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Invited Review

Vehicular influence on transdermal drug penetration

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Key words: Lipid structure; Stratum corneum; Concentration profile; Drug solubility; Penetration mechanism; Penetration enhancement; Ointment base; Capillary action

Summary

The realization of the composition and structure of the intercellular multiple bilayers of the horny layer lipids has created a basis for understanding the diffusive control of transdermal xenobiotic permeation and the enhancing effects of vehicles and other ingredients added to dermal preparations. The measurement of concentration profiles in the stratum corneum and deeper skin layers yields insight into the transdermal permeation mechanisms. Lipid solubility is a predominant criterion for the transdermal permeation of substances which enter via the lipid route. Applying lipid vehicles, liquid components carrying dissolved drug substances enter the intercellular spaces of the stratum corneum disjunctum by spreading and by capillary action. Drugs and certain vehicle constituents diffuse into the stratum corneum conjunctum and deeper skin layers. There, they lower the diffusion resistance of the horny layer by interfering with the molecular packing of the dermal lipids and by disturbing their order. Furthermore, the dissolving power of the horny layer lipids may be changed.

Introduction

For almost a century, it has been known that transdermal drug absorption is influenced by the vehicle in which the applied drug is incorporated (Bourget, 1893). Furthermore, the use of certain substances which are able to enhance transdermal drug penetration has been practised for a long time (Ritschel, 1973). However, it is only during the course of the last decade that an awareness of the mechanisms that control these effects has developed. It follows from reasons of diffusion kinetics in multilayered systems that the diffusion flux is limited by that region having the highest diffusion resistance, which is characterized by the steepest concentration gradient occurring in a steady-state system (Fig. 1). Many experiments have shown that the intact stratum corneum is the barrier restricting skin permeability (Berenson and Burch, 1951; Blank, 1953; Monash, 1957; Malkinson, 1958). To understand this fact, the histological and histochemical structure of the horny layer has to be taken into consideration.

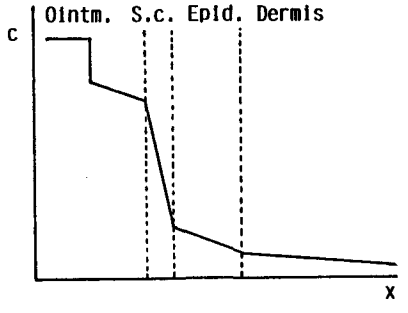

Fig. 1. Schematic concentration profile of a drug diffusing from a suspension ointment into the skin.

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Composition and Structure of Horny Layer Lipids

Round about 1980, several papers concerning the composition of horny layer lipids were published, for example, by Gray et al. (1982), Elias (see Lampe et al., 1983), Yardley (1983), Nugteren (see Bowser et al., 1985), Bowser and White (1985) and Wertz (1986). Summarizing these investigations, the lipid components of human abdominal horny layer are shown in Table 1. Special interest is demanded by the sphingolipids, a group of substances to which the acylceramides belong. By reason of their chain lengths, they play an important role in stabilizing the lipid layer structure (Wertz and Downing, 1983).

Despite the melting point depression of mixtures, the horny layer lipid phase which consists of a great number of components shows endothermic phase transitions at the relatively high temperatures of 72 and 85 °C measured by DSC. They were interpreted as melting processes or as transitions from the liquid-crystalline to the isotropic state by van Duzee (1975) and Barry (1987). These high melting temperatures indicate that the lipids of the horny layer are arranged with a high degree of order and in a high density (Fig. 2). They form multiple bilayers which are further stabilized by the above-mentioned acylceramides having a chain length long enough to connect two bilayers like a rivet. In addition, the intercellular lipids are probably attached to the envelopes of the horny cells by lipoproteins which contain ceramides (Wertz et al., 1989). The diffusion resistance of the stratum corneum, therefore, comes close to that of solids.

