflammation tests, assessment of antimitotic effects, response to
drug application in disease states and the assessment of adverse effects following drug application. The one in vivo assessment method for corticoids that was first reported in 1957 and is still widely used today is the skin blanching or vasoconstriction assay. Over the last 30 years, the basic methodology of McKenzie and Stoughton has been practised in many forms, not all of which are accepted as ideal. The major criticism of the assay has been the subjective nature of the visual assessments. The applicability of several instrumental methods has been investigated in this regard: currently the reflectance chromameter appears to be the instrument most likely to augment visual grading and provide more meaningful statistical analysis of the results.

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Irritation Potential in Transdermal Drug Delivery
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Skin irritation that occurs during transdermal delivery has been an obstacle to the
development of many transdermal systems. Irritation can be caused by the system or it can be
the result of mass transport. Irritation due to the system is usually minor and appears to be the
result of occlusion or mechanical factors. These can be minimized by selecting the proper
system configuration and application site. Mass transport-induced irritation can involve the
drug, the permeation enhancer, the adhesive, or the excipients. Irritation not linked to the
drug generally can be decreased by proper selection of system components or drug
formulation. Transdermal electrotransport delivery is a special case in which ionic flux may
cause irritation. Adjustment of electrical parameters may minimize this problem. Reduction
of irritation caused by drugs and other permeating species can be accomplished by
controlling flux, or local skin concentrations of the compounds. The former can be effectively
achieved using rate-controlling membranes. Such membranes also result in a reduction in
inter-subject flux variability and maintenance of zero-order delivery. Local skin
concentrations can be altered by co-delivery of vasoactive agents. An understanding of the
irritation mechanism(s) of drugs can also lead to inhibition strategies. Using in vitro models,
we have demonstrated that a decrease in the cytotoxic and irritation potential of several
amine-containing drugs could be accomplished
by modifying their accumulation gradients within the cell. Therefore, strategies can be
developed to mitigate skin irritation occurring during transdermal delivery.

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Transdermal Iontophoresis: Effect of Penetration Enhancer and Iontophoresis on Drug
Transport and Surface Characteristics of Human Epidermis
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The possible synergistic effect of a chemical penetration enhancer and iontophoresis on the
transdermal transport of drugs has only been minimally explored. The exact mechanism by
which most penetration enhancers work is largely unknown. The visualization of the changes
on the surface of human skin due to the application of these penetration enhancers, either
alone or in combination, on iontophoresis may help in predicting the possible mechanism of
their action. In this study, the effect of penetration enhancers and iontophoresis on the in vitro
transport of methotrexate (MTX) and the surface characteristics of the human epidermis have
been investigated. The penetration enhancers, e.g., dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), dimethyl acetamide (DMA) and Azone® increased the passive diffusion of MTX across human epidermis. Transport was further increased when iontophoresis was used in combination with these enhancers. The penetration enhancers in conjunction with iontophoresis enhanced the flux of MTX in the following order: Azone® > DMA > DMF > DMSO.

Scanning electron microscopy of the skin samples treated with these enhancers showed that DMSO, DMF and DMA acted predominantly by swelling the cells. Azone® acted differently by increasing the intercellular space. Iontophoresis in combination with Azone® further increased the intercellular space.

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Prediction of Drug Concentration in Blood after Topical Application
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Data published on the drug permeability of shed snake skin, hairless mouse skin and human cadaver skin were compared with permeability coefficients for the same substances calculated as a function of molecular weight and partition coefficient. The permeability coefficients of drugs having a partition coefficient in the region of 1.5 were similar between the shed snake skin and human skin. Hairless mouse skin on the other hand had permeability coefficients ten times greater than those of human skin. This suggests that snake skin could be used to predict permeability in relation to human skin of drugs with a partition coefficient of between 1 and 2. Following this, a computer program to predict the blood concentration of drugs after topical application was developed using the convolution method with data from an in vitro experiment and pharmacokinetic parameters obtained through intravenous administration. Predicted blood concentrations were found to agree well with results from actual tests in which Frandol® tape-S (isosorbide dinitrate) was used as a model transdermal patch. It would thus seem that this new program will be useful in predicting drug concentrations in the bloodstream and will be a valuable tool in developing new transdermal patches.

amongst Propionibacteria and, to a lesser degree, amongst Micrococcaceae.

