The Effect of Occlusion on Epidermal Penetration of Parabens from a Commercial Allergy Test Ointment, Acetone and Ethanol Vehicles

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The efficacy of topical allergy screening systems relies on the ability of test agents to effectively penetrate the stratum corneum from applied vehicles and reach the viable cells involved in the cutaneous immune response system. There is very little evidence in the dermatologic literature to justify the choice and suitability of vehicles used in many allergy test systems and the effectiveness of occlusion, reported to have variable effects on solute penetration, often employed in combination with these systems. In this study we evaluated the *in vitro* human epidermal penetration of a mixture of paraben ester preservatives from a commercially available test ointment and two commonly employed solvent vehicles (acetone and ethanol), together with the effect of occlusion on the rate of delivery from these systems. Parabens were applied as finite doses (5 mg per cm^2) to epidermal membranes mounted in horizontal Franz-type diffusion cells. At intervals of 2 h for a total of 10 h the receptor phase (20% ethanol in distilled water) was completely removed and replaced. Occlusion was effected by the placement of a piece of high density polyethylene (20 μm) over the application site immediately after dosing. Concentrations of parabens in receptor fluid and remaining in the epidermis at the end of the study were determined by high-performance liquid chromatography. There was a significant change in the epidermal flux of parabens from each of the vehicles following occlusion. Whereas increases were observed for the acetone and ethanol vehicles a decrease was seen following occlusion of the ointment formulation. Changes in flux appeared to result from a significant decrease in the epidermal partitioning of the esters following occlusion of the ointment and primarily by an increase in paraben epidermal diffusivity (estimated from changes in flux/retention) following occlusion of the solvent vehicles. These studies show that the effects of occlusion are strongly vehicle dependent, having wide implications for optimization of this technique with a range of topically applied solutes. ***Key words: vehicle occlusion. J Invest Dermatol 115:914–918, 2000***

Efficacy of topical allergy screening systems relies on the penetration of allergens from the applied vehicle through the stratum corneum (SC). Within the viable cells of the upper epidermis allergens come into contact with Langerhans cells and other mediators of the cutaneous immune response system (Bos, 1997). The ideal vehicle for patch test systems is far from optimized, being traditionally based on ease of use involving ointment or emulsion formulations incorporating empiric doses of allergens. Test site occlusion has also been suggested to provide better penetration of topical agents (Bucks *et al*, 1988; Qiao and Riviere, 1995). The predominant effect of occlusion is hydration, increasing the water content of SC from 5 to 15% to 50% (Potts, 1986). Occlusion was first suggested to increase the clinical efficacy of topical steroids (Garb, 1960; Scholtz, 1961; Sulzberger and Witten, 1961; McKenzie, 1962). Work in the 1980s with perfumes showed that, although increased penetration was observed for many structures, hydration enhancement of permeability was independent of solute lipophilicity; however, if hydration was merely decreasing the viscosity of the SC intercellular domain by increasing bilayer fluidity (Alonso *et al*, 1996), then penetration of all solutes should be equally enhanced, which is not the case. As enhancement appears to be compound specific, the most likely contribution of occlusion has been suggested to be the alteration in the partitioning of solutes between the SC and viable epidermis (Guy *et al*, 1987; Treffel *et al*, 1992). These authors, in finding that occlusion did not necessarily increase percutaneous penetration, also highlighted the need for further studies into the compound-specific effects of occlusion.

In this study we chose to examine the *in vitro* human epidermal penetration and retention of paraben esters (methyl, ethyl, propyl, and butyl), used widely as preservatives in cosmetics and topical formulations and to which approximately 1% of eczema sufferers demonstrate sensitivity. We compared a standard commercial test ointment with application of parabens in either acetone or ethanol, previously shown better than petrolatum in the elicitation of steroid hypersensitivity (Wilkinson and Beck, 1996), under open and occluded conditions.

