Clinical and laboratory studies

Effects of petrolatum on stratum corneum structure and function

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Background: Ointments (e.g., petrolatum) are thought to be occlusive, thereby blocking transcutaneous water loss and trapping water under the skin's surface. If this premise is correct, then petrolatum should delay barrier recovery after barrier perturbation, as shown previously in occluded murine skin.

Objective: We reexamined the assumption that Vaseline Petroleum Jelly (VPJ) is occlusive, ascertaining both its site and mechanism of action.

Methods: Barrier recovery was measured in VPJ-treated versus untreated sites after acetone-induced barrier disruption in human volunteers. Moreover, VPJ was localized within the stratum corneum (SC) with tracers and ruthenium tetroxide staining, which allowed visualization of the depth of VPJ penetration and its relation to intercellular membrane structures.

Results: VPJ accelerated, rather than impeded, barrier recovery. Moreover, VPJ was present within the interstices at all levels of the SC, where it replaced intercellular bilayers.

Conclusion: VPJ neither forms nor acts like an epicutaneous impermeable membrane; instead, it permeates throughout the SC interstices, allowing normal barrier recovery despite its occlusive properties.

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Because of its hydrating properties, petrolatum, a mixture of long-chain aliphatic hydrocarbons, has long been considered the standard against which other moisturizers are compared. Although it is generally assumed that petrolatum works by forming an occlusive layer on the outer surface of the stratum corneum, neither its site nor its mechanism of action in the epidermis is known. To date, most studies that have addressed the mechanism of action of petrolatum and other moisturizers have analyzed their effects on normal skin. To achieve greater relevance, such studies should be performed after various types of acute or chronic skin damage, which represents the more relevant clinical setting in which moisturizers are used. Moreover, it may not be justifiable to extend conclusions drawn from normal skin to acutely damaged, chronically irritated, or "sensitive" skin. Furthermore, evaluation of the dynamics of barrier repair after experimental perturbation, with or without moisturizer treatment, would bring out functional differences that might otherwise not be apparent under basal conditions. To date, this approach has been applied to detergent-damaged skin, but detergent treatment evokes a series of potentially unrelated responses attributable to lipid extraction, toxicity, and/or cell removal. In contrast, applications of organic solvents, such as acetone, to the skin (1) selectively remove stratum corneum lipids, (2) are accompanied by minimal cytotoxicity, and (3) induce a well-characterized repair response.

Despite the acknowledged importance of the intercellular lamellar bilayers of the stratum corneum for barrier function, knowledge about their structure was limited until the application of ruthenium tetroxide fixation, in lieu of osmium tetroxide, resulted in clear images of these bilayers at all levels of the stratum corneum. To date, the ruthenium technique has been used to characterize the mem-
brane structural alterations in essential fatty acid deficiency and in other human and rodent disorders of cornification. This method should possess equally great potential for the study of the site and structural basis of moisturizer activity in the stratum corneum. Therefore in this study we have both assessed the impact of petrolatum on barrier recovery in acetone-treated human and murine skin and localized this agent in normal and acetone-treated murine stratum corneum by histochemistry and electron microscopy.

MATERIAL AND METHODS
Experimental protocols

Five human volunteers, with no history of skin disease, participated in this study after first providing informed consent (protocol approved by the University of California San Francisco Human Research Committee). Contralateral sites on the volar aspect of each forearm were treated with acetone-soaked cotton balls with gentle rolling until elevated rates of transepidermal water loss (TEWL) occurred (range 16.5 to 39.7 gm/m²/hr), as measured with the evaporimeter. During the course of all experiments, the relative humidity range was 42% ± 4%, and the temperature remained at approximately 22° ± 1° C. Although the EP1 Evaporimeter (Servomed, Stockholm, Sweden) is a widely used method for measuring TEWL, it does not always distinguish water content from transcutaneous water loss, that is, barrier function. In contrast, the electrolytic water analyzer measures only TEWL but is relatively cumbersome and time-consuming. Therefore TEWL was measured by two methods. With the evaporimeter, readings were taken 30 seconds after application of the probe to the skin. In four of the five subjects measurements also were performed simultaneously with an electrolytic water analyzer (Mseco, Inc., Warrington, Pa.), as described previously.

