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Evaluation of Barrier Creams: An *In vitro* Technique on Human Skin

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A method was developed to measure in vitro on human skin the effectiveness of barrier creams against three dyes (eosin, methyl-violet and oil red O) with different n-octanol/water partition coefficients (0.19, 29.8 and 165, respectively). Some galenic properties (water washability, water content and viscosity) of the products were also evaluated to try to understand the mechanisms of such a protection. The barrier creams were assayed by measurements of the dyes in the epidermis of protected skin samples after an application time of 30 min. Whereas some products showed some degree of protection, as claimed on the packaging, we demonstrated in several cases disagreement with the manufacturer's information. Surprisingly, petrolatum was found to provide the best protection of all tested products in our in vitro model. There was no correlation between the galenic parameters of the assayed products and the level of protections, indicating that neither the water content nor the consistence of the formulations influenced the protection effectiveness. In conclusion, regarding the possible skin effects of some irritants, our results stress that barrier creams should be used with caution, knowing the protection limits of some of the formulations marketed. ***Key words: Percutaneous absorption; Irritant; Stratum corneum.***

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Barrier creams would appear to be a logical approach to the problem of protection of the skin against chemicals, but in fact their use is subject of a lively debate concerning the prevention of contact dermatitis (1). The lack of evidence of their efficacy is in part due to the dearth of suitable standardized techniques for evaluation of their protective activity and perhaps also to their classification as cosmetics, precluding proof of efficacy as requested for registered drugs. Only few studies have been published earlier about in vivo evaluation of such products, e.g. on guinea pigs (2–6) or on human beings (7–9), but these methods are not suitable for the screening of a large number of candidate formulations during experimental development. The aim of this study was to develop an in vitro model to test the ability of a barrier cream to avoid skin contact with a chemical, as only a few in vitro methods are available (10). In addition, the mechanisms of such a protection were studied. We worked with human skin and in order to have good simulations of several irritants (hydrophilic and lipophilic) we assessed the protection efficacy using three dyes (8) with different physicochemical properties.

MATERIALS AND METHODS

Tested products

Twenty products were tested (Table I), 16 of them being marketed specially as barrier creams. The other four were not claimed as barrier creams.

Properties of the studied products

Three parameters were assessed for each product.

Water washability: The water washability was determined by applying 0.5 ml of the product on the back of the hand and cleaning it under cold running water for 15 s by hand rubbing. The product was said to be water washable if after this period of time the amount remaining on the skin was negligible, as measured by immediate afterfeel of the fatty residue.

Percentage of water in the formulation: We evaluated this parameter by measuring the loss of weight of the products spread on plastic support and stored at 60°C until weight constancy. This measurement did not take into account the presence of any other volatile compound in the formulation.

Viscosity: As a viscosity parameter we assessed at 30°C the point at which the sample was flowing (flow limit) with the controlled stress flow method using a cone and plate viscometer (Rheolab MC 10, Physica, Stuttgart, Germany).

Table I. *Products assayed*

The 16 first listed products were barrier creams, the others (*) not. Q and R were w/o and o/w emulsions respectively, marketed as moisturizers. The lotio alba aquosa was made in our laboratory as described in the Formularium Helveticum.

	Name	Batch	Company
A	Kerodex 1	0170392	Turimed, Wallisellen, CH
B	Turexan	0559106	Turimed, Wallisellen, CH
C	Stokoderm	not lisible	Stockhausen, Krefeld, D
D	Arretil	510006	Stockhausen, Krefeld, D
E	Travabon	036278	Stockhausen, Krefeld, D
F	Stokolan	40036669	Stockhausen, Krefeld, D
G	Kosmosan	604005	Stockhausen, Krefeld, D
H	Marly skin	not found	Trimex, Zug, CH
I	Silicoderm-F	LV041	Bayer, Leverkusen, D
J	Softguard	A257A	Neolab, Heidelberg, D
K	Pro 1 isolex	1Y122	Isolex, Paris, F
L	Pro 2 isolex	1Z67	Isolex, Paris, F
M	Antixol	202202	Laphi, Paris, F
N	Deltasol B	77431	Laphi, Paris, F
O	Cutanel hygiène	22691	Deb, Chalette / Loing, F
P	Cutanel industrie	16801	Deb, Chalette / Loing, F
Q*	Excipial fatty cream	0010295	Spirig, Egerkingen, CH
R*	Excipial cream	0121095	Spirig, Egerkingen, CH
S*	Lotio alba aquosa	–	–
T*	Petrolatum	–	–