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Table I. The effect of occlusion on the permeability, quantitated as initial flux and total amount penetrating epidermal membranes during the 10 h study period, of each paraben following topical application of mixtures in either the commercial ointment formulation, acetone or ethanol. Mean ± SE, n = 3–10

Paraben and vehicle	Unoccluded		Occluded			
	Initial flux (μg per cm^2 per h)	Total penetrating in 10 h (μg)	Initial flux (μg per cm^2 per h)	E*	Total penetrating in 10 h (μg)	E
Methylparaben						
Ointment	1.9 ± 0.1	27.0 ± 1.3	0.9 ± 0.1	0.5	11.9 ± 0.6	0.4
Acetone	7.2 ± 1.2	86.4 ± 15.7	49.0 ± 6.4	6.8	531.6 ± 68.6	6.2
Ethanol	8.0 ± 2.8	90.3 ± 28.3	51.5 ± 2.0	6.5	593.2 ± 43.0	6.6
Ethylparaben						
Ointment	7.3 ± 0.4	87.1 ± 6.0	2.2 ± 0.3	0.3	28.4 ± 3.1	0.3
Acetone	4.8 ± 0.9	57.6 ± 12.3	87.8 ± 0.5	18.4	976.7 ± 21.2	17.0
Ethanol	8.2 ± 3.0	93.1 ± 30.5	81.9 ± 4.4	10.0	894.7 ± 46.1	9.6
Propylparaben						
Ointment	6.7 ± 0.4	78.0 ± 5.8	1.9 ± 0.3	0.3	24.4 ± 3.1	0.3
Acetone	3.1 ± 0.8	36.8 ± 9.5	42.1 ± 0.7	13.6	494.6 ± 16.0	13.5
Ethanol	4.5 ± 1.8	50.2 ± 19.0	40.6 ± 2.0	9.1	450.1 ± 22.1	9.0
Butylparaben						
Ointment	6.6 ± 0.4	75.7 ± 6.2	1.9 ± 0.3	0.3	25.1 ± 2.8	0.3
Acetone	6.9 ± 1.6	84.8 ± 22.7	54.9 ± 3.5	7.9	650.1 ± 38.6	7.7
Ethanol	10.2 ± 4.8	111.3 ± 49.0	60.6 ± 2.6	6.0	684.3 ± 39.1	6.1

*E, enhancement ratio.

MATERIALS AND METHODS

Materials Paraben cosmetic test series no. 17, containing 3% of each paraben in an ointment base was obtained from Ralza (Perth, Australia). Acetone and ethanol were purchased from Sigma (Sydney, Australia). All other reagents were of analytical grade.

***In vitro* epidermal diffusion studies** Human epidermal membranes were prepared from female abdominal skin using the heat separation technique (Kligman and Christophers, 1963). Membranes were mounted, SC side uppermost, in horizontal Franz-type diffusion cells (surface area 1.3 cm^2) and the receptor chamber (3.5 ml) filled with degassed 20% ethanol in distilled water, which was continuously stirred with a magnetic flea. Diffusion cells were semisubmerged in a water bath (35 ± 0.1°C). Finite doses (5 mg per cm^2) of parabens in the chosen vehicle (ointment or saturated solutions in ethanol or acetone) were added to the donor chamber and gently spread over the skin surface. In occlusion studies a piece of high-density polyethylene (HDPE, 20 μm) was immediately placed over the membrane and clamped in place. At intervals of 2 h (for 10 h) the receptor phase was completely removed and replaced with fresh solution.

Paraben concentrations in the receptor phase were determined by high-performance liquid chromatography. At the end of the study any remaining donor solution/ointment was wiped from the surface of the skin and the epidermal membrane tape stripped once to remove any residual vehicle. Skin exposed to the donor vehicle was excised with a punch into preweighed vials, 0.5 ml ethanol was added and the samples minced with scissors, vortexed, and centrifuged. The supernatant was then assayed for paraben concentration.

Estimate of the effect of occlusion on epidermal membrane hydration

Uptake of 3H-water from receptor phase Water content increase with occlusion was estimated using a radiolabel tracer technique. Briefly, the uptake of 3H-water (Amersham Australia, Sydney, Australia) from the receptor phase (0.5 μCi per ml) into epidermal membranes was determined over 10 h. The degree of hydration was expressed relative to unoccluded epidermal membranes.

Transepidermal water loss (TEWL) Occlusivity of HDPE and ointment treatments was quantitated on the forearm skin of three human volunteers from relative changes in TEWL before and 30 min after application of either the HDPE membrane or ointment (5 mg per cm^2) and compared with untreated sites. TEWL measurements were taken in triplicate at 2 × 2 cm marked sites along the forearm using a Tewameter 210 (Courage and Khazaka, Köln, Germany). Relative changes in TEWL were calculated by dividing the measurement 30 min after treatment by pretreatment values.

