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Aspects of the transdermal delivery of prostaglandins

Adam C. Watkinson [1], Jonathan Hadgraft [1] and Alan Bye [2]

[1] *The Welsh School Of Pharmacy, University of Wales, Cardiff (U.K.) and* [2] *Upjohn Ltd, Fleming Way, Crawley, W. Sussex (U.K.)*

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Summary

The skin penetration of prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ has been studied in vitro in human skin. The absorption rates are influenced by the presence of the enhancers Azone® and Transcutol. The results indicated different potential interactions which were structure related. These were further investigated using both bilayers and monolayers of dipalmitoylphosphatidylcholine (DPPC) to mimic stratum corneum lipids. Pretreatment of skin with both Azone and Transcutol was found to facilitate drug fluxes. This was especially the case with the 2-series prostaglandins where, both with, and without enhancer pretreatment, the actual amounts penetrating were greater than for the 1-series. A bilayer model (DPPC multilamellar vesicles) indicated that the 2-series compounds have a greater disruptive effect on structured lipids than their 1-series counterparts. Monolayer experiments demonstrated the mechanism of disruption to be non-penetrative, implying interaction between the 2-series prostaglandins and the DPPC head-group region. The major barrier to prostaglandin penetration appears to reside in the lipid region of the stratum corneum bilayers and the 2-series prostaglandins may interact with polar head-groups in this region in such a way as to ease their passage through them. The reason why the 1-series prostaglandins are absorbed comparatively poorly probably lies in their lack of compatibility with the skin membrane constituents and their consequent inability to interact with and destabilize the head-group regions of the stratum corneum lipid bilayers.

Introduction

Prostaglandins are a large and much investigated group of compounds known to have a wide variety of physiological effects (Oesterling et al., 1972). However, clinical application of their recognized therapeutic potential has been hindered by factors such as the absence of oral efficacy (due to extreme first pass metabolism), a limited duration of action and a lack of specificity. These factors have initiated recent investigation into the delivery of prostaglandins via the transdermal route (Chan et al., 1983; Spilman et al., 1984; Crandall et al., 1985; Eriksson et al., 1988; Nicolau et al., 1989).

The purpose of the present investigation was to examine the factors influencing the percutaneous delivery of this group of compounds by the use of E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ as representative compounds. It was considered that the structural and consequent physicochemical trends within

Correspondence: J. Hadgraft, The Welsh School of Pharmacy, University of Wales, Cardiff, U.K.

this group might be reflected in their interactions with and passage through human skin.

The passive and enhanced (skin pretreatment with Azone® and Transcutol) permeation of the model compounds through excised human skin has been studied in vitro. Light scattering and Langmuir film techniques were utilized to investigate any interactions between prostaglandin and the structured lipids in liposomes and monolayers of DPPC, respectively.

Materials and Methods

Materials

DL-α-Dipalmitoylphosphatidylcholine (DPPC), 2-bromo-4′-nitroacetophenone and *N*,*N*-diisopropylethylamine were purchased from Sigma. Azone, Transcutol and prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ were gifts from Whitby Research Inc., Gattefosse and The Upjohn Co., respectively. The subphase water used for the Langmuir trough work was double distilled from an all-glass apparatus and further purified by a Milli-Q Plus filtration unit (Millipore).

Permeation studies using excised human skin

The diffusion of prostaglandins across excised human skin (obtained mostly from abdominal surgical reductions) was studied using a static diffusion cell based on the Franz design. Dermatomed skin (300 μm) was placed between the donor and receptor compartments of the diffusion cell (cross-sectional area ~ 0.5 cm^2) and clamped into position. The receptor phase in the case of the 2-series prostaglandins was approx. 13.5 ml filtered pH 7.4 isotonic phosphate-buffered saline (PBS). Because of the lower water solubility of the 1-series prostaglandins the receptor phase used contained 20% ethanol in pH 7.4 PBS. The cells were placed on a multi-position stirring block in a water bath set at 37 °C. The skin surface temperature was found to be 32 ± 0.5 °C. Before any compound was applied to the skin the cells were allowed to equilibrate for 1 h in the water bath.