TABLE 1

Composition of the horny layer lipids of human abdominal skin (Lampe et al., 1983)

Polar lipids: (4.9%)	phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, sphingomyelin, lysolecithin
Neutral lipids: (74.8%)	free sterols (14%), free fatty acids (19.3%), triglycerides (25.2%), sterol and wax esters (5.4%), squalene (4.8%), *n*-alkanes (6.1%)
Sphingolipids: (18.1%)	ceramides I (13.8%), ceramides II (4.3%), glucosylceramides I and II (traces)
Cholesterol sulphate (1.5%)	

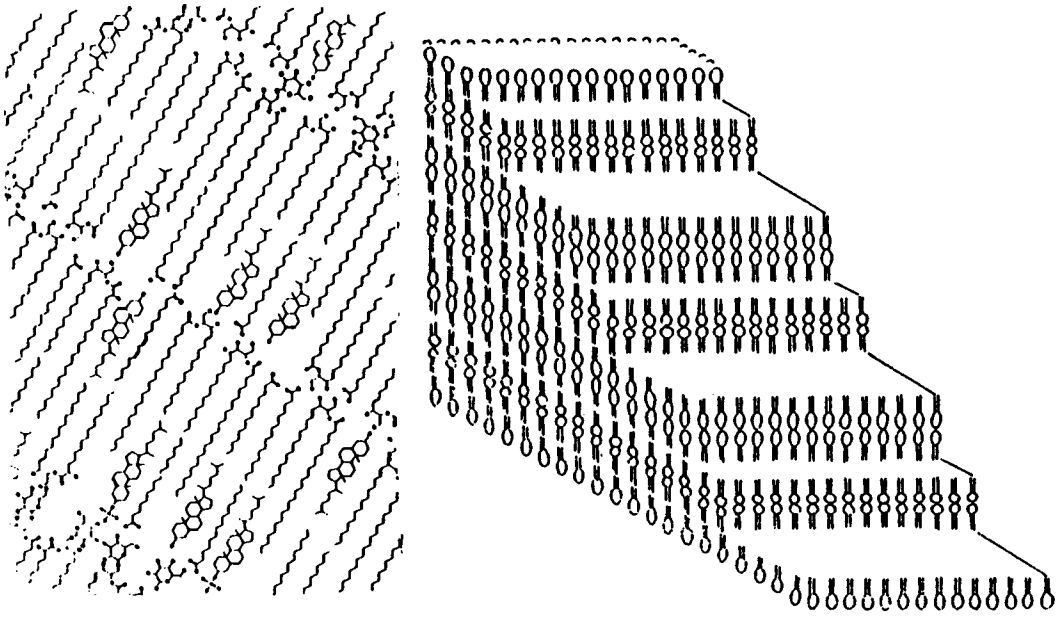

Fig. 2. Scheme of multiple bilayers of the horny layer lipids (according to Wertz (1986) and Landmann (1986)).

The formation of these structures is part of the differentiation processes in the stratum granulosum, where the so-called membrane-coating granules extrude their contents into the intercellular spaces (Fig. 3). There, accompanied by biochemical modifications, the lipid structures are rearranged to form the multiple lipid bilayers of the stratum corneum. In 1981, Elias and Elias et al., supported by some convincing facts, suggested that this intercellular lipid domain is the favoured pathway for xenobiotic penetration. Further evidence may be given by the following observations:

(1) Using hydrophilic tracer techniques, Squier (1973), Squier and Rooney (1976) and Elias and Friend (1975) observed a zone between the upper stratum granulosum and the upper stratum corneum that was free of tracer.
(2) Disturbed lipid layer structure (for instance, because of the deficiency of linoleic acid) enhances the permeability (Elias et al., 1980; Bowser et al., 1985).
(3) Lipid solubility is a prerequisite for a good permeation through the horny layer.
(4) Certain lipid compounds are able to enhance transdermal absorption.