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Table II. *Individual data of absorbances (×10⁻³) obtained from the control skin samples*

Inter and intra-series coefficients of variation are given in the last column and on the last line, respectively. Indexed letters are the skin sources.

Eosin

Series	1[a]	2[a]	3[a]	4[b]	Inter-series c.v.
Absorb.	18	31	29	15	34
	31	26	39	16	34
	52	31	37	22	35
Intra-series c.v.	50	9	15	21	

Methyl-violet

Series	1[b]	2[b]	3[b]	4[b]	Inter-series c.v.
Absorb	98	143	182	90	33
	142	123	131	97	15
	106	245	103	76	57
Intra-series c.v.	20	38	28	12	

Oil red O

Series	1[b]	2[b]	3[c]	4[c]	Inter-series c.v.
Absorb.	119	79	108	117	17
	167	89	130	109	26
	201	77	106	114	42
Intra-series c.v.	25	7	11	3	

Dyes

The following dye solutions were used: 0.1% eosin scarlet (Fluka, Buchs, Switzerland) in water, 0.1% methyl violet 2 B (Sigma, St Louis, MO, USA) in water and 0.1% oil red O (Sigma, St Louis, MO, USA) in ethanol. The 0.1% concentration was chosen because it was in the region of the concentrations already used (8). The partition coefficients (n-octanol/water) of these dyes were measured as previously described (11). Briefly, we performed a water/n-octanol and an n-octanol/water mutual saturation with 3 h of mechanical stirring of a vol/vol mixture. Fifty µl of each solution were deposited in glass tubes, evaporated and 3 ml of each solvent were added. About 100 inversions in 5 min of the stoppered test-tubes were performed before centrifugation for 10 min at 3000 rpm. The tubes were left overnight at room temperature and we assessed the dyes in both phases at their absorption maximum with a Lambda 5 UV/vis spectrophotometer (Perkin Elmer, Ueberlingen, Germany). The partition coefficient P was calculated using the absorbance ratios of both phases.

Dye penetration into the stratum corneum of unprotected skin samples

We evaluated in vitro the penetration depth of the dyes into the stratum corneum by stripping the isolated skin samples. After dye application and washing procedure as described below for the skin sample controls, 20 strips (Cellux, Rorschach, Switzerland) were made. Each strip was stuck on a white paper sheet in the order of removal and the staining was measured using the Chromameter CR 300 (Minolta, Osaka, Japan) which was calibrated to a standard white plate. Each determination was related to a control (strip without dye application) and we calculated the Δ total colour change (ΔTCC) by the following calculation: $\Delta TCC = \Delta L^* - \Delta a^* + \Delta b^*$. The final results were expressed as percentage of a second ΔTCC represented by the difference between the dye solution applied on a white paper sheet and the white paper control. The strips were made in triplicate and assessed once.

Barrier cream efficacy assessments

Fresh skin samples were obtained from breast reductions of three Caucasian women. Immediately after the surgical operations the sub-

Table III. *Studied product measurements and efficacy of the protection against different dyes (mean ± sd)*

Information given by the manufacturer concerning the product are the following: 1 = lipophilic product, 2 = hydrophilic product, 3 = containing silicone, 4 = without information. Concerning the type of protection: a = barrier against aqueous substances, acid, alkali, b = barrier against lipid solvent material and c = specific indication not given or too imprecise.