Three acetone-treated areas, each exhibiting TEWL rates in excess of 16 gm/m²/hr, were marked on each forearm. A thin layer of petrolatum (Vaseline Petroleum Jelly) was applied to all three sites in one area; the three sites on the contralateral arm were left untreated. TEWL measurements were made immediately after barrier perturbation, and 3, 6, 24, 48, 72, and 96 hours after barrier perturbation at all three sites on both the treated and control sides. Petrolatum was reapplied after each measurement as well as each morning and night. Measurements were taken at least 3 hours after the most recent application of petrolatum at which time there was no evidence of residual petrolatum on the skin surface. Subjects were allowed to bathe as usual, but they avoided the direct application of soap and other moisturizers or emollients to study areas.

Finally, we assessed the site-to-site variation in both the rates of normal barrier recovery and the response to petrolatum applications. Two human subjects were treated with acetone over both the arms (as already described) and the shins. One side was then treated with petrolatum; the opposite side was left untreated.

Effects of exogenous petrolatum versus endogenous lipids on barrier repair

Studies in hairless mice have shown that cooling of the skin with ice water completely blocks barrier recovery after acetone treatment. This model then allows a comparison of exogenous lipids alone on barrier function.

To distinguish the exogenous versus endogenous components of petrolatum-induced barrier recovery, we also compared barrier recovery after acetone treatment in (1) mice whose skin was allowed to recover (up to 24 hours) otherwise normally; (2) mice in which the skin was exposed to ice immediately after application of petrolatum for 1 hour and thereafter observed for 6 hours at room temperature; and (3) a group allowed to recover at room temperature with petrolatum treatment. Petrolatum was gently applied to the skin immediately after acute barrier disruption with acetone, and the treated areas then were chilled with topically applied ice-cubes (3.0 cm²; skin temperature reduced to ~ 10° C). After ice exposure for 1 hour, the skin was blotted lightly with an absorbent tissue to remove remaining droplets of water and the ice-exposed side was warmed for 15 minutes to normal skin temperatures (32°C) to allow bound water to evaporate from the stratum corneum before TEWL measurements were performed. Pilot studies showed that TEWL in normal hairless mice (n = 5) returned to normal 10 to 15 minutes after exposure to ice for 1 hour.

Statistical comparisons in all human and animal studies were performed with the paired t test.

Light microscopy

To assess the depth of penetration of petrolatum into normal murine epidermis, two different types of tracers were suspended in the petrolatum immediately before topical application (not treated with acetone): (1) the lipid-soluble dye, fat red 7B (25% by volume); and (2) a water-soluble tracer, lead nitrate (25% by volume; both stains from Sigma Chemical Co., St. Louis, Mo.). Control sites either were treated with an aqueous suspension of the tracer (no petrolatum) or left untreated. Applications were repeated twice daily for 3 days to one flank of normal hairless mice (hr/hr, Simonsen Laboratories, Gilroy, Calif.), and the opposite flank was treated an equivalent number of times with the control preparation.

After 3 days, mice were killed by cervical dislocation. Specimens were embedded in OCT Compound (Miles Scientific, Naperville, Ill.) and frozen sections of unfixed skin (6 to 10 μm) were prepared with a Tissue-Tek RII cryostat and examined under a Leitz Ortholux II light microscope. Specimens in the petrolatum with fat red 7B group were examined directly after first counterstaining.
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Fig. 1. Effect of petrolatum on barrier recovery. Note accelerated rates of barrier recovery after repeated applications of petrolatum after acute barrier disruption with acetone. Measurements of TEWL were made with the Servomed Evaporimeter. Data points shown represent the mean ± standard error of the mean (n = 5). Asterisks indicate p < 0.05 with the paired t test.

with hematoxylin. Petrolatum with lead nitrate specimens were treated first with dilute ammonium sulfide to convert colorless lead to brown precipitates of lead sulfide and then counterstained with hematoxylin.

To assess penetration of petrolatum into perturbed versus intact skin, a second set of mice were treated on one flank with acetone until TEWL rates exceeded 5.0 mg/cm²/hr, followed immediately by a single application of petrolatum, whereas the opposite flank was left untreated. Samples were taken for light and electron microscopy 2 and 6 hours after petrolatum applications to acetone-treated versus untreated sites.