Stratum corneum for diffusion experiments was obtained by placing full-thickness skin on a filter paper soaked with 0.5% trypsin solution in pH 7.4 buffer for 24 h. The stratum corneum was then peeled away from the underlying tissue and soaked in 0.01% trypsin inhibitor for 10 min. After washing the stratum corneum in distilled water to remove any remaining epidermal cells it was mounted in diffusion cells on a plastic gauze for support. Diffusion experiments were then carried out as outlined for dermatomed skin.

Solutions (either the enhancer pre-treatments or drugs) were applied to the skin in 50 μl of ethanol which evaporated within 3 h of application leaving a film of drug and/or the pre-treatment constituents on the surface (the composition of the pretreatments being as follows: control, ethanol; Azone, 1% (v/v) Azone/ethanol; Transcutol, 18% (w/v) Transcutol/ethanol; Azone/Transcutol, 1% (v/v) Azone and 18% (w/v) Transcutol/ethanol). Samples (0.5 ml) were removed from the receptor phase via a covered sampling arm at regular intervals over a 48 h period (this was considered the maximum time period permissible before PG degradation became an important factor and the barrier function of the skin was compromised) and replaced by fresh pre-thermostatted pH 7.4 PBS. The samples were frozen and stored at −20 °C until assay by the HPLC methods outlined below.

Analysis

Prostaglandins E_1 and E_2 The method used was based on that of Herman et al. (1987) and relies on conversion of the prostaglandins to PGB_2.

A 0.5 ml sample of receptor phase was extracted into chloroform (E_2) or ethyl acetate (E_1) after acidification. The solvent was pooled and removed gently in vacuo, the residue then being reacted with NaOH in ethanol. The pH of the reaction mixture was then reduced to 3.0 by the addition of a few drops of 2 M HCl and extracted into chloroform (E_2) or ethyl acetate (E_1).

The solvent volume was reduced to approx. 1 ml in vacuo, transferred to a vial and the rest of the solvent removed under a stream of nitrogen gas. HPLC was carried out on a Zorbax ODS column (4.6 mm × 25 cm) with a mobile phase of acetonitrile/water/acetic acid (70:30:1) at a

flow rate of 0.5 ml min^{-1} (E_2) and 1.0 ml min^{-1} (E_1). UV detection was carried out at 280 nm.

Prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ The method used relies on the conversion of the prostaglandins to their respective *p*-nitrophenacyl esters and is based on a method of Roseman et al. (1976).

Initial sample isolation was carried out as above using chloroform ($F_{2\alpha}$) and ethyl acetate ($F_{1\alpha}$) as extraction solvents. Samples for HPLC were reacted with a solution of 2-bromo-4'-nitroacetophenone in acetonitrile in the presence of a base (diisopropylethylamine) for 15 min. The HPLC system used consisted of an Apex silica (5 μm) column (Jones Chromatography) with a mobile phase of chloroform/acetonitrile/water (50:50:1) provided at a flow rate of 1.0 ml min^{-1}. UV detection was carried out at 254 nm.

Preparation of multilamellar vesicles (MLV) and determination of phase transition temperature (T_m)

MLV were prepared by the following method: DPPC (0.5 mg ml^{-1}) in chloroform was mixed with solutions of prostaglandin (in chloroform for E_2 and $F_{2\alpha}$ and in ether for E_1 and $F_{1\alpha}$) in a 5 ml round-bottomed flask to yield the relevant molar ratio of lipid to drug. The solvent was then removed in vacuo producing a thin film around the flask. Hydration of the lipid films was carried out with 3 ml of water, about 5 min prior to use (to minimise prostaglandin degradation), by repeatedly heating the mixture above the phase transition temperature ($\sim 42\,^{\circ}C$) and cooling in ice.