Products	Water washable	Water content %	Flow limit (eta/Pa)	% of protection against		
				Eosin	Methyl-violet	Oil red O
A[4,a]	y	54	38	83±8	68±3	40±10
B[4,a]	y	52	174	71±3	78±2	66±2
C[4,a,b]	y	68	98	74±9	96±4	74±3
D[2,b]	y	62	298	88±8	99±1	69±2
E[2,b]	y	55	1002	43±15	93±1	73±3
F[2,c]	y	61	123	59±8	52±8	81±1
G[1,a]	n	50	1495	80±9	87±3	66±7
H[4,a,b]	y	90	–	66±9	15±18	65±7
I[3,a,b]	y	50	241	72±4	70±7	64±6
J[3,c]	y	79	25	19±58	91±3	61±2
K[4,b]	y	51	14	31±12	33±26	2±18
L[4,a]	n	54	244	30±11	61±8	52±9
M[4,b]	y	87	194	44±9	78±3	57±15
N[4,a]	n	60	255	53±18	44±12	55±5
O[4,a]	y	79	119	17±27	9±19	57±14
P[4,b]	y	71	89	–73±25	81±9	53±3
Q*	n	44	229	89±10	58±13	73±4
R*	y	67	135	20±20	–7±20	89±1
S*	y	65	–	–202±171	66±7	45±8
T*	n	0	609	100±0	95±6	79±9

* not claimed to be barrier cream products.

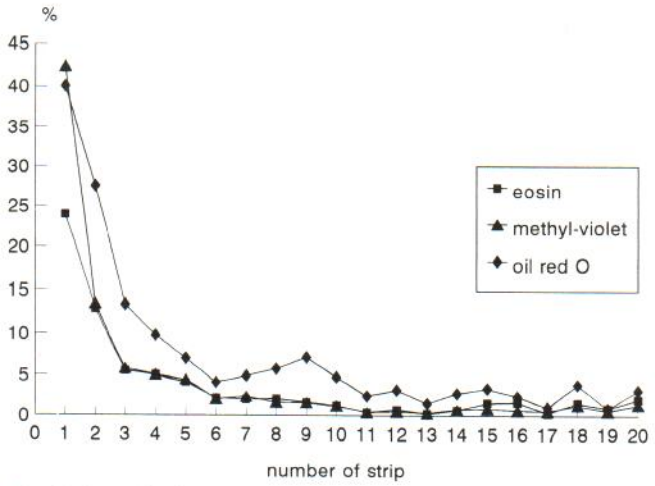

Fig. 1. Δ total colour change measurement in the stratum corneum expressed in percentage.

cutaneous fat was carefully removed and pieces of skin (2–3 cm^2) were cut out of the surgical specimen, washed with physiological saline, placed individually in aluminium foil and stored in frozen state at −18°C for a period not exceeding 3 months. Before use, the samples were placed for 2 h at room temperature on a marble plate. To avoid excessive dehydration, the dermal side was in contact with physiological saline. The skin was washed once more before the experiments and cut into pieces of 8 × 15 mm.

Except for the controls (not pretreated with barrier cream), 3–4 mg/cm^2 of product were applied with a small metallic spatula and the skin samples were laid in diffusion cells for 0.5 h. These diffusion cells were made of glass chambers, in which a teflon ring with a piece of wire mesh in the middle was deposited. The receptor phase (physiological saline) was stirred mechanically at 300 rpm and kept at a constant temperature by a water bath (37°C). After this 0.5 h period of time we applied on all skin samples, without having washed the surface, two filter papers type AA-Discs, 6 mm of diameter (Whatman, Maidstone, UK), each soaked with 30 μl of the eosin, 30 μl of the methyl-violet or 20 μl of the oil red O solutions. Fifteen min later, or 10 for oil red O, the solutions were pipetted again on the filters so that the total applied quantity was 40 μl. These different application schedules were realized to minimize the evaporation effect of the ethanolic solution. The filters were removed after a total contact time of 0.5 h and the skin surface was washed three times with a 0.1% triton X 100 water solution to wipe off the excess of product and dye. The epidermis (stratum corneum + viable epidermis) was separated from the dermis after heating the skin samples on a hot-plate at 50°C for approximately 2 min. The tissue was homogenized in 1.5 ml of ethanol or for eosin in 1.5 ml of a 80/20 (vol/vol) ethanol water solution sonicated for 5 min. The homogenates were filtered through a 0.2 μm filter type minisart SRP 15 (Sartorius, Göttingen, Germany) and the absorbances were determined at the absorption maximum for each of the dyes, with as zero value a homogenate of untreated epidermis. We worked on series (one type of dye per series) of six diffusion cells in which each cell received at once three skin samples. Four series of measurements were made with each dye and during each of them skin samples not treated with the barrier creams (controls) were exposed to the dyes on one cell. Each product was tested in triplicate.

