

An *in vivo* model to evaluate the efficacy of barrier creams on the level of skin penetration of chemicals

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The reservoir function and the barrier function are important properties of the skin. The reservoir function is dependent on the barrier function which, however, needs support by protective measures, in particular under working conditions. Barrier creams represent a possibility to protect the skin.

In the present study, a method was developed to investigate the effectiveness of reservoir closure by different formulations. Patent Blue V in water was used as a model penetrant. Its penetration, with and without barrier cream treatment, was analyzed by tape stripping in combination with UV/VIS spectroscopic measurements.

The investigations showed that the stratum corneum represents a reservoir for topically applied Patent Blue V in water. Furthermore, the barrier investigations showed that vaseline and bees wax form a 100% barrier on the skin surface. The third barrier cream, containing waxes and surfactant, only partially showed a protective effect against the penetration of Patent Blue V in water. Strong interindividual differences were observed for this barrier product.

In conclusion, it was assumed that the application of barrier creams cannot replace other protective measures and should be maximally used to inhibit low-grade irritants or in combination with other protectants or in body areas where other protective measures are not applicable.

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The human skin is a very complex organ which achieves several functions. The barrier function of the skin is one of its important properties. Healthy skin forms a good natural barrier against chemicals and other agents, microorganisms and dangerous substances; furthermore, it retains moisture (1, 2). Otherwise, it is known that the stratum corneum (SC) serves as a reservoir for penetrated substances (3). This reservoir function depends on the physico-chemical properties of the penetrant and on the status of the skin barrier. Thus, chronic exposure to solvents such as water and detergents, which is inevitable in many occupations, may lead to an impairment of this barrier, so that inter-alia toxic substances are allowed to reach the reservoir of the SC or even deeper layers of the skin (4, 5).

Several prophylactic measures for the protection of the skin are known, such as gloves, moisturizing creams and barrier creams (5); however, all of them have advantages and disadvantages. Gloves are often considered to represent an

absolute and 100% barrier for the penetration of substances into the skin. However, various recent investigations demonstrate that, in this case, the users have a false feeling of safety. Many gloves do not resist the penetration of low molecular weight chemicals (6). Some allergens are soluble in rubber gloves and can penetrate the glove and induce severe dermatitis (7). Furthermore, the glove membrane can be structurally modified by a solvent; this may lead to changes in permeation behaviour (1). Many people prefer creams rather than gloves because they do not want the hands to be sealed inside the gloves (6), as the occlusive conditions of gloves can lead to maceration of the skin and may disturb the natural skin barrier (8). Moisturizing creams or barrier repair creams may act on three different levels of the epidermis. An immediate effect is provoked by adding a lipid mixture to the skin surface (occlusion), an intermediate effect by adding a lipid mixture to the intercellular spaces and a delayed effect by

providing lipids to the epidermal cells (9), which should restore the natural barrier function of the skin (8). The advantage of barrier creams over bland emollients is still being vehemently debated (10). Theoretically, skin barrier creams reduce or even prevent the penetration into the skin by building up a physical barrier, like a thin film, between the skin and the irritant (4, 6, 8, 11). Practically, controversial experiences concerning the effectiveness of barrier creams exist. Various *in vivo* and *in vitro* investigations on animal and human skin have been performed to evaluate the efficacy of barrier creams. A frequently used *in vivo* technique is the repetitive irritation test to induce an irritant contact dermatitis and compare occurrence in barrier cream pretreated and untreated skin areas (4, 12, 13). As a model irritant, sodium lauryl sulfate is often used (8). In addition, *in vitro* skin models and diffusion cells were utilized (4, 14). Further techniques measured the penetration of topically applied substances after pretreatment with different barrier creams utilizing skin reflectance measurement, chromameter or laser-induced breakdown spectroscopy (2, 5, 11, 15).