On the other hand, there is a general experience that the stratum corneum can incorporate water under swelling and that the permeability of drugs depends on the degree of hydration. Further, some substances having considerable polarities also enhance the permeability of the horny layer (Barry, 1987). Hence, the question may arise as to whether or not these facts are in accordance with the lipid pathway. Indeed, the effective mechanisms are

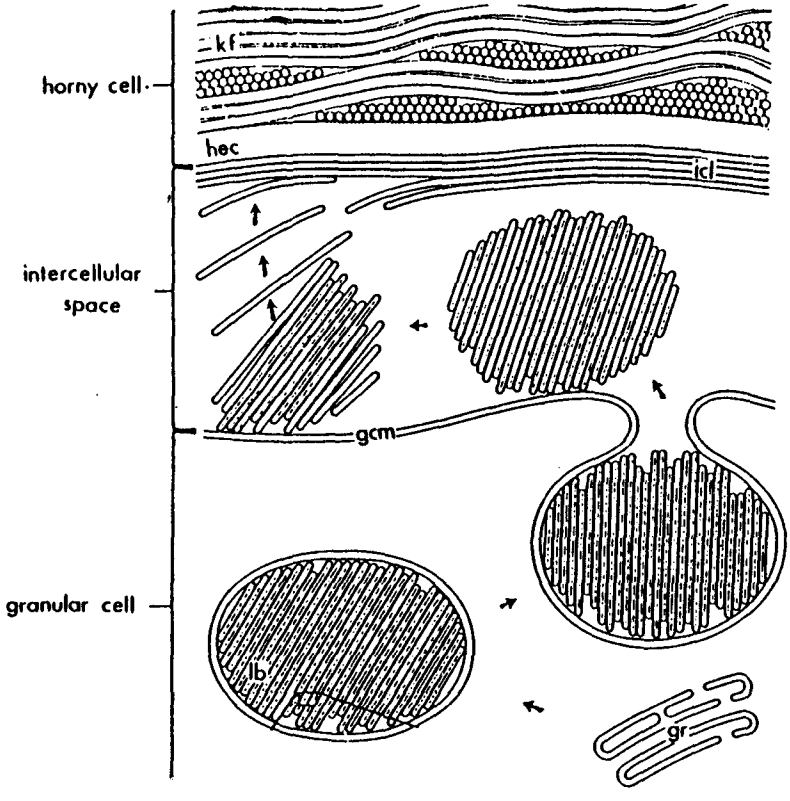

Fig. 3. Formation of the intercellular lipid domain by lipid extrusion from membrane coating granules into intercellular spaces of the stratum granulosum (Wertz and Downing, 1982).

closely linked with the alternating arrangement of non-polar and polar laminae that characterizes the multiple bilayer structure (Fig. 4):

(1) The water molecules can hydrate the polar head groups of the lipids forming thinner or thicker films between the lipid bilayers.