Calculations and statistics

The data were expressed as percentage of protection related to the corresponding control (0% protection), from the following calculation: [(control absorbance − sample absorbance) / control absorbance] × 100. The products were ranked according to their percentage of protection as blocker (100%), as giving a good protection (>70%), a weak protection (70–30%) and no protection (<30%).

Means were compared by analysis of variance with the one-way procedure, followed, if statistical significance was obtained ($p < 0.05$), by the Duncan's multiple-range test. Correlation analyses were performed by calculating the Pearson correlation coefficient.

RESULTS

Validity of the method

The individual data of the assessment of the epidermal staining of the unprotected skin samples (controls) are shown in Table II. The absorbance means ($\times 10^{-3}$; $n = 12$) were 29, 128 and 118 for eosin, methyl-violet and oil red O, respectively. The staining generated by eosin was found to be the lowest ($p < 0.001$). The inter-series coefficient of variation (32.5%) was greater than the intra-series one, which was 19.9% ($p < 0.05$).

Properties of products and dyes

Products: Table III indicates the information given by the manufacturers and the measurements we have made on the product itself. Thirteen of the barrier creams were found to be water washable. The water contents in the formulations were between 44 and 90% except for petrolatum. The flow limits of the formulations (viscosity parameter) were measured from 14 to 1495 (eta/Pa). Two measurements were not possible because one product was a foam (H) and the other was too consistent (S).

Dyes: The n-octanol/water partition coefficients of the dyes, expressed as mean ± sd ($n = 5$), were $0.19 \pm 2. 10^{-3}$ for eosin, 29.8 ± 21 for methyl-violet and 165 ± 99 for oil red O.

Penetration depth of the dyes into the stratum corneum

Fig. 1 shows the ΔTTC (%) related to the number of the strips made from the skin sample controls. The dyes were present in high amounts in the superficial layers of the stratum corneum; the ΔTCC due to eosin in the first strip was lower than those obtained with both the other dyes but this was not statistically significant ($p = 0.098$). Furthermore, our results show oil red O to penetrate in greater amounts into the deep layers of the stratum corneum (Table IV). The amount of the three dyes at the bottom of the stratum corneum remains, however, low.

Barrier cream efficacy

Against eosin: It is apparent that there was some degree of protection in each of the experiments, as indicated by the values showed in Table III. Nevertheless, a number of the barrier creams showed weak (E, F, H, K, L, M, N) or no protection (J, O, P) against this hydrophilic dye, but E, K, M and P were not

Table IV. *Cumulated percentage of Δ total colour change for each of the dyes*

Indexed letters indicate the significance of the dye comparisons: a = 0.0502, b = 0.0082, c = 0.0384 and d = 0.1418. The strips were pooled in the order of removal, i.e. 1 to 5 represent the superficial stratum corneum, 16 to 20 the deep layers.

Strips	Eosin	Methyl-violet	Oil red O
1–5	51 ± 17	71 ± 18	98 ± 17[a]
6–10	9 ± 5	9 ± 1	27 ± 7[b]
11–15	3 ± 3	3 ± 3	13 ± 6[c]
16–20	6 ± 4	4 ± 1	11 ± 5[d]

claimed to protect against aqueous substances. On the other hand, H, L, N and O should have been good protectors but this was not the case in our in vitro model. Concerning the non-barrier creams, petrolatum (T) was found to be an absolute blocker and the lotio alba aquosa (S) enhanced dramatically the epidermal staining. The w/o emulsion (Q) showed barrier properties greater than the o/w one (R).