The aim of the present study was the investigation of the penetration and reservoir behaviour of a hydrophilic dye (Patent Blue V) in water as model penetrant as prerequisite to investigate the effectiveness of reservoir closure by three different barrier formulations. The tape-stripping technique, combined with UV/VIS spectroscopic measurements [as described previously by Weigmann et al. (16) and Surber et al. (17)], appeared to be an appropriate method to investigate the penetration and reservoir behaviour of the dye and to evaluate the effectiveness of barrier creams, as tape stripping allows both the determination of the penetration depth in correlation with the amount of corneocytes and the quantification of the amount of topically applied substance penetrated into the SC.

Materials and methods

Pre-treatment of volunteers

The present study was performed *in vivo* on 12 healthy volunteers, male and female, aged between 25 and 30 years utilizing the tape-stripping technique. Additionally, biopsies were taken from ear skin of one freshly slaughtered pig (6-month-old, German domestic pig). Porcine skin is known to represent a suitable model for human skin (18), showing a similar penetration for topically applied substances (19, 20). Approval had been obtained from the Ethics Committee of the Charité and from the Veterinary Board of

Control, Berlin Treptow-Köpenick. The study was conducted according to the ethical rules stated in the Declaration of Helsinki Principles. The volunteers participating in the study had given their informed written consent.

The flexor forearms of the volunteers were prepared by washing, drying with soft tissue and abscising the hairs. Then, defined skin areas of 4 cm × 5 cm were demarcated using a permanent marker. Subsequently, a silicone barrier (Window Art Paints, LACUFA AG, Nerchau Mal- und Künstlerfarben, Nerchau, Germany) was built around the application areas in order to avoid lateral spreading of the formulations (21–23), which were applied afterwards.

Study designs

- (1) Study design A: Investigation of penetration and storage behaviour of a hydrophilic dye in water (Patent Blue V) using the tape stripping technique and histological investigations.
- (2) Study design B: Investigation of the effectiveness of the skin reservoir closure by three different barrier creams using the tape stripping technique.

Study design A. The reservoir investigations were carried out on 6 volunteers according to the method described by Teichmann et al. (21). Increasing concentrations and amounts of Patent Blue V ($C_{54}H_{62}CaN_4O_{14}S_4$, Fluka Chemie GmbH, Buchs, Switzerland) in water were applied using a pipette: 10 $\mu\text{g}/\text{cm}^2$ and 40 $\mu\text{g}/\text{cm}^2$ of the 0.5% Patent Blue V-containing formulation were applied and 40 $\mu\text{g}/\text{cm}^2$ of the 2% Patent Blue V-containing formulation. The homogeneous distribution was realized using a spatula. After a 1-hr penetration time (the volunteers rested in order to avoid sweating and contact with textiles), the amount of substances, which had not penetrated into the SC (excess), was removed by using a spatula in order to avoid the effect of occlusion (24, 25). After an additional four hours, the tape stripping procedure was performed as described below.

The histological investigation was performed on one pig-ear. After application of Patent Blue V in water, biopsies of 7 mm in diameter were punched out. The samples were fixed in formaldehyde solution and embedded in paraffin. Sections of 4- μm thickness were cut vertical to the surface using a microtome. Images were taken using a microscope (System Microscope BX60, Olympus Optical Co., Hamburg, Germany)

equipped with a digital camera (SIS Color View 12, Soft Imaging System GmbH, Münster, Germany).

Study design B. The barrier cream investigations were performed on a further 6 volunteers. 3 mg cm^{-2} of 3 different barrier formulations were applied homogeneously within the demarcated skin areas B, C and D using a blunt syringe and saturated glove fingers. Area A served as a reference skin area. Investigations were performed using vaseline (H. Hagner GmbH & Co., Freudenstadt, Germany) on area D and bees wax (Foot Locker Europe B.V., Vianen, the Netherlands) on area C as model substances; additionally, a commercial barrier cream (o/w emulsion containing waxes and surfactants) was tested on area B.

Application of Patent Blue V

After a 30-min penetration time of the barrier formulations, Patent Blue V-saturated filter papers were placed onto the skin areas A, B, C and D for a further 15 min. Subsequently, the filter papers were removed, and the skin areas were rinsed for 10 s using running hand-warm water.