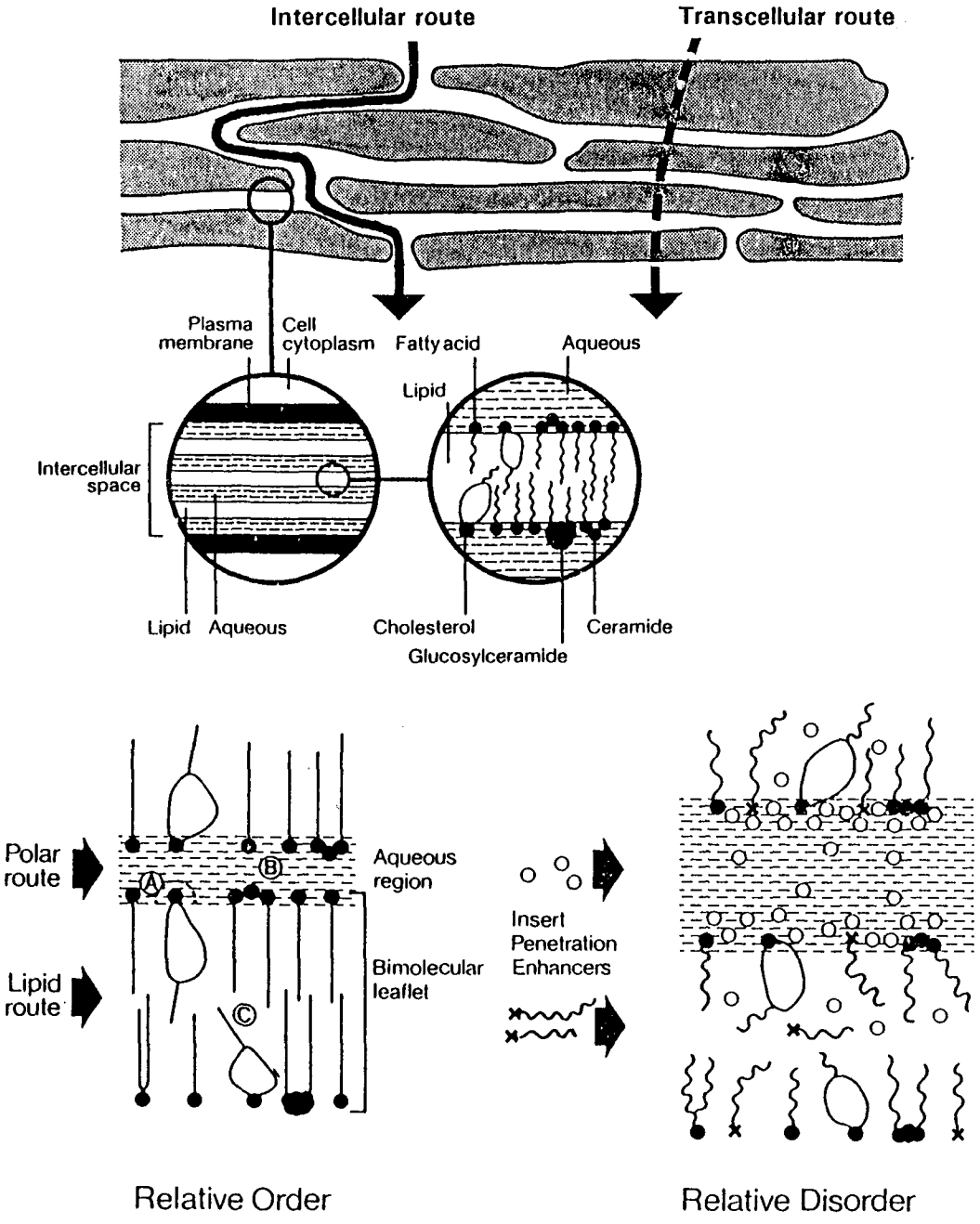

Fig. 4. Polar and lipid routes through the intercellular lipid domain (Barry, 1987).

(2) Polar penetration enhancers also interact with the polar head groups of the lipids influencing their hydration and the relative order of molecular packing.

The lipid penetration enhancers are effective in an analogous way by being intercalated into the non-polar region of the bilayers and by disturbing their molecular packing. As a prerequisite, these substances have to be able to diffuse into the lipid layers of the whole stratum corneum in such an amount that the diffusion resistance is markedly reduced. As a result, the concentration of a penetrating drug in the skin increases. Further, the solubility of the drugs in the horny layer lipids may be changed more or less. Therefore, drug concentration profiles in the human skin can be used to indicate the efficiency of penetration enhancers.

Influence of Ointment Bases and Enhancers on Drug Permeation through the Horny Layer

To verify this last mentioned concept, Blasius (1985) used indomethacin as a model drug, penetrating from an ointment into human skin. The following ointment bases have been applied: Vaseline, a hydrocarbon gel according to the German Pharmacopoeia, equivalent to soft paraffin or petrolatum; wool alcohol ointment, consisting of Vaseline and wool wax alcohols also according to the German Pharmacopoeia; semisolid triglycerides, a preparation with the trade name Softisan 378 (Dynamit Nobel AG, Troisdorf-Oberlar, Germany). First, to choose a suitable ointment base, indomethacin was incorporated into Vaseline and into semisolid triglycerides. These preparations were applied to excised human skin composed of dermis, epidermis and horny layer. Comparing the concentration profiles after a certain penetration time (16 h), the indomethacin levels obtained with semisolid triglycerides were considerably higher than those obtained with Vaseline, showing a clear influence of the vehicle. For that reason, semisolid triglycerides were chosen as the standard base to which the penetration enhancers were admixed. The reference level of drug penetration, therefore, is already elevated, but substances used as enhancers should be more potent. Some liquid lipids (see Table 2) described in the literature as having penetration enhancing effects (Ritschel, 1973) were selected in order to be incorporated with the ointment base in the proportion of 20%.