**Electron microscopy**

Biopsy specimens from petrolatum with lead nitrate-treated versus control mice (non-acetone-treated) were processed for electron microscopy. Samples were minced in pieces 1 mm³ or smaller and fixed overnight (16 hours) at 4°C in 2% glutaraldehyde, 2% paraformaldehyde with 0.06% calcium chloride in 0.1 mol/L sodium cacodylate buffer, pH 7.3. Specimens then were washed in 0.1 mol/L sodium cacodylate buffer before further processing. Tissue sections were placed in either (1) 0.2% ruthenium tetroxide (Polysciences, Warrington, Pa.) with 0.5% potassium ferrocyanide in 0.1 mol/L sodium cacodylate, pH 7.4, at room temperature in the dark for 0.5 hour; or (2) 1% osmium tetroxide with potassium ferrocyanide (1.5%) in 0.1 mol/L sodium cacodylate at room temperature in the dark for 1 hour. After rinsing in cacodylate buffer, tissue samples were dehydrated in a graded ethanol series and subsequently embedded in a low viscosity epoxy resin containing DER 736 and Epon 812. Thin sections were examined both unstained and/or after staining with uranyl acetate and lead citrate in a Zeiss 10A electron microscope.

**RESULTS**

**Assessment of barrier function by evaporimetry versus electrolytic water analysis**

A comparison of data obtained with the evaporimeter and the electrolytic water analyzer in the human subjects showed close correlation (r = 0.807, p < 0.025) (data not shown). Furthermore, com-
Thereafter the slope of the recovery curve decreases, and barrier repair proceeds more slowly. Complete normalization of barrier function requires from 5 to 7 days (data for last time points not shown).

**Effect of petrolatum treatment.** We next assessed the effects of repeated topical applications of petrolatum on barrier repair in acetone-perturbed skin. The differences in petrolatum-treated vs. control groups were most apparent in the early phases of barrier repair. As shown in Fig. 1, a significant improvement of barrier function was evident at 6, 24, and 48 hours ($p < 0.05$). At 72 hours, there were still some apparent differences in recovery rates between petrolatum-treated mice and controls but the number of data points was too small to allow statistical analysis. Thus petrolatum enhances the initial stages of barrier repair after acute injury, an effect that becomes less noticeable at later stages of barrier repair.

**Site-to-site variation.** To determine the site-to-site variation of the above finding, the same experiment was performed simultaneously on both the anterior aspect of the legs and the volar forearms of three subjects. As seen in Fig. 2, petrolatum also improved barrier function in the leg sites just as in the volar forearms.

**Fig. 2. Effect of petrolatum on barrier recovery: site-to-site variation.** Recovery rates are compared for forearm and shin for two subjects. Data represent mean of three different sites on each subject. Petrolatum application resulted in more rapid recovery on the leg as well as the arm.

A comparison of the recovery curves on both petrolatum-treated and control sides were comparable with either method (data not shown). Therefore, because of its relative ease of operation, the Servomed evaporimeter was used in subsequent experiments with the human subjects.

**Barrier recovery after acetone treatment in human subjects**

**Untreated controls.** When the barrier was disrupted in normal human skin, baseline TEWL measurements immediately after acetone applications ranged from 16.5 to $39.7 \text{ gm/m}^2/\text{hr}$. Baseline values before acetone treatment ranged from 1.8 to $4.0 \text{ gm/m}^2/\text{hr}$. In normal human skin, acetone treatment is followed by a rapid recovery phase, leading to 70% recovery by 72 hours (Fig. 1). Thereafter the slope of the recovery curve decreases, and barrier repair proceeds more slowly. Complete normalization of barrier function requires from 5 to 7 days (data for last time points not shown).

**Barrier recovery after acetone treatment in hairless mouse: effects of exogenous lipids**

By a comparison of barrier repair in precooled versus noncooled skin, it is possible to assess separately the occlusive effects of petrolatum without any additive (or negative) impact on barrier repair mediated by endogenous lipid synthesis. As seen in Fig. 3 (curve a), petrolatum dramatically reduced TEWL immediately after application. In addition, petrolatum did not interfere with normal recovery: When petrolatum was applied to acetone-treated, cooled skin, a positive effect also was seen at early time points, followed by a slight deterioration of the barrier during the next 2 to 3 hours (curve b). Thereafter, barrier function recovers in parallel with the recovery rate for acetone-treated animals left at room temperature. These data demonstrate that petrolatum produces a dramatic reduction in TEWL immediately after application without any influence from endogenous lipid synthesis and does not impede barrier recovery in murine epidermis, as shown for human epidermis (see Fig. 1).
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Fig. 3. Comparison of effect of petrolatum in acetone-treated murine skin (with or without cooling). Barrier recovery after single topical application of petrolatum (curve a) and petrolatum followed by cooling on ice for 1 hour (curve b). Curve c shows normal recovery curve after acetone treatment plus air exposure. Note that application of petrolatum significantly (p < 0.001) reduces TEWL even when increased lipid synthesis is blocked by cooling (time point, 1.25 hours). After warming, TEWL recovery rates decrease insignificantly during the next 2 to 3 hours (p < 0.10); thereafter they increase in parallel with the air-exposed control (endogenous lipid synthesis allowed to occur from time 0). Recovery rates in curve a represent early occlusive effect of curve b plus repair from normal or supernormal endogenous lipid synthesis (curve c; p < 0.02 at all time points).