Tape-stripping procedure

Then, the penetration of the Patent Blue V in water was investigated in the case of study designs A and B performing the tape-stripping procedure, as described previously (16). The SC was stripped using an adhesive tape (tesa no. 5529, Beiersdorf, Hamburg, Germany). The tapes were pressed onto the pretreated skin areas using a roller, which stretches the skin surface and brings the tape strip into contact with the entire flat skin surface, which is otherwise structured by wrinkles and furrows. 20 tape strips were removed from each skin area, as previous investigation have shown that, in general, the highest concentration of topically applied substances can be found on the skin surface and within the superficial layers of the SC (23, 26–28).

In the case of study design B, pictures were taken from the stripped skin areas utilizing a Canon EOS 50/50 (Canon Inc., Tokio, Japan) combined with the macro lens MP-E 65 mm F 2.81-5X, after the removal of 20 tape strips.

Determination of the amount of corneocytes and the concentration of Patent Blue V on each removed tape strip

The determination of the amount of corneocytes and the concentration of Patent Blue V on the

removed tape strips were realized spectroscopically using a modified UV/VIS spectrometer (Lambda 20, PerkinElmer, Überlingen, Germany) with a quadratic beam of $10 \text{ mm} \times 10 \text{ mm}$. The spectra of each tape strip were recorded between 400 nm and 900 nm with an empty tape strip in the reference beam. Normally, the pseudo-absorption measured at 430 nm corresponds with the amount of corneocytes on the removed tape strip (16, 29, 30). In the present investigation, the pseudo-absorption of the corneocytes at 430 nm was influenced by the absorption of Patent Blue V; therefore, the pseudo-absorption measured at 850 nm was utilized to determine the amount of corneocytes on the removed tape strips. Subsequently, the tape strips were extracted in 3.5 ml ethanol (Uvasol, Merck, Darmstadt, Germany) and 1 ml 1 M sodium hydroxide (Aldrich Chemical Company, Milwaukee, WI, USA) using ultrasound (Sonorex Super RK102H, Bandelin Electronic, Berlin, Germany) and centrifugation (at $\text{ref} = 1487.36 \times g$ for 10 min at 20°C , Centrifuge MR1812, Jouan GmbH, Unterhaching, Germany) followed by the determination of the concentration of Patent Blue V using spectroscopic measurement at the absorption maximum at 620 nm. For quantitative determination, a calibration curve of Patent Blue V extracted with ethanol and an alcoholic solution containing sodium hydroxide was established.

Analysis of the data and calculation of the penetration profiles

For a better comparison of the obtained data of study design B, the recovery rates of Patent Blue V of the reference skin area of each volunteer were standardized to 100%. Provided that the reference skin areas represented a recovery of 100% (area A; volunteers 1–6), they were compared with the barrier-treated skin areas (areas B, C, D; volunteers 1–6). A percentage less than 100% for the pretreated skin areas indicated a reduced penetration of Patent Blue V into the skin.

The penetration profiles take into consideration both the amount of corneocytes and the concentration of the Patent Blue V removed with each tape strip in a common figure. The amount of corneocytes is the basis for the calculation of the horny layer profile, as the concentration of the removed dye is related to the depth of the SC from where the tape strips were removed (26).

The amount of SC removed by tape strips is characterized by the distance between the

horizontal lines in the penetration profile. The lengths of the bars in the penetration profile correspond to the concentration of the active agent in the corresponding depth of the SC. Therefore, the penetration profiles can be seen as a cut through the SC (21).

Statistical analysis

For statistical analysis of the data, we calculated the mean values and standard deviations in the case of study design A, and in the case of study design B, we utilized the Kolomogorov–Smirnov Test (spss 11.0[®], Illinois, USA) to demonstrate that the obtained data were not not-normally distributed ($P > 0.05$) and to calculate the mean values and standard deviations. Then, we utilized the Wilcoxon test (spss 11.0[®]) for the comparison of the data obtained from the pretreated skin to those obtained from the reference skin areas in order to determine the significance P . Values for $P < 0.05$ were interpreted as significantly different.