TABLE 2

Relative increase of the amount of indomethacin penetration from ointments containing penetration enhancers (19.8%) into excised human skin (Blasius, 1985)

Penetration enhancer	Relative increase of indomethacin in the skin (%)		
	Total	Epidermis	Dermis
Glycerol monooleate	−6	−5	−1
Oleyl oleate	+10	+12	−2
Medium-chain triglycerides (liquid)	+48	+49	−1
Isopropyl myristate	+29	+21	+8
2-Octyldodecanol	+78	+47	+31
Cetyl and stearyl 2-ethylhexanoate	+86	+41	+45
n-Octanol	+90	+7	+83

Reference, ointment free of enhancer; ointment base, semisolid triglycerides (Softisan 378).

Table 2 shows the percentage increase in the amount of indomethacin penetrating from the ointment into the skin related to the drug penetration from semisolid triglycerides free of enhancers. Looking at the total increase, all substances except glycerol monooleate seem to enhance the indomethacin penetration as compared to pure Softisan. However, a more differentiated viewpoint is obtained if the amounts penetrating into the epidermis with the horny layer included are separated from those penetrating into the dermis. The increase in drug concentration is restricted to the epidermal area, if oleyl oleate and medium-chain triglycerides are used. This means that both substances reduce the diffusion resistance of the horny layer lipids to about the same extent as do the triglycerides of Softisan, but not to a higher degree; this will be discussed later. With the last four substances only, the increase in drug concentration in the dermis is greater than with pure semisolid triglycerides. This indicates that these four penetration enhancers reduce the diffusion

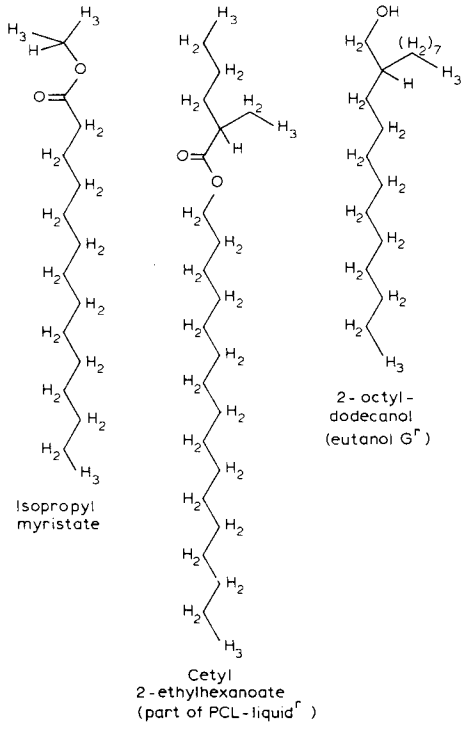

Fig. 5. Lipophilic penetration enhancers.

resistance of the epidermal barrier to a greater degree than do triglycerides. It is noticeable, that three of these compounds have branched hydrocarbon chains (Fig. 5).

These experiments indicate that the influence of vehicles and enhancers on the diffusion resistance of the horny layer may not differ in principle but in intensity; they should work by the same mechanism. The penetration enhancers and any liquid components of the vehicles, as well, migrate into the intercellular lipid layers, disturb their order of packing and possibly liquefy the intercellular material. The result is a decrease in the diffusion resistance. Differences in the intensities can be caused by the ability to disturb the packing order or by the rate of penetration of the enhancer molecules into the horny layer. So, *n*-octanol having a relatively low molecular weight may migrate rapidly and may have a great capacity to liquefy the lipid layer. The conclusion can be drawn that the differences are quantitative rather than qualitative. The relevant mechanisms, therefore, are the focus of interest.