The liposomal suspensions were cooled in ice prior to loading into a stirred, jacketed cuvette where the absorbance was monitored at 500 nm using a spectrophotometer. A temperature/time gradient of approx. $10\,^{\circ}C\ min^{-1}$ was produced by passing water heated to $60\,^{\circ}C$ through the cuvette jacket whilst the temperature within the cuvette was monitored with a thermocouple. The equipment was interfaced to a computer as described elsewhere (Beastall et al., 1988).

As the temperature of the liposomal suspension is increased the absorbance slowly decreases until there is a sharp drop which marks the phase transition. The value of T_m was taken as being the intersection of tangents to the absorbance/temperature curve.

Measurement of DPPC pressure/area isotherms

Pressure-area compression isotherms were obtained at $25\,^{\circ}C$ using a Langmuir film balance (Nima Technology Ltd) equipped with a pressure sensor and filter paper Wilhelmy plate giving pressure measurements to ± 0.1 mN m^{-1}. The trough was interfaced to an Amstrad P.C. equipped with the relevant software for the collection and manipulation of the data. The surface of the subphase was cleaned before any materials were deposited.

Equimolar solutions (8.0×10^{-4} M) of DPPC and prostaglandin were mixed in different ratios before deposition to ensure a homogeneous spread of molecules, and were applied to the surface dropwise from a syringe. For E_2 and $F_{2\alpha}$ the spreading solvent used was chloroform but for E_1 and $F_{1\alpha}$ a diethyl ether/ethanol, 85:15 mixture had to be used because of their low solubility in chloroform. The normal volume of solvent used was 100 μl. Once applied, each film was left for 10 min at an initial area per molecule of about 150 $Å^2$ to allow the solvent to evaporate. Compression to a surface pressure of 35 mN m^{-1} was carried out at a rate of 50 $Å^2$ $molecule^{-1}$ min^{-1}. The stability of monolayers was confirmed by holding them at a fixed pressure of 15 mN m^{-1} and monitoring the area per molecule with time.

Results and Discussion

In vitro skin permeation studies

PGE_2 and $PGF_{2\alpha}$

The unenhanced penetration of these compounds was found to be very slow (not more than 10% permeation in 48 h) but pretreatment of the skin surface with the penetration enhancers Azone and Transcutol was found, in both cases, to facilitate drug flux. The magnitude of the enhancing effect was found to be greatest when Transcutol was used as the pretreatment with approx. 80% penetrating in 48 h. Azone pretreatment gave rise to a smaller but significant enhancing effect, producing 20–30% penetration over the 48 h period (the marked levelling out

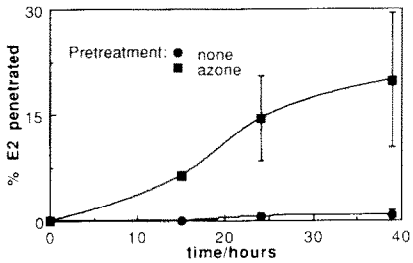

Fig. 1. Effect of Azone pretreatment on the percutaneous penetration of PGE_2.

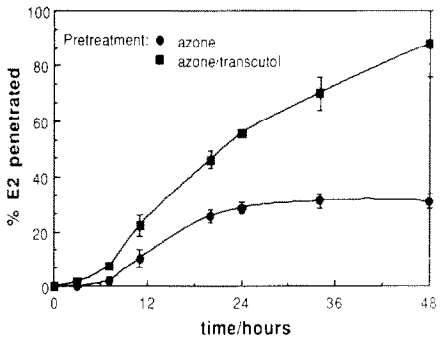

Fig. 2. Comparative effects of Azone and an Azone/Transcutol mixture on the percutaneous penetration of PGE_2.

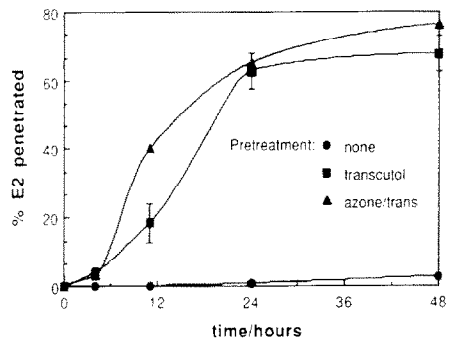

Fig. 3. Comparative effects of Transcutol, an Azone/Transcutol mixture and no pretreatment on the percutaneous penetration of PGE_2.

effect is thought to be due to some prostaglandin-Azone association within the skin). The combined effect of the two compounds was found to be virtually identical to that of Transcutol alone. The results achieved with PGE_2 are shown graphically in Figs 1–3; those gained for $PGF_{2\alpha}$ were very similar.