Results

Study design A

Study design A investigated the reservoir of the SC quantitatively. The reservoir capacity was investigated by varying either the amount of the topically applied formulation (10 $\mu\text{g}/\text{cm}^2$ and 40 $\mu\text{g}/\text{cm}^2$) or the concentration of the active agent Patent Blue V (0.5% and 2%).

After the application of 10 $\mu\text{g}/\text{cm}^2$ of 0.5% Patent Blue V in water and the removal of 20 tape strips, 5.1 $\mu\text{g}/\text{cm}^2 \pm 1.25 \mu\text{g}/\text{cm}^2$ were recovered on average ($n = 6$). In this case, no excess (amount of substances which had not penetrated into the SC after a 1-hr penetration time) was developed. After the application of 40 $\mu\text{g}/\text{cm}^2$ of 0.5% Patent Blue V in water, the recovery rate averaged at 21.5 $\mu\text{g}/\text{cm}^2 \pm 1 \mu\text{g}/\text{cm}^2$. The excess averaged at 6.7 $\mu\text{g}/\text{cm}^2 \pm 2.8 \mu\text{g}/\text{cm}^2$.

The application of 40 $\mu\text{g}/\text{cm}^2$ of 2% Patent Blue V in water resulted in an average recovery rate of 27.7 $\mu\text{g}/\text{cm}^2 \pm 1.5 \mu\text{g}/\text{cm}^2$. From the excess, 5.5 $\mu\text{g}/\text{cm}^2 \pm 1.5 \mu\text{g}/\text{cm}^2$ were recovered.

The penetration profiles of Patent Blue V in water showed the highest concentration of Patent Blue V on the skin surface and the upper part of the SC independent of the amount and concentration of topically applied Patent Blue V (data not shown). However, the recovered concentration of Patent Blue V increased if either the amount or concentration of Patent Blue V in water was increased. Figure 1 represents the

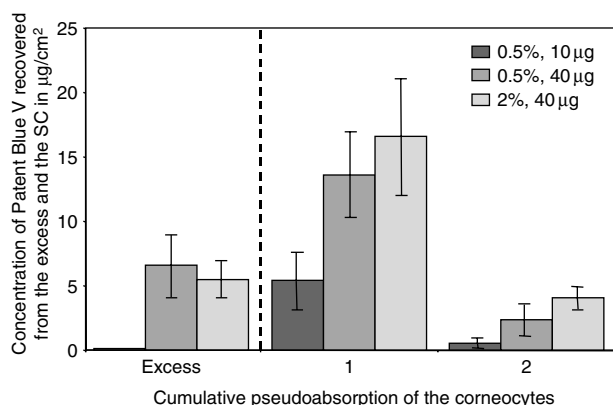


Fig. 1. Concentrations of Patent Blue V recovered from the excess and different depths of the stratum corneum (SC) (a cumulative pseudo-absorption of 1 generally corresponds to 4 layers of corneocytes).

concentrations of Patent Blue V recovered from SC sections, which correspond to a cumulative pseudo-absorption of 1. Usually, 4 layers of corneocytes correspond to a cumulative pseudo-absorption of 1 (31). An increase of the applied amount and concentration of Patent Blue V leads to increased recovery rates in the excess and the upper layers of the SC.

The histological investigation of the reservoir showed that the largest amount of topically applied Patent Blue V in water is located on the skin surface and the upper parts of the SC. Furthermore, a larger amount of substance is located in the valleys of the furrows, as can be seen in Fig. 2.