To gain a better insight, some details of the methods were changed (Wild, 1988). As model drugs, three substances with similar structures and molecular weights were used: flufenamic acid, niflumic acid and mefenamic acid; they show graduated solubilities in lipids and water. Further, the technique of stripping the stratum corneum was applied in such a manner that the concentration gradient was analyzed in finer steps.

By comparison, Fig. 6 shows the usual concentration profiles in the epidermis and dermis with steep gradients in the stratum corneum and stratum granulosum. The higher flufenamic acid concentrations were obtained with semisolid triglycerides as ointment base, the lower ones with wool alcohol ointment. With Vaseline, which is not recorded in the diagram, the concentration levels are still lower. The thin layers of the stratum corneum and stratum granulosum are condensed to a very narrow section of the abscissa in this figure. Hence, one cannot glean much information about this extremely important region. As a consequence, it is necessary to analyse the drug concentrations in the stratum corneum in layers that are as thin as possible, and to enlarge the scale of the local coordinate axis of the diagram.

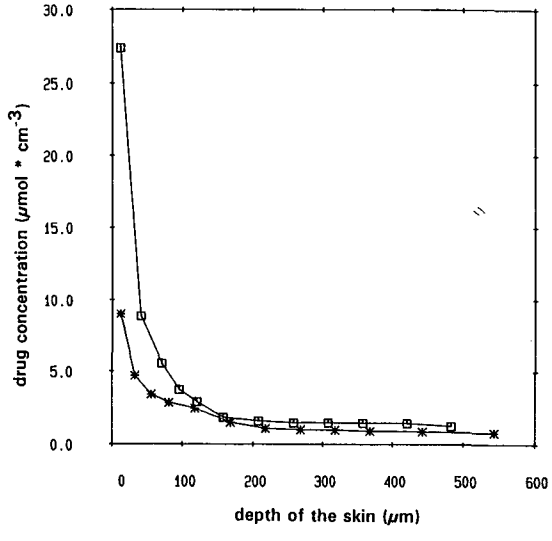

Fig. 6. Concentration profiles of flufenamic acid in excised human skin using several ointment bases (Wild, 1988).

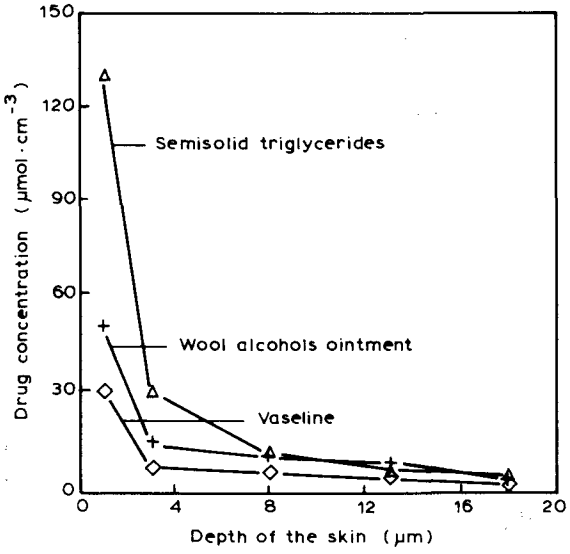

Fig. 7. Concentration profiles of flufenamic acid in the human stratum corneum using several ointment bases (Wild, 1988).

At first sight, the diagram in Fig. 7 appears to be like the preceding one. However, the horny layer only is represented and the graduations on the axes are changed. As one perceives, there are two different concentration gradients, and the sudden change in the concentration gradient corresponds to the transition from the stratum corneum disjunctum to the stratum corneum conjunctum. However, there is no indication or reason to assume that intercellular lipids alter their biochemical and biophysical nature within the horny layer to a sufficiently great extent that large variations such as these are caused. Therefore, vehicle effects must be sought.