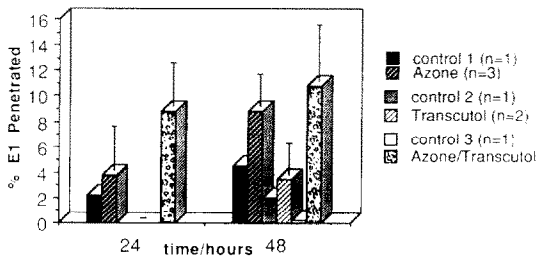

Fig. 4. Effect of Azone, Transcutol and an Azone/Transcutol mixture on the percutaneous penetration of PGE_1.

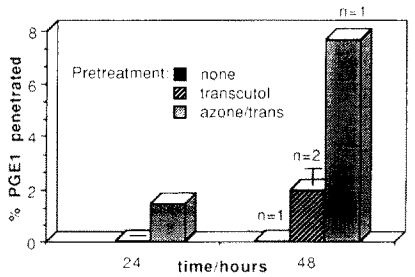

Fig. 5. Comparative effect of Transcutol, an Azone/Transcutol mixture and no pretreatment on the percutaneous penetration of PGE_1.

PGE_1 and $PGF_{1\alpha}$

The results for the PGE_1 experiments are shown as histograms in Figs 4 and 5 (samples were only taken at 24 and 48 h because of the very slow rates of penetration). Again, unenhanced penetration was very slow (not more than 5% in 48 h) and pretreatment with Azone and Transcutol individually did not seem to produce as large a flux as seen with the 2-series prostaglandins above. The greatest enhancement found was after pretreatment with a mixture of Azone and Transcutol (~ 10% at 48 h). Results

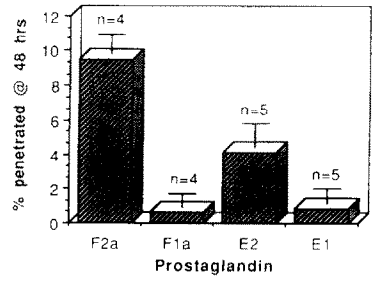

Fig. 6. Comparison of unenhanced penetration of prostaglandins at 48 h.

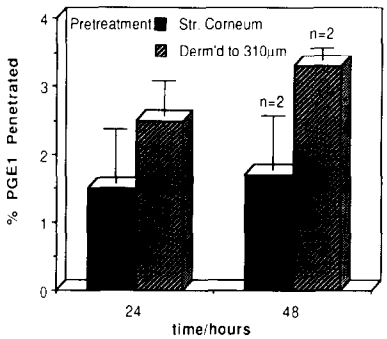

Fig. 7. Comparison of PGE_1 penetration through stratum corneum and dermatomed (310 μm) skin.

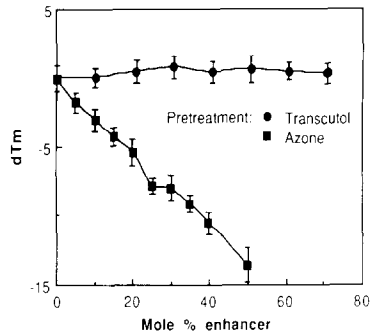

Fig. 8. Differing effects of Azone and Transcutol on the phase transition temperature of DPPC liposomes.

achieved with $PGF_{1\alpha}$ showed exactly the same trend as observed with PGE_1. Fig. 6 shows a histogram comparing the relative amounts of each prostaglandin penetrated after 48 h. It is clear from this graph that even under unenhanced conditions the 2-series compounds are more readily absorbed than the 1-series.