Depth of penetration of petrolatum in stratum corneum

**Application of fat 7B in petrolatum.** After application of petrolatum, containing the lipid-soluble tracer fat red 7, to normal hairless mice twice daily for 3 days, red dye could be visualized in membrane domains at all levels of the stratum corneum (Fig. 4, A). However, no dye was visible within the nucleated cell layers of the epidermis. When an aqueous suspension of the dye was applied to one flank, the whole mouse soon appeared bright red on gross examination. However, in contrast to the petrolatum-suspended dye, microscopic examination revealed that the dye penetrated solely into the pilosebaceous units with no evidence of permeation into the stratum corneum intercellular spaces of the interfollicular epidermis (Fig. 4, B). These studies suggest that repeated applications of petrolatum lead to permeation of this substance throughout the intercellular domains of the stratum corneum.

**Application of lead nitrate in petrolatum.** Lead is a water-soluble tracer, employed both at a light and an electron microscopic level to assess diffusional pathways. Therefore, we used this technique to independently substantiate the penetration of petrolatum to different levels within the stratum corneum. By light microscopy, brown to black precipitates of lead sulfide were visible in the upper layers of the stratum corneum, with extension evident focally to depths as low as one cell layer above the stratum corneum–stratum granulosum interface (Fig. 5). Again, no precipitate was visualized within the nu-
Fig. 4. Application of fat red 7B in petrolatum. A, Twice-daily application for 3 days of fat red 7B, suspended in petrolatum resulted in presence of red dye in membrane domains at all levels of stratum corneum. B, In contrast, when dye was applied as aqueous suspension, stain appeared only within pilosebaceous orifices, and in untreated controls no staining is seen in the stratum corneum (C). (Counter-stained with hematoxylin; X400.)

Fig. 5. Application of lead nitrate in petrolatum. Lead was precipitated with ammonium sulfide and sections were counterstained with hematoxylin. Precipitates of lead sulfide are seen within all layers of stratum corneum. However, no precipitate is evident in nucleated layers of epidermis. (X400.)

Ultrastructural localization of petrolatum

Both to assess the depth of penetration of petrolatum and to determine its subcellular location in the stratum corneum, samples were examined by electron microscopy after both osmium tetroxide and ruthenium tetroxide fixation. As anticipated, osmium tetroxide fixation of petrolatum-treated (non-acetone-perturbed) skin did not reveal the presence of either membrane bilayers or stainable lipid in murine stratum corneum. In contrast, ruthenium tetroxide fixation of control, petrolatum-treated skin revealed large quantities of flocculent, moderately electron-dense material limited to the stratum corneum intercellular spaces and extending to the lower layers of the stratum corneum (Fig. 6, A). Moreover, the normally compact intercellular domains, characteristic of untreated stratum corneum, appeared greatly expanded because of separation of individual bilayer structures. Likewise, abundant flocculent, electron-dense, amorphous material was also seen at all levels of acetone-treated stratum corneum, both 2 and 6 hours after petrolatum application (Fig. 6, B). However, comparable electron-dense material was not visible in untreated controls (Fig. 6, C), in agreement with previous descriptions of ruthenium-stained murine stratum corneum. In acetone-treated and untreated stratum corneum.

As can be seen in Fig. 6, A, petrolatum induced cleft formation within the intercellular lamellar bilayers. Moreover, these clefts could be seen to span, and in some cases to obliterate, both electron-lucent and electron-dense lamellae, inducing the formation of progressively larger lacunae within the membrane bilayers.