It is generally known that the cells of the upper stratum corneum after the loss of the desmosomes are loosely packed so that they can desquamate. This loose packing of the horny layer cells includes the existence of intercellular capillary spaces which show capillary action. On the other hand, ointment bases contain liquid components which can escape from the semisolid gel to a greater or lesser extent. If they wet the horny layer lipids, they will enter the intercellular spaces by spreading and by being soaked up by capillary forces as well, and they will carry solute molecules into the spaces. Applying suspension ointments, the vehicle is saturated with respect to the dissolved drug. This results in relatively high drug concentrations in the upper horny layer.

The left side of the diagram in Fig. 7 represents the stratum corneum disjunctum. In this part, substantial differences in flufenamic acid concentration result from treatments with different vehicles. The sequence of the concentrations is determined by the solubility of flufenamic acid in the different ointment bases and by the degree to which the horny layer is wetted by the vehicles. The triglycerides having a higher polarity than the hydrocarbons wet the polar horny layer lipids and dissolve the drug to a greater degree than Vaseline. Wool alcohol ointment shows intermediate behaviour.

So far, the proposed mechanisms and experimental results are in accordance. Summarizing, the transport of xenobiotics applied as ointments, oils or the like through the stratum corneum disjunctum is performed in part by spreading of liquid components and their contents of dissolved substances into the intercellular spaces under the participation of capillary action and in part by diffusion. Diffusion, however, is the only transport mechanism in the stratum corneum conjunctum and in the viable epidermis. This results in the sharp change in concentration gradients in the region of the transition from the stratum corneum disjunctum to the stratum corneum conjunctum, where the gradients are flatter. Nevertheless, in the deeper layers of the skin, i.e. in the epidermis and in the dermis, the drug concentrations differ according to the applied vehicle. The reason is that vehicle components also diffuse in different amounts into the intercellular lipid layers of the stratum corneum conjunctum and reduce the diffusion resistance to a greater or lesser extent. In this respect, triglycerides are more effective than hydrocarbons.

To confirm this concept, two further types of penetration experiments have been implemented. First, to demonstrate the vehicle effect in a modified way, three concentration profiles obtained in a different manner were compared (Fig. 8): According to the facts, which have been already discussed, high flufenamic acid concentrations are obtained if semisolid triglycerides (ST) are used as

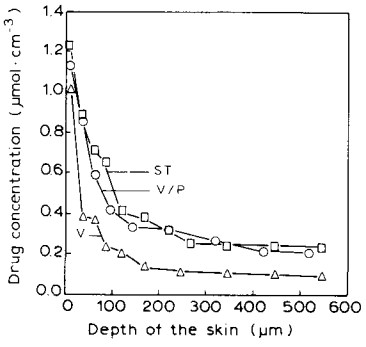

Fig. 8. Concentration profiles of flufenamic acid in excised human skin without and with pretreatment (Wild, 1988).

the vehicle, whereas Vaseline (V) gives low concentrations. The third concentration profile of this diagram (V/P), which is equivalent to the profile produced by the triglyceride ointment, is also obtained from a flufenamic acid-Vaseline ointment, but by the use of special conditions: Initially, the skin is pretreated with semisolid triglycerides which do not contain any drug substance. Therefore, the triglycerides are allowed to penetrate into the intercellular lipid layers influencing the diffusion resistance and the dissolving power of the horny layer lipids. After 2 h, the flufenamic acid-Vaseline preparation is applied, and the drug permeates the horny layer and penetrates the viable skin layers to the same extent as if it were incorporated into the triglyceride base. This result clearly shows the influence of vehicles on the skin permeability.