Data analysis The flux of a solute (J, μg per cm^2 per h) through a membrane is defined as: J = (K.Cv.D)/h where K is the membrane/solvent partition coefficient, Cv is the concentration of solute in the vehicle, D is the diffusion coefficient of the solute in the membrane and h is the diffusional path length. In this study, estimations of paraben flux were made using the initial linear portions of the receptor concentration *vs* time curves by linear regression. Diffusivity (cm h−1), was approximated as J/Rm, where Rm is the epidermal membrane retention of the paraben, corrected to per cm^2.

RESULTS

Flux There was a significant change in initial flux and total amount of each paraben penetrating the epidermal membranes following occlusion (**Table I**). Increases were observed following occlusion of the volatile solvent vehicles, acetone and ethanol, but a significant decrease in penetration followed occlusion of the ointment.

Retention Rm of parabens from the commercial ointment was significantly decreased by occlusion (**Fig 1*A***). The decrease in Rm was, however, not linear. No significant change in paraben Rm was observed following occlusion of the acetone and ethanol vehicles (**Fig 1*B*, *C***).

Diffusivity Significant increases in paraben diffusivity were observed following occlusion of the acetone and ethanol vehicles (**Fig 2*B*, *C***), but not the ointment (**Fig 2*A***). The increases in diffusivity of those parabens seen with occlusion were linear and approximately 8- and 16-fold, respectively, for acetone and ethanol (**Fig 3**).

Hydration Although no statistical difference could be found between the total ^{3}H-water uptake into membranes occluded with HDPE, dosed with ointment or dosed with ointment and occluded with HDPE relative to unoccluded epidermis, **Fig 4(*A*)** shows that there was a trend towards greater hydration under the occluded membranes. The TEWL studies in volunteers, however, showed a statistically significant occlusion (ANOVA, $p < 0.01$) for both the ointment and HDPE-treated sites relative to untreated controls (**Fig 4*B***).

DISCUSSION

This study using the paraben ester series showed that, as well as the compound-specific variations reported previously, a vehicle-