Addition of lead nitrate as an electron-dense tracer to the petrolatum confirmed that the electron-dense material represents petrolatum (Fig. 7). As in
Fig. 6. A, Ruthenium tetroxide staining of petrolatum-murine stratum corneum. Note expansions of intercellular space (brackets), filled with flocculent, amorphous material at several levels within the stratum corneum (asterisks). (×60,000.) B, Ruthenium tetroxide staining of petrolatum treated–acetone wiped murine stratum corneum. Note similar expansions of intercellular space with excess amounts of lipid material within the space (arrows). C, Ruthenium tetroxide staining of normal murine stratum corneum. Normal bilayer structure consists of alternating lucent and dense bands (arrows). Electron-dense foci in control samples correspond to desmosomes and/or sites of desmosomal degradation.¹⁰ (×40,000.)
Fig. 7. Ruthenium tetroxide staining of lead-containing, petrolatum-treated murine stratum corneum. Note expansion of intercellular space by large amounts of nonlamellar, floculent material (asterisks), with lamellar bilayers displaced to one side of intercellular spaces. In addition, note lead deposits decorating lamellar bilayers (arrows). (×80,000.)

the non-lead-containing samples (Fig. 6, A and B), petrolatum caused expansion of intercellular domains, permeating to as low as one layer above the stratum granulosum–stratum corneum interface. However, no tracer reached the nucleated layers of the epidermis or the stratum granulosum–stratum corneum interface. These observations confirm that petrolatum reaches all levels of the stratum corneum and that petrolatum penetrates through the stratum corneum via intercellular domains.

Finally, the lead-labeling experiments provided still further insights into the possible mechanisms of petrolatum action. First, in some cases the petrolatum itself demonstrated a lamellar substructure. Because this pattern was noted only in lead tracer-containing petrolatum (Fig. 7), the lamellae could represent intercellular membrane bilayers in various stages of dispersal and/or fragmentation. Second, the lead-labeled petrolatum often segregated into extensive nonlamellar domains; that is, the petrolatum appeared to produce phase-separation, inducing the formation of distinct lamellar and nonlamellar domains in the intercellular spaces (Fig. 7).

Finally, close examination of the apparently undisturbed lamellar domains revealed lead precipitates at frequent intervals within the compact multilayers (Fig. 7). This may correspond either to lead nitrate molecules that have partitioned out of the petrolatum into the lamellar bilayer system or to the addition of small amounts of petrolatum (plus lead) to the lamellar domains. These tracer studies provide additional information about the relation of topically applied petrolatum to the intercellular lamellar bilayers in the stratum corneum.

DISCUSSION

Our results showed a close correlation between evaporimetry and electrolytic water analysis. Therefore they confirm earlier reports that, despite concerns about issues of stratum corneum water content versus water loss, evaporimetry provides reliable data about cutaneous barrier function in the perturbed state.13, 15 Because evaporimetry is more rapid, more portable, and can be used to measure TEWL from any exposed surface, it may be preferable to electrolytic water analysis for most evalua-
tions of barrier recovery in humans and experimental animals.

Previous studies have suggested that petrolatum exerts its effects on skin function by forming an inert, epicutaneous, occlusive membrane (reviewed in Flesch\(^1\)). Indeed, a prior in vitro study that used the ventral skin of hamster ears in a diffusion chamber demonstrated decreased transcutaneous water flux during a 24-hour period after applications of petrolatum.\(^3\) However, a thick, epicutaneous layer of petrolatum on normal, undisturbed skin in a diffusion chamber is unlikely to reflect the in vivo situation, in which the amount of moisturizer quickly diminishes because of absorption, contact with other surfaces, and washing of the skin. An occlusive effect also was suggested in one in vivo study, in which a single application of petrolatum to normal skin caused TEWL rates to fall from 0.5 to 0.1 gm/m\(^2\)/hr, an effect that disappeared by 2 hours after application.\(^4\) Likewise, we showed previously that a single application of petrolatum to acetone-treated murine skin significantly decreased TEWL rates,\(^6\) as the data in Fig. 5 demonstrate again in greater detail.