Finally, the skin is treated with three drug substances with differing lipid solubilities incorporated into semisolid triglycerides in a concentration (6%) sufficing to give suspensions (Fig. 9). In the stratum corneum disjunctum, represented on the left side of the diagram, flufenamic, niflumic and mefenamic acids show differing concentration profiles, which rank in the order of their lipid solubilities, according to the mechanisms discussed before. Again, a sharp change in the gradients near the transition from the stratum corneum disjunctum to conjunctum is found. In the stratum corneum conjunctum also, the height of the drug concentrations follows the lipid solubility of the substances, and this order is continued in the deeper skin layers not represented in the diagram.

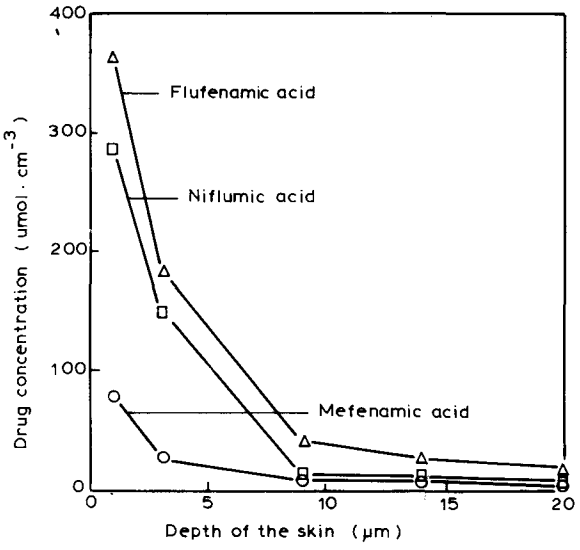

Fig. 9. Concentration profiles in the horny layer obtained from structurally related drugs showing differing lipid solubility (Wild, 1988).

Using wool alcohol ointment and Vaseline as vehicles, the concentrations of these three drug substances in all the skin layers are lower than with the triglyceride base, due to the vehicle effects. In each case, the concentrations of the substances, however, decrease corresponding to the solubilities in the vehicles. Hence, the order of the concentrations and of the solubilities is flufenamic acid, niflumic acid and mefenamic acid (Table 3). In contrast to this sequence, the values of the

TABLE 3

Solubilities and distribution coefficients (Wild, 1988)

Solvent	Flufenamic acid	Niflumic acid	Mefenamic acid
Solubility (mmol/l)			
Medium-chain triglycerides	194	35	16
n-Octanol	850	230	50
Water	0.019	0.11	0.002
Buffer (pH 7.4)	7.3	13	0.49
Distribution coefficients c_0/c_w			
Medium-chain triglycerides/water	10300	310	7800
n-Octanol/water	45000	2070	23800
n-Octanol/buffer (pH 7.4)	117	18	100

partition coefficients decrease in the order flufenamic acid, mefenamic acid and niflumic acid, which is determined by the relatively high water solubility of niflumic acid.

Conclusions

It follows from these observations that the lipid solubility rather than the partition coefficient may be the parameter determining xenobiotic permeation through the horny layer. This relates closely to the suggested lipid pathway through the stratum corneum on the basis of physicochemical causalities: The higher the lipid solubility, the steeper can the concentration gradient in the horny layer be, especially if suspension preparations are applied, and the steeper the gradient, the higher is the permeation flux. This system may be influenced by dermal vehicles performing interconnected and overlapping procedures.

Liquid components carrying dissolved drug substances enter the intercellular spaces by spreading and by capillary action. Subsequently, they diffuse into the intercellular lipid layers of the stratum corneum and of the stratum granulosum interfering with the molecular packing of the lipids and disturbing their order. This results in a decrease in the diffusion resistance and in a change of the dissolving power of the intercellular lipids affecting the drug distribution within the skin tissues; this means that reservoir and occlusion effects are concerned. These effects influence the permeability, which is confirmed by the evaluation of concentration profiles and by permeation measurements, as well. In this context, it is not unimportant and should therefore be realized that both types of transdermal diffusion measurements have different experimental conditions, especially with respect to the intake of water by the skin. The degree of skin hydration can alter the permeation rate in general, but not the sequence of the permeation rates of certain compounds or the order of vehicle effects, as far as it is possible to conclude from these investigations.

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