Study design B

Study design B investigated the effectiveness of reservoir closure by 3 different barrier formulations. The penetration behaviour of Patent Blue

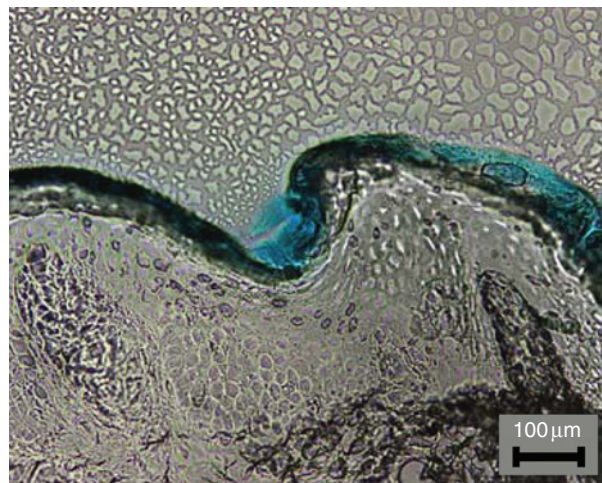


Fig. 2. Histological image of a biopsy of porcine skin after application of Patent Blue V in water.

V in water was studied after pretreatment of skin areas with different barrier formulations using the tape-stripping technique. Varying results for the different barrier creams were obtained.

In Fig. 3, the penetration behaviour of Patent Blue V is represented for untreated and pretreated skin. In the case of the reference skin area (area A) and the area pretreated with the commercial barrier cream (area B), the penetration behaviour of Patent Blue V was similar. No penetration of Patent Blue V was observed when bees wax (area C) and vaseline (area D) were applied in advance. The same results were obtained for all 6 volunteers.

Figure 4 demonstrates the mean values of the recovered amounts of Patent Blue V in all 6 volunteers. Provided that the reference area represented a recovery of 100%, a mean percentage of $104.3 \pm 56.1\%$ of Patent Blue V was recovered from the skin areas B, pretreated with the commercial barrier cream. In the case of skin areas C and D (pretreated with bees wax and vaseline), no Patent Blue V was detected in the SC.

In the case of the commercial barrier cream (area B), no significant difference was revealed between untreated skin (area A) and the pretreated skin ($P > 0.05$), whereas, for the pretreatment with bees wax (area C) and vaseline (area D) and untreated skin, a significant difference was observed ($P < 0.05$).

In Fig. 5, pictures of skin areas after pretreatment, the penetration of Patent Blue V and the

removal of 20 tape strips are demonstrated. Skin areas A and B are again comparable. After the removal of 20 tape strips, the blue dye is still visible in the furrows of the skin. Regarding skin areas C and D, after the removal of 20 tape strips, Patent Blue V cannot be observed on the skin.

Discussion

The aim of the present study was the investigation of the penetration and reservoir behaviour of a hydrophilic dye (Patent Blue V) in water as prerequisite to investigate the effectiveness of reservoir closure by 3 different barrier creams.

The investigation was performed on 6 volunteers. It has been shown that a long-term reservoir of the SC exists for the investigated topically applied substance, as after a period of 5 hr the Patent Blue V was detectable in the SC. However, it has to be mentioned that the reservoir capacity is dependent on the polarity of the topically applied substance as previous investigation have shown (32–34). A definition of the SC reservoir has been established by Waibler (35) who defined that a reservoir develops if a concentration gradient of a topically applied substance arises inside the SC in the direction of the viable epidermis. From the reservoir of the SC, the topically applied substances penetrate in direction to deeper layers of the skin. The reservoir is complete if a steady state is reached, which means when