In the present investigation, topical therapy with erythromycin over at least 8 weeks was found to lead to resistance of Propionibacteria in 44% of cases, and with clindamycin in 31% of cases. Resistance to tetracycline (14%) or minocycline (4%) was much more rare. To date, we have found no case of resistance to chloramphenicol. All subjects, including the untreated control group, showed resistance to metronidazol (100%). In up to 13% of erythromycin-resistant patients, cross-resistance against clindamycin developed with no prior treatment.

The incidence of resistance amongst Micrococcaceae after 8 weeks of treatment was much higher: 100% for erythromycin; 64% for clindamycin, and 87% for tetracycline. By means of concomitant treatment with local antiseptics, e.g. benzoyl peroxide, it is possible to inhibit
the development of resistance and to achieve an inhibition of cell proliferation in the sebaceous glands.

In contrast to current opinion, antibiotic resistance in Propionibacteria and Micrococcaceae does not seem to be spontaneously reversible, since 5.5 months after cessation of therapy, 83% of patients still showed resistant Propionibacteria and 100% resistant Micrococcaceae. We were able to detect a relationship between resistance in Micrococcaceae and Propionibacteria in our investigations. The results would appear to support the conjecture that the rapidly inherited antibiotic resistance of the Micrococcaceae may be genetically transferable to the Propionibacteria, and thus complicate the antibiotic treatment of acne.

25 Antibiotic Resistance amongst Propionibacteria and Micrococcaceae in Systemically and Topically Treated Patients with Acne Compared to an Untreated Control Group

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Since many lines of evidence indicate an effect of Propionibacteria on acne pathogenesis, antibiotics are used both systemically and topically in acne therapy. A significant factor in the efficiency of an antibiotic in this context is the frequency of resistance induction.

26 Facilitated Drug Delivery during Transdermal Iontophoresis

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Transdermal iontophoresis (TI) may be defined as the transport of solutes across the skin under an appropriate electrical potential gradient. For low molecular solutes, TI effectively overcomes the stratum corneum barrier so that solute clearance by the local cutaneous microcirculation may in some cases become the limiting factor. The presence of an intact dermal microcirculation in experimental models used to study iontophoretic transport may be desirable. Low levels of electric current induce local vasodilatation at the site of electrode application. Strategies may be designed to maximize either systemic or local drug delivery by TI.

27 Skin Irritation

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Irritant contact dermatitis is generally accepted to be a multifactorial disease and may be classified in several distinct syndromes. Contact with irritants may lead to different cutaneous symptoms. Clinical expression may vary with the presence of enhancing or suppressing factors. Primary irritants have also different mechanisms in inducing cell damage and irritation thus contributing to the wide variation in clinical expression. Different mechanisms and mediators of inflammation stay behind the several patterns of irritant dermatitis. Individual skin susceptibility, mostly related to the efficacy of the skin barrier, is an important factor determining the onset of irritant contact dermatitis and results as a combination of endogenous and exogenous variables. Bioengineering techniques help in
investigating and quantifying these mechanisms allowing the identification of high-risk individuals.

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Skin and Organic Solvents
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Occupational handling of organic solvents frequently involves skin contact. Due to their lipophilic nature the solvents delipidize the skin and occasional skin contact may induce dryness of the skin. If contact is continued and appropriate occupational hygienic procedures are not implemented, these light skin changes may progress erythema and edema which develops into nonallergic contact eczema. Clinical experience shows that extensive skin exposure to organic solvents will result in irritative contact dermatitis usually followed by long periods of sick leave before using.