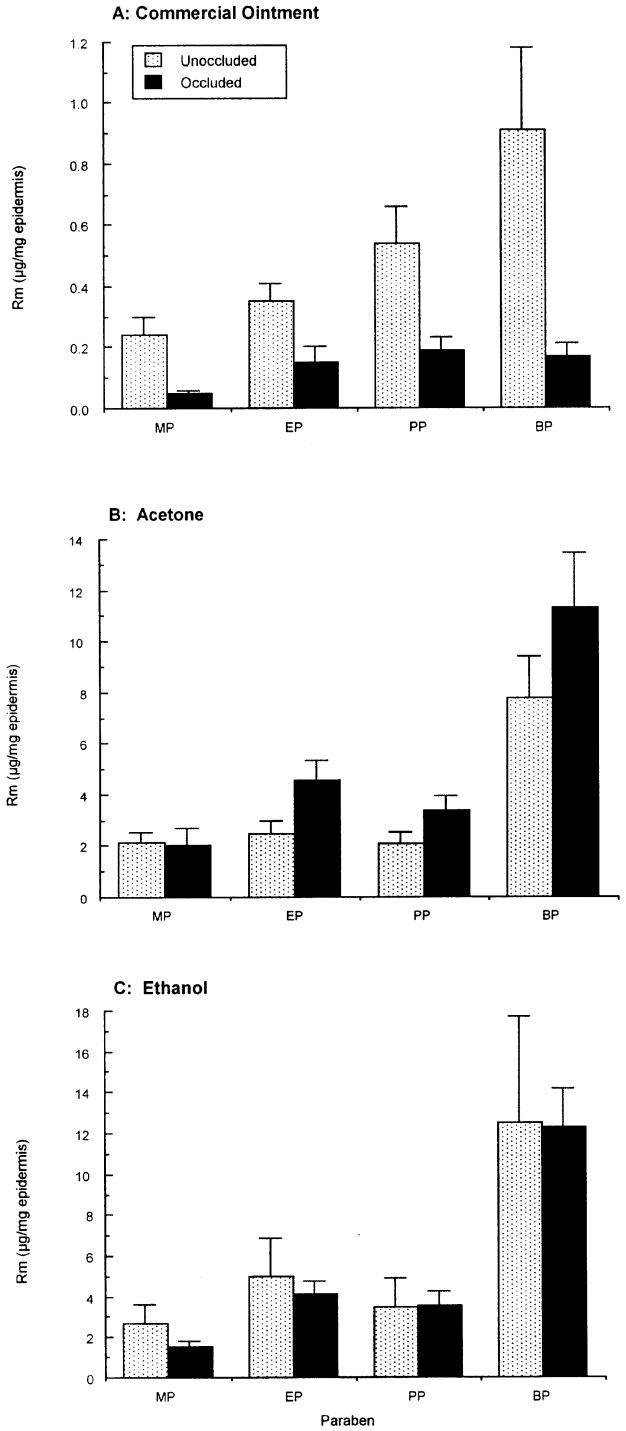

Figure 1. Epidermal retention of parabens. The effect of occlusion on the in-vitro epidermal retention (Rm, µg per mg tissue) of paraben esters (MP, methyl paraben; EP, ethylparaben; PP, propylparaben; and BP, butylparaben) following topical application of finite doses of: (*A*) a commercial ointment formulation; (*B*) a saturated solution in acetone; and (*C*) a saturated solution in ethanol. Mean ± SE, n = 3–10.

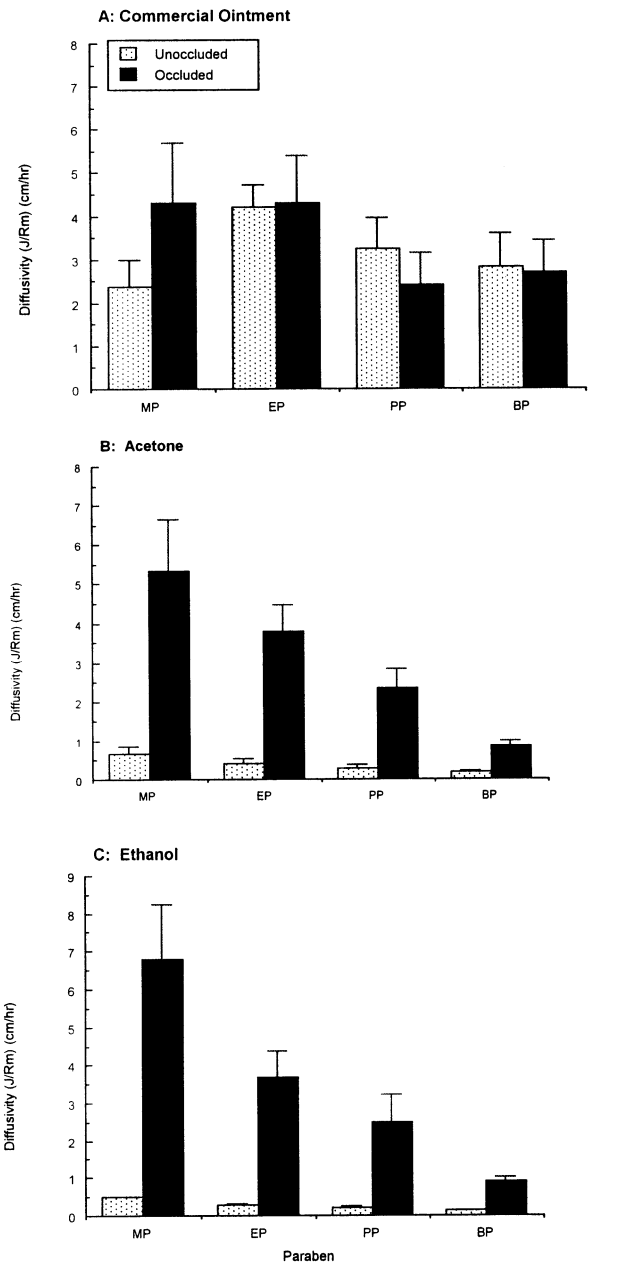

Figure 2. Estimated epidermal diffusivity of parabens. The effect of occlusion on the *in vitro* epidermal diffusivity (J per Rm) of paraben esters (MP, methyl paraben; EP, ethylparaben; PP, propylparaben; and BP, butylparaben) following topical application of finite doses of: (*A*) a commercial ointment formulation; (*B*) a saturated solution in acetone; and (*C*) a saturated solution in ethanol. Mean ± SE, n = 3–10.

dependent variation in relative epidermal penetration in the presence of occlusion occurs, with the occlusion of an ointment formulation even causing a significant retardation of penetration.