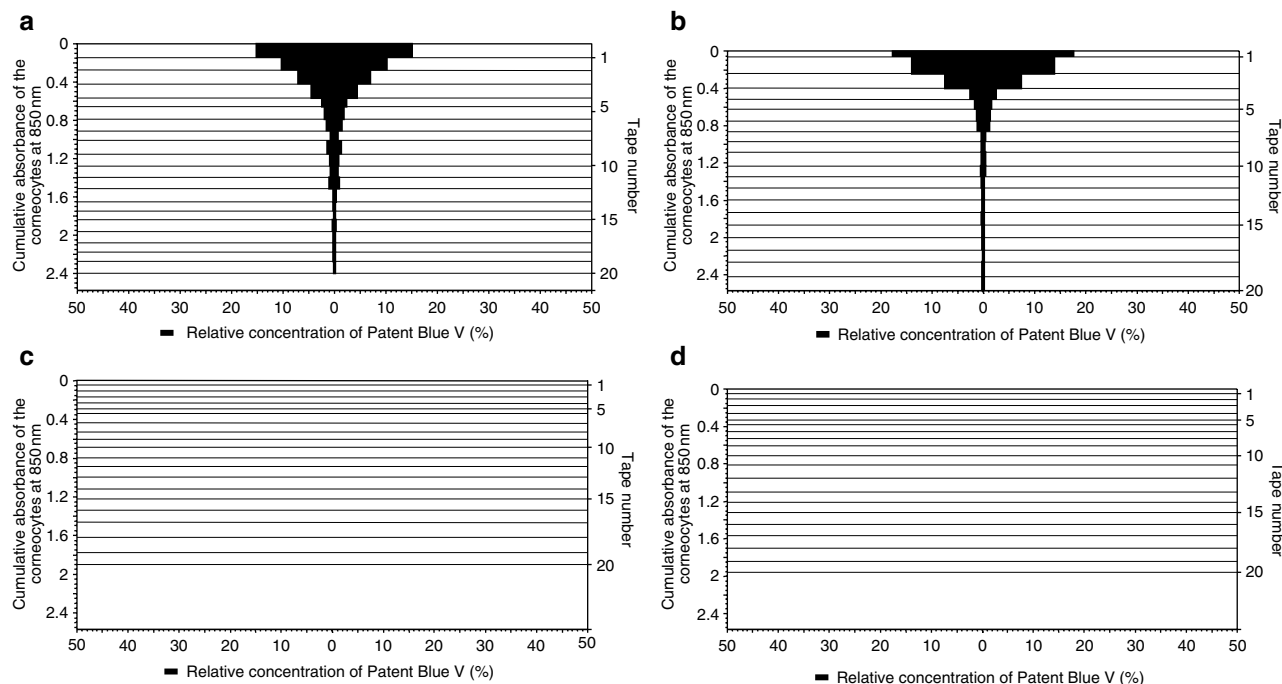


Fig. 3. Penetration profiles of Patent Blue V obtained by tape stripping, penetration time 15 min, volunteer no. 4; a) area A: reference skin area; b) area B: commercial barrier cream c) area C: bees wax; d) area D: vaseline.

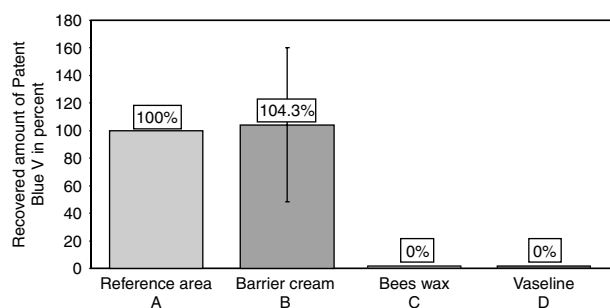


Fig. 4. Concentrations of Patent Blue V recovered from the stratum corneum of skin areas A, B, C and D by tape stripping in percentage (mean values of volunteers 1–6), providing a 100% recovery from the reference skin areas.

inflow from the excess into the SC and outflow are balanced. However, this state is time-unstable and indicates the storage of a topically applied substance over a certain period followed by continuous delivery.

For Patent Blue V in water, it has been shown that the reservoir is mainly located in the upper part of the SC, the more loose SC disjunctum. In previous investigations, it has been demonstrated that the SC disjunctum plays an important role in reservoir function (36, 37).

Furthermore, it was discovered that the reservoir of the SC increased if either the amount or the concentration of topically applied Patent Blue V in water was increased. This is in accordance

with previous investigations (21); whereas, at that time, a saturation effect of the SC reservoir was also observed after the application of very high amounts of substances. In the present investigation, the increase from $10 \mu\text{g}/\text{cm}^2$ to $40 \mu\text{g}/\text{cm}^2$ Patent Blue V in water led to an increase in the recovered Patent Blue V at a factor of 2.5 on the upper part of the SC. In the deeper parts, only small differences were detected. Therefore, it can be assumed that the increase of a topically applied hydrophilic substance only provokes an increase in the concentration on the skin surface (excess) and the upper part of the skin. For substances of other polarities, this may be completely different.