The most evident difference between these results and those obtained with the 2-series prostaglandins is the enormous difference between the actual amounts of drug diffusing through the skin even under enhanced conditions. It was thought that this might have been due to a difference in the location of the main barrier but Fig. 7 shows the percentage PGE_1 penetrated at 24 and 48 h through stratum corneum alone to be no more than that through dermatomed skin. It therefore seems likely that the main barrier to penetration of both series resides within the stratum corneum despite the low water solubility of the 1-series compounds.

Effect of Transcutol on the phase transition temperature of DPPC liposomes

Azone is known to reduce the phase transition temperature of DPPC liposomes (Beastall et al., 1988) and the effect was confirmed here. Fig. 8 shows a plot of dT_m (the change in phase transition temperature) vs mol% Transcutol and Azone. The change in the value of T_m in the presence of Transcutol was found to be negligible.

On considcration of this result it would seem that there are two distinct mechanisms by which the enhancement of percutaneous penetration of these compounds may be achieved. Firstly, by increasing the drug mobility in the lipid bilayer regions (the action of Azone) and secondly by raising its solubility within those regions (the solubilizing power of Transcutol).

Effect of the prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ on the phase transition temperature of DPPC liposomes

Log P (octanol/water) partition coefficients (Table 1) were calculated with the Hansch group contribution method using Medchem software (version 3.54, January 1989, Daylight Chemical Information Systems Inc.). The data indicate that partitioning into the lipid regions of liposomes may be in the order $E_1 > F_{1\alpha} > E_2 > F_{2\alpha}$. The same trend is observed in partition coefficients measured in a cyclohexane/buffer system (Uekama et al., 1978), (Table 1). The latter values may give a clearer overall picture as cyclohexane provides an environment more similar to the pure

TABLE 1

Comparison of some physicochemical properties of the prostaglandins studied (see text for source references)

Prostaglandin	Log P (octanol/water)$_{calc.}$	Log P (cyclohexane/buffer)	Aqueous solubility (mg/ml)	Approximate lipid solubility (mg/ml)
E_1	2.151	0.642	0.0075	0.1
$F_{1\alpha}$	1.799	–	–	–
E_2	1.607	0.576	1.222	2.0
$F_{2\alpha}$	1.255	0.435	1.478	3.2

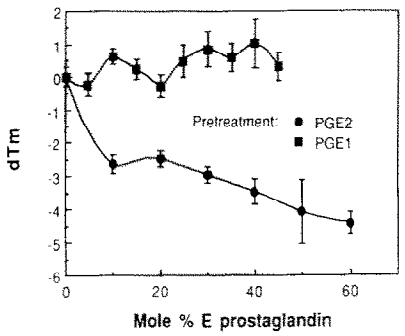

Fig. 9. Comparison of the effect of PGE_1 and PGE_2 on the phase transition temperature of DPPC liposomes.

alkyl chain region of the liposomal lipids. If increased partitioning into the lipid environment were to produce a greater decrease in the phase transition temperature, it would seem that the order of effect on T_m of these compounds might be $E_1 > F_{1\alpha} > E_2 > F_2\alpha$.

The changes in T_m with increasing prostaglandin concentration for the four compounds are shown in Figs 9 and 10, and clearly indicate that the order of effect on T_m is $E_2 \sim F_{2\alpha} >> E_1 \sim F_{1\alpha}$, the reverse of what was nominally predicted above. Although the maximum decrease in T_m produced by the 2-series prostaglandins is only approx. 5 °C previous workers have reported such small changes in the value of T_m as significant (Rowe, 1983). In addition, consideration should be given to the absolute solubilities of the compounds involved (Table 1). Even though the partition coefficients of the 1-series are greater than those of the 2-series, it is predicted that there would be less actual 1-series drug present

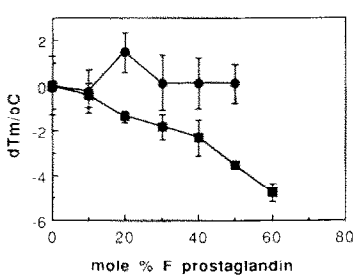

Fig. 10. Comparison of the effect of $PGF_{1\alpha}$ (●) and $PGF_{2\alpha}$ (■) on the phase transition temperature of DPPC liposomes.

in the lipid phase because of a decreased lipid solubility. In fact, the overall presence (in the whole solution system) of the 1-series prostaglandins will be very much less than that of the 2-series because of the large differences in water solubility (~ 200 fold).