Mukherji *et al* (1994) reported no increase in the bioavailability of 2′,3′-dideoxyinosine, a nucleoside analog, compared with unoccluded controls following occlusion in rats. The log p octanol/water of 2′,3′-dideoxyinosine is approximately −1.2 therefore as relatively polar molecules, according to the SC-viable epidermis partitioning theories put forward by Treffel *et al* (1992) no great enhancement with occlusion would have been expected. The parabens however, are more lipophilic, log p-values 1.96–3.57, and their penetration would have been expected to have benefited from the change in partitioning between the SC and viable epidermis. These results suggest that occlusion must also be affecting the kinetics of release of the parabens from the ointment vehicle into the SC. Changes in paraben formulation release could occur either as a result of interactions, possible sorption, between the ointment and the HDPE used to occlude the area, or due to increases in the moisture layer between the ointment and the SC, effectively forming an extra hydrophilic layer into which the parabens must partition before entering the SC. The ^{3}H-water and TEWL studies show that the ointment also has occlusive properties (**Fig 4*A*, *B***) and, compared with unoccluded controls, confirming

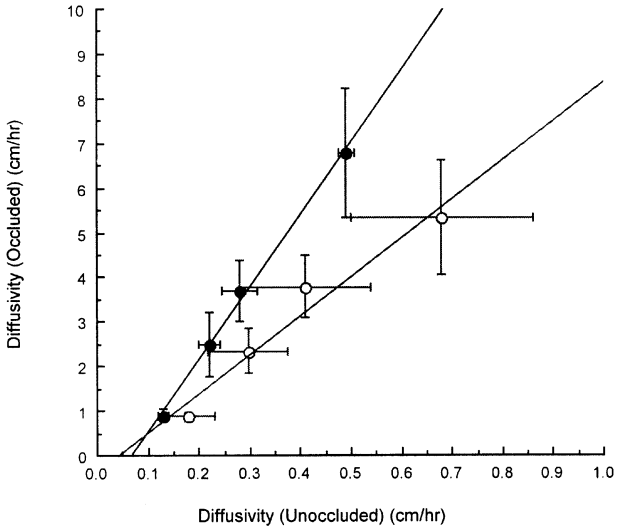

Figure 3. Relative diffusivity of esters ± occlusion. The linear increase observed in the diffusivity of paraben esters within the epidermis following occlusion of acetone (●) and ethanol (○) saturated solutions. Mean ± SE, n = 3–5.