A few solvents have been reported to have skin-sensitizing properties. Among those balsam turpentine is best known from clinical experience. Recently, however, in experimental studies, another terpene, limo-nene, has been shown to have potent sensitizing properties after air oxidation.

The major route of entry for solvents into the body during occupational exposure is via the respiratory tract. However, due to their lipophilicity several solvents are also readily absorbed into the lipid domain of the epidermis and diffuse down through the epidermis to be absorbed into the systemic circulation. This exposure may be the major route of entry for some solvents, thus presenting a serious occupational hazard. Particularly under extreme work or ambient conditions. Skin absorption may also be an additional route adding to the total body burden acquired via respiratory absorption. Some solvents are known to enhance percutaneous absorption of compounds dissolved in them. Exposure to such solutions may be hazardous.

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Immunomodulation of Contact Dermatitis
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Contact dermatitis, allergic (ACD) or irritant (ICD), is a readily inducible inflammatory skin disorder, providing a useful model for the study of compounds affecting the immune system. We used it as a model for the study of a novel immunosuppressant, tacrolimus (FK506), as a possible topical treatment for skin inflammation. Hairless guinea pigs were chosen as animal models. FK506 was applied topically either before, during or after elicitation of ACD to dinitro-chlorobenzene (DNCB). FK506 suppressed ACD, especially when skin sites were pretreated with it. This finding is consistent with the earlier in vitro findings that FK506 has its most prominent suppressive effects on the early activation genes of T lymphocytes. Topical FK506 was also able to suppress ICD, suggesting that immune responses also play a key role in the pathogen-
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esis of ICD. The results of this study provide further evidence that topical FK506, which also seems to be effective topically in humans [Lancet 1992;340:556], may be an immunosuppressant that can be used locally for inflammatory skin diseases. Further, the efficacy of FK506, a drug acting narrowly on immune responses shows that both ACD and ICD are predominantly immune diseases. Additionally, the study shows the strength of contact dermatitis as a model for the study of immunomodulation in the skin.

The Allergen Bank: The Idea behind It and the Preliminary Experience with It

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The allergen bank was established to give dermatologists easy access to special test materials in order to make it possible for them to diagnose early special cases of allergic contact dermatitis. The allergen bank comprises a computer system to register patch test results and about 540 contact allergens in appropriate patch test concentrations available at the allergy laboratory. The allergen bank may supply to dermatologists in practice special contact allergens on request for aimed patch testing of contact dermatitis patients. The organization of the allergen bank and the procedure of use is described. During the first 23 months of use 28 dermatologists have asked for 2,209 allergen samples for testing 386 patients, an average of 6 allergens/patient and 14 patient/dermatologist (range 1-162). A total number of 164 positive reactions have been registered (7.4%), and 440 of the 540 allergens (81%) have been in use. One third of the positive reactions (51/164) were caused by the 16 most frequently ordered allergens, which amounted to 340 allergen samples. The allergens included plant chemicals, acrylates, animal feed additives, fragrance chemicals and preservatives. The relevance of the reactions in relation to the actual dermatitis of the patients is not yet evaluated. Among the rare allergens giving the highest yield of positive reactions were: sodium omadine, tripropyl-ene glycol diacrylate, chloroacetamide, Eusolex 6300 and diethyl thiourea, all requested only a few times.

Protective Gloves

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For human, social, and economic reasons, it would be of great benefit if people exposed to harmful chemicals and products could be protected from developing contact dermatitis. The multiple prophylactic means available can be grouped under subheadings: chemicals, including identification of the allergen and predictive testing; individuals identified at pre-employment examination; avoidance of direct contact with products and materials, i.e. protective gloves; skin care program, and miscellaneous, including legislation, labelling, safety sheets, etc. The use of protective gloves is then one of several possibilities to avoid developing a contact dermatitis or a relapse. The best results are achieved when several of the prophylactic means are combined in a wise and fruitful way.
Current protective gloves are not perfect. Some are permeable to various chemicals and do not provide the promised protection. Their protective effect can be tested in vivo (man, experimental animals) and in vitro. Glove test data are compiled in a database (DAISY) at our Department. Side effects, such as irri-tancy, contact urticaria from latex and allergic contact dermatitis from rubber chemicals, are common and are sometimes reasons for discontinuance of their use by patients and exposed workers. Gloves that will give more efficient protection and less side effects are then highly desirable.