The combination of both low water and lipid solubility ensures that the 1-series do not associate with either phase to a great extent. This may partly explain why their transdermal absorption is considerably less than the 2-series prostaglandins which have a much greater all-round compatibility with the system.

It is not clear from these results whether the reduction in T_m by the 2-series compounds is due to a penetrative or non-penetrative interaction with the liposomal lipids and it was for this reason that the film balance experiments were carried out. The results indicate that the greater transdermal absorption of the 2-series compounds could be due to both their ability to ease their passage through the bilayer regions of the stratum corneum and their more favourable solubility characteristics.

Langmuir film work

The shape and characteristics of the DPPC isotherm are well documented in recent literature. The main feature of note is that four phases are present; these correspond to the gas, liquid-expanded (LE), liquid-condensed (LC) and solid-condensed phases. The transition between LE and LC phases has been compared with the liquid crystal-gel transition seen in DPPC liposomes (Blume, 1979) by a number of different methods (absorbance measurements, ESR, DSC and FTIR). It is thought that the change occurring between these two phases is a decrease in the number of *gauche* conformations of the DPPC alkyl chains to form a more ordered system with predominantly *trans* chain conformations (Jain, 1988) and consequently more favourable packing capabilities. It therefore follows that the abolition of this transition is indicative of an increase in the number of *gauche* conformers and a decrease in order of the system. Compounds that remove the LE/LC transition in mixed monolayer systems might be expected to have a disordering

effect on skin lipids and thus improve their rate of penetration.

None of the prostaglandins were found to be capable of forming monolayers in their pure form. There is a steady shift to lower area per molecule with increasing percentage of prostaglandin in the mixture. This occurs because either none or only a small amount of the prostaglandin is inserting into the monolayer. A plot of area per molecule vs mol% prostaglandin at a fixed surface pressure (30 mN m^{-1}) deviated little from the theoretical plot for correspondingly decreasing amounts of DPPC for all the compounds.

However, despite this apparent lack of penetration of prostaglandin into the monolayer the LE to LC phase transition is abolished in the case of the 2-series prostaglandins. This transition was clearly visible in isotherms of monolayers consisting only of decreasing amounts of DPPC deposited from both of the solvent systems used.

The abolition of the phase transition suggests some interaction between the DPPC and prostaglandin molecules. Because it seems that no prostaglandin enters the monolayer this interaction may be occurring between the DPPC head-groups and the prostaglandins. Some association between the polar groups on the prostaglandins and the zwitterionic head-group of DPPC would be expected and prostaglandins have previously been shown to destabilize stearic acid monolayers by non-penetrative mechanisms (Sims and Holder, 1974). The interaction of prostaglandins with the lipid head-groups can be used to rationalise the greater effect of the 2-series prostaglandins on the isotherms. Because E_2 and $F_{2\alpha}$ possess two double bonds the extra electron density may lead to greater association with the DPPC head-groups than that experienced by the mono-unsaturated E_1 (the differing potential for electrostatic interaction is also reflected in the large difference in the water solubilities of the 1- and 2-series; Table 1).

The ability of these compounds to abolish this transition merely by head-group interaction sheds further light on the mechanisms by which penetration enhancers may work. Azone is a known enhancer and its incorporation into a DPPC monolayer leads to both expansion from ideality and the abolition of the phase transition at low concentrations (Lewis and Hadgraft, 1990). The mechanism by which Azone works is thought to involve disruption of the cooperativity of the transition by both the presence of its chain in the hydophobic regions of the monolayer and the interaction of its head-group