The histological investigation of porcine skin revealed that the furrows of the skin also play an important role in reservoir function of the skin. Figure 2 shows an image of a biopsy of porcine skin after application of Patent Blue V according to study design A. It can be seen that most of the blue dye is located on the skin surface and in the upper part of the skin. A further important point is that a large amount of substance is also located in the valleys of the furrows. So that in these areas, an enhanced and elongated penetration can be assumed. This has to be taken into account when considering skin protection and reservoir closure.

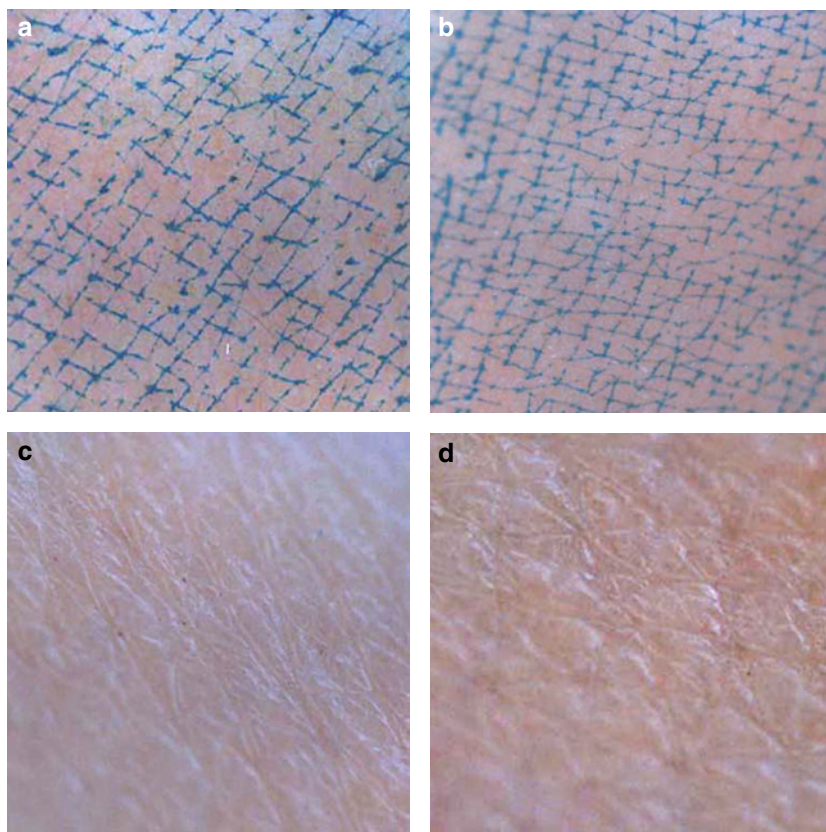


Fig. 5. Pictures of skin areas after pre-treatment, a 15-min penetration time of Patent Blue V, and the removal of 20 tape strips, volunteer no. 5; a) area A: reference skin area; b) area B: commercial barrier cream; c) area C: bees wax; d) area D: vaseline.

The investigation concerning reservoir closure was likewise performed on human volunteers by comparing the effectiveness of three different barrier formulations with untreated skin by means of tape stripping. Varying results for the 3 different barrier creams were obtained. First, the results showed that the different barrier creams had an influence on the amount of corneocytes removed with the tape strips. In the case of no treatment or treatment with the o/w emulsion, more corneocytes were removed by 20 tape strips than in the case of vaseline or bees wax. This is due to the reduced adhesive potency of the tape strips caused by the fatty formulations vaseline and bees wax. However, the penetration profiles of the Patent Blue V are comparable as the concentrations of the topically applied substances were correlated to the penetration depth determined by the cumulative absorbance of the corneocytes at 850 nm.