Keratinocyte Monolayer Culture System and Its Application in Skin Irritancy
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An in vitro technique of evaluating skin irritancy, a cell culture method, has been gradually developed, initially using fibroblasts and later on other cells including normal human keratinocytes. The monolayer culture system of normal human keratinocytes seems to be an ideal cell for evaluating skin irritancy because irritants contacted with keratinocytes first in skin. Whether this in vitro keratinocyte culture system can replace in vivo methods is an interesting subject.
Several cytotoxic studies have been performed including MTT and lactic dehydrogenase assay, and these data were compared with in vivo human patch test responses measured by laser Doppler flowmetry. Cytotoxicity studies using oral keratinocyte culture were also compared with skin keratinocytes.
In conclusion, the immersed cell culture method cannot directly replace in vivo methods and data obtained by the cell cultures should be interpreted carefully.

Polar Pathway for Percutaneous Absorption
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Passive transport of ions across stratum corneum is still a subject of controversy. The mechanism of permeation based on partitioning and diffusion in the intercellular lipid domain of the stratum corneum is well established, but this model does not explain the phenomenon of transcutaneous, passive or iontophoretically enhanced transport of ions. Amino acids, ionized in the whole range of pH, are good models to investigate permeation of skin to ions. In vitro passive transport of lysine, aspartic acid and histidine from aqueous solutions was observed. Permeability coefficients $1.2-4.7 \times 10^{-8}$ cm s$^{-1}$ were independent of the degree of ionization. Long T $\approx$ 20-30 h indicates that intact stratum corneum, not skin appendages, is the site of the ionic transport. Flux of aspartic acid from 30% ethanol was 20-fold smaller and flux of histidine was comparable to that from water. This cannot be expected if the partitioning mechanism is obtained; an increase in transport would have been expected since ethanol reduces the solubility of amino acids by an order of magnitude. A porous mechanism of membrane transport is then proposed. Further evidence for this results from the experiment where permeation of lysine and histidine from hyper-tonic solutions was studied and permeation of lysine was substantially reduced under these conditions.
Further extensive studies are required to characterize the pores in stratum corneum, but one can expect that they are formed by tortuous microchannels, probably situated intracellularly, and filled with water or an other hydrophilic solvent present in the vehicle.

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The Pityrosporum Yeasts and Skin Diseases: A Review
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Pityrosporum ovale is a lipophilic yeast belonging to the normal human cutaneous flora. It is also an opportunistic pathogen associated with: pityriasis versicolor Pityrosporum folliculitis, seborrhoeic dermatitis and some forms of atopic dermatitis. In pityriasis versicolor P. ovale changes from the blastospore to the mycelial form under the influence of predisposing factors. The great problem is recurrence and to avoid this prophylactic treatment is mandatory. Pityrosporum folliculitis is a chronic disease characterized by pruritic follicular papules and pustules located primarily on the upper trunk, neck and upper arms. Under the influence of predisposing factors P. ovale increase in numbers in the hair follicles. The main differential diagnosis is acne vulgaris. The effect of antifungal treatment is often dramatic. There are now many studies indicating that P. ovale plays an important role in seborrhoeic dermatitis. Many of these are treatment studies showing a good effect of antimycotics paralleled by a reduction in the number of P. ovale. Severe seborrhoeic dermatitis which is often difficult to treat is associated with AIDS. In a recent study we have evidence of a slight T-cell defect in many patients with seborrhoeic dermatitis. Adult patients with atopic dermatitis located on the head, neck and scalp are in 75-80% prick test positive to a P. ovale antigen and they respond much better to antifungal therapy in combination with corticosteroids than to corticosteroids alone.

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