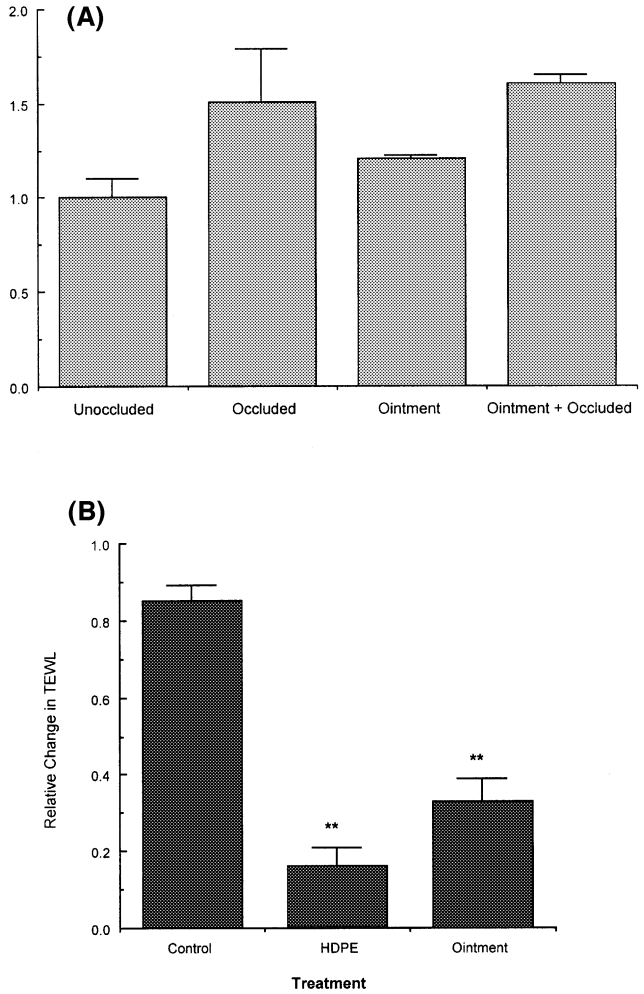

Figure 4. The effect of occlusions and formation on epidermal hydration and TEWL. (*A*) The hydration of epidermal membranes relative to unoccluded controls, estimated from ^{3}H-water content after 10 h and treatments of occlusion with HDPE, ointment application, or ointment application and occlusion (mean ± SE, n = 5). (*B*) The relative changes in TEWL observed on forearm sites in three volunteers treated with either ointment or HDPE compared with controls (mean ± SEM).

that moisture does not readily pass through the ointment layer and must accumulate within the skin or on the skins surface.

Differences in allergen penetration between vehicles was attributable interactions between the vehicles and the skin and not to variations in the concentration or thermodynamic activity of the paraben esters, as each was at saturation. In order to effect increases in epidermal penetration, an increase in either the amount of material partitioning into the epidermis or diffusivity within the epidermal membrane is required. In this study, we saw that occlusion had significantly different effects on both membrane partitioning and diffusivity following occlusion, depending on the vehicle in which they are applied. In the case of the ointment, a decrease in penetration following occlusion appeared to accompany a significant decrease in the epidermal retention (**Fig 1*A***), without any apparent change in paraben diffusivity within the epidermis (**Fig 2*A***). Whereas, the increased penetration observed with occlusion of both the acetone and ethanol vehicles did not reveal any significant change in epidermal retention, and therefore partitioning, but was associated with large increases in diffusivity within the epidermis. Differences in the effect of occlusion on the two types of vehicles, ointment and volatile solvents, lies in the ability of the solvent vehicles to enter the epidermal membrane. By occluding these solvents, not only is epidermal hydration likely to occur, but the amount of solvent diffusing into the SC will be dramatically increased as its evaporation is significantly reduced.

Ethanol was seen to have greater ability to enhance paraben epidermal diffusivity of the parabens under occlusion compared with acetone. The slopes of the penetration relationships, unoccluded *vs* occluded conditions (**Fig 3**) were 16.3 and 8.8 for the ethanol and acetone vehicles, respectively. The linearity in the unoccluded *vs* occluded diffusivity plot for the acetone and ethanol vehicles (**Fig 3**) also shows that the mobility of each of the parabens was equally enhanced by the effect of the solvent on the membrane. This finding suggests that an overall change in the SC barrier occurred which was not compound specific. This is consistent with the assumption that these solvents were acting by extracting lipids from within the SC (Grubauer *et al*, 1989; Goates and Knutson, 1994) creating a looser structure that allowed greater solute movement within the membrane. The results also indicate that the resultant fluidizing ability of ethanol is twice that of acetone for the passage of these particular compounds through the epidermis.

The increases in diffusivity were not, however, reflected to such an extent in the overall epidermal fluxes of the parabens applied in the two solvents with and without occlusion. Therefore, in the case of the acetone, the overall effect on flux of the paraben esters was being contributed to by both a small increase partitioning and a large change in diffusivity.

CONCLUSIONS

This study has shown that a significant change in the epidermal flux of allergens from ointment and solvent vehicles occurs following occlusion. Changes in flux appeared to result from a significant decrease in the epidermal partitioning of the esters following occlusion of the ointment and primarily by an increase in paraben epidermal diffusivity (estimated from changes in flux/retention) following occlusion of the solvent vehicles. The demonstration that the effects of occlusion are strongly vehicle dependent has wide implications for optimization of the use of occlusive techniques with a range of topically formulated solutes.

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