Furthermore, the results show that for the penetration of Patent Blue V in water, the commercial product (area B) did not represent a barrier. Both the penetration behaviour (see Fig. 3) and the average recovery rate (see Fig. 4) of Patent Blue V were not influenced significantly by the commercial barrier cream; no significant differences between the penetration of Patent Blue V in untreated and pretreated skin (area B) could be detected ($P > 0.05$). However, considering the results of every single volunteer, strong interindividual differences appear. In the case of volunteer nos. 1 and 2, the barrier cream had a protective effect. In the case of volunteer nos. 3 and 5, the protective effect became negative. For both these volunteers, the penetration of Patent Blue V in water was obviously enhanced after application on pretreated skin. For volunteer nos. 4 and 6, neither a notable protective nor an enhancing effect could be observed. This effect may be explained by different skin conditions of the single volunteers, such as hydration and transepidermal water loss of the skin, which is even known to influence the reservoir function of the skin (21). Dry and rough skin may inhibit the barrier cream to act as a physical barrier but helps penetration into the probably enlarged SC reservoir accompanied by the topically applied irritant. This aspect was not considered in the present investigation but must be kept in mind for further barrier cream studies.

On the contrary, bees wax and vaseline showed a 100% protection against the penetration of the applied dye. This is in concordance with the results of Treffel et al. (15) who ranked petrolatum as a blocker in their evaluation of barrier creams. Also Zhai et al. saw protective effects of

paraffin wax and bees wax (38, 39). Therefore, vaseline and bees wax, respectively, can be seen as the 'gold standard' for our barrier cream test method against the penetration of a hydrophilic substance. Both seem to build up effective physical barriers, which also fill out the furrows of the skin, so that a reservoir closure is reached.

In the literature, an ambivalent effectiveness of barrier creams is also described. A detailed investigation of the efficacy of barrier creams by Frosch et al. (13, 40–42) showed that some of the investigated barrier creams reduced skin irritation induced by some of the utilized irritants, but not all; other barrier creams showed no protective effect or even enhanced irritation induced by several irritants. Treffel et al. (15) found varying protective effects for different barrier creams. They also observed enhancing effects with some products and assumed that they were due to a high affinity of the topically applied substance on the barrier formulation.

Considering the results of the present investigation and the results described in the literature, it must be assumed that a prediction on the effectiveness of a barrier cream cannot be made solely on the basis of the barrier formulation, but both the penetrant applied and the skin conditions must be taken into account. The still often cited dogma that, theoretically, o/w emulsions are recommended against lipophilic irritants or penetrants and w/o emulsions against hydrophilic irritants or penetrants (4, 6, 8) has practically already been disproved in different studies (13, 41). Other authors used further classifications of barrier creams: Chilcott et al. (14) divided the barrier creams into non-specific (passive) and chemically neutralizing (reactive) barrier creams, De Fine Olivarius et al. (5) divided the barrier creams into 2 main groups, with or without silicone.

A further problem represents the model penetrant. For each single real penetrant or irritant, an adequate model penetrant will not be found (frequently used are hydrophilic and lipophilic dyes and irritants), furthermore, in most cases, under in-use conditions the danger does not originate from a single substance but from an unpredictable mixture, which cannot be simulated adequately.

Nevertheless, the barrier cream test method presented in this paper offers several advantages. Primarily, it is applicable *in vivo* and in humans. This is very important, as animal models are not suitable in this case because their skin has a different permeability (14). Furthermore, the method is applicable for different barrier creams and model penetrants and allows a quantitative

ranking of the effectiveness of different barrier formulations. Vaseline and bees wax can be utilized as a 'gold standard' concerning the effectiveness of barrier substances for this method, although, naturally, their application under working conditions is not practicable on account of their slithery properties.

Finally, on the basis of the present investigation and the review of the literature, we assume that the application of barrier creams cannot replace other protective measures and should be maximally used to inhibit low-grade irritants or in combination with other protectants or in body areas where other protective measures are not applicable. However, tape stripping offers an efficient method to rank quantitatively the effectiveness of different barrier creams.

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