Against methyl-violet: In agreement with the manufacturer's information, C, D, E, J, M and P showed a good protection against this amphiphilic or slightly lipophilic dye. Two of the barrier creams did not protect (H, O; <30%). In the case of H, this disagreed with the manufacturer's information. The product R (not a barrier cream) enhanced weakly the epidermal staining.

Against oil red O: Only one barrier cream (K) did not show any protection. Most of these barrier creams should have been ranked as having a good protection but this was not always the case. K for instance was in total disagreement with the manufacturer's information. Surprisingly, the o/w moisturising emulsion (R) showed the highest protection and the lotio alba aquosa was not found to be a good protector.

Correlation with the product properties: We did not find any correlation between the percentage of protection against the three dyes and the water content in the formulation or the viscosity parameter (flow limit).

DISCUSSION

Our study proposes a method of rating the effectiveness of various topical barrier cream preparations against a standardized staining assessed in the full epidermis. The validity of the method was investigated, and we showed the intra-series variation to be lower than the inter-series one. This shows that the reproducibility (inter-series variation) of the method could be improved, although this would be difficult with isolated skin samples. In vitro regenerated skin from keratinocytes and fibroblasts culture could be a future model to consider.

The partition coefficients of the dyes we found indicate that the dyes chosen were hydrophilic (eosin), slightly lipophilic (methyl-violet) and lipophilic (oil red O). These different properties should well reflect the behaviour of hydrophobic (acid, alkali) or lipophilic (some cutting oil, lipid solvent material) chemicals, especially when partition of the dye between the barrier cream and the stratum corneum is considered. The results we obtained by the stripping technique demonstrated that only a small quantity of the dyes reached the lower part of the stratum corneum. This emphasizes the fact that the staining measured in the full epidermis was mainly due to the presence of the dye in the upper stratum corneum layers (Fig. 1) but gives the model a possible relevance to the in vivo situation. In addition, it appeared that the more lipophilic dye (oil red O) penetrated deeper into the stratum corneum. As measured in the overall epidermis (Table II) the stratum corneum results showed the staining due to eosin to be the lowest.

Two ways of ensuring that an irritant or an allergen does not reach the epidermis are the prevention of its penetration by a barrier product or its sequestration by the barrier product in order to avoid its uptake by stratum corneum. In the first case, prevention must be obtained by formulations in which the foreign compound is not soluble and in the second by the contrary; thus water washable formulations have shown in several cases a good efficacy against eosin (hydrophilic) in our study. The enhancing effects we observed with some products (P, R, S) must be explained by a great affinity of the dye for the applied formulation with following preferential partitioning of the dye to the stratum corneum.

The limits fixed for classifying the protection effectiveness may be regarded as arbitrary but were defined with respect to the variation coefficients we found. They could be of relevance in the case of an irritative compound, as an irritant reaction largely depends on the dose of the irritant penetrated. They may be of little value in the case of an allergen if sensibilization to this compound has already occurred, as only a minor quantity is needed to trigger the allergic reaction. Although the application time of the dyes was short, we did not find any absolute skin protection except with petrolatum against eosin. Petrolatum was the only product ranked as a blocker or giving a good protection in each case. Furthermore the degree of protection afforded by Q (only a moisturizing w/o emulsion) relativizes the efficacy of the barrier creams. In previous studies, Antixol (M) showed a slight protection against toluene as determined in vivo by blood flow measurements (5), while against epoxy resin Arretil (D) was ineffective and Kerodex (A) slightly effective as measured by allergic skin reactions. We measured Arretil as a good protector in vitro against the three dyes, and we think that this different result shows the difficulties in objectively evaluating protection. Actually, Arretil could have been an excellent barrier, but as stated above, only a minor amount of the chemical could have started an allergic reaction as measured by these authors. Finally, the weakness of the correlations we found indicates that neither the water content nor the consistence of the formulations influenced the level of the protection.

In conclusion, our model enabled us to evaluate some aspects of the efficacy of several barrier creams in vitro. While most of them showed a good protection after half an hour, none of them demonstrated an absolute efficacy. Regarding the possible long-time effect of some irritants (12), our results stress that such products should be used with caution, knowing the protection limits of some of the formulations marketed at present.

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