These studies also show that, despite being occlusive, petrolatum does not impede and may improve barrier function. If petrolatum were solely occlusive, it would be expected to impair rather than improve barrier function. Grubauer et al.\(^18\) showed that applications of vapor-impermeable membranes prevented the recovery of barrier function and blocked the return of stainable lipids to the stratum corneum after acetone treatment. Moreover, vapor-permeable wraps, such as Op-Site or Gore-Tex, permit barrier recovery but lipid biosynthetic rates are slower than those in skin exposed to air alone.\(^18\) In contrast, although petrolatum applications to acetone-treated murine skin also reduced TEWL rates to near normal levels,\(^6\) we have shown here that petrolatum does not interfere with and may actually improve (rather than impair) barrier function in human skin in comparison to sites left untreated and exposed to air. These ameliorative and seemingly paradoxical effects of petrolatum on barrier recovery are consistent with our earlier observation of stimulation of lipid biosynthesis in acetone-treated murine skin after petrolatum applications.\(^5\) Thus the most striking finding of these studies is that petrolatum neither inhibits nor delays endogenous processes that lead to barrier recovery. Moreover, our data are inconsistent with current concepts of petrolatum as solely forming an inert, occlusive layer on the outer surface of the skin. Furthermore, the early improvement of barrier function may have important clinical and therapeutic implications. Such a high degree of protection may reduce the risk of both further injury and superinfection. Petrolatum comprises a mixture of aliphatic hydrocarbons not synthesized by mammalian tissues. Aliphatic hydrocarbons provide an effective barrier in terrestrial plants and insects.\(^19\) Hence the ability of petrolatum to form an effective barrier in damaged human and murine skin may involve mechanisms similar to those operative in these unrelated taxa (see later).

Our search of the literature for information about the localization of petrolatum in the stratum corneum bore no fruit. Hydrophobic dyes, such as fat red 7B, partition into neutral lipids, including aliphane-containing mixtures such as petrolatum. Light microscopy of fat red 7B suspended in petrolatum demonstrated dye throughout the stratum corneum whereas fat red 7 in aqueous suspensions did not penetrate the stratum corneum. Moreover, the water-soluble tracer, lead nitrate, when suspended in petrolatum, also extended in a patchy distribution down to one layer above the stratum corneum–stratum granulosum interface. Thus these histochemical findings also support the concept that petrolatum does not simply form an inert, epicutaneous covering, but rather that it permeates to all levels of the stratum corneum.

However, the light microscopic histochemical stains themselves do not totally establish the depth of permeation of petrolatum because either or both of the tracers could, in part, partition out of the petrolatum carrier into the almost equally hydrophobic mixture of stratum corneum intercellular lipids. Thus it remains theoretically possible that the petrolatum could simply act as a vehicle, remaining in the outer layers of the stratum corneum, while the stain could penetrate toward the depths of this layer, independent of the petrolatum carrier. Hence to gain further insights into both the extent and the pathway of petrolatum penetration, we performed ultrastructural studies of petrolatum-treated versus control sites. Not surprisingly, osmium tetroxide staining did not provide information about the subcellular localization of petrolatum, because osmium tetroxide typically does not deposit the relatively hydrophobic, lamellar bilayers in the mid to upper stratum corneum.\(^9,10\) However, ruthenium tetroxide, with and without added lead tracer, provided further proof that petrolatum penetrates to the depths of the stratum corneum and additional, potentially important insights into possible mechanisms of action of petrolatum in the stratum corneum. With ruthe-
nium staining, flocculent, electron-dense material, was found in foci and clefts within the intercellular species at all levels of the stratum corneum. We conclude that this material represents petrolatum because (1) it is not present in controls; (2) the lead label was localized to the electron-dense material and absent in ruthenium-stained controls; and (3) this material is not osmiophilic, but still reactive with a more powerful oxidizing agent, ruthenium tetroxide. In vitro studies have shown that ruthenium tetroxide, but not osmium tetroxide, will react with paraffins, such as those in petrolatum.

However, despite its intercellular location, the bulk of the ruthenium-stained material did not appear to be incorporated into the lamellar bilayers. Instead, the electron-dense material formed clefts by (1) splitting hydrophilic and hydrophobic lamellae; (2) forming a separate nonlamellar phase, which appeared to displace contiguous, intact bilayers; and/or (3) apparently obliterating one or more lamellae during the formation of clefts within the lamellar multilayers. Nevertheless, it seems clear that petrolatum is localized to intercellular domains and that it interacts with the bilayer system in some loci, while in other locations it forms separate nonlamellar domains. If the petrolatum in the latter sites contributes to the improved barrier function after acetone treatment, then this would suggest that certain intercellular lipids can form an effective barrier, independent of a lamellar arrangement. This observation would be consistent with the observation that n-alkanes (i.e., long-chain hydrocarbons, such as those found in petrolatum), deposited both in bulk and without a visible lamellar substructure, provide an effective barrier in both insect and plant cuticle. Indeed, the demonstration that petrolatum in part incorporated within the bilayer structure is compatible with the concept that attributes barrier function to the location and organization, rather than the composition, of the intercellular bilayers. However, it should be noted that many recent studies have shown that specific lipids, for example, cholesterol, fatty acids, and sphingolipids, appear to be required for barrier homeostasis.

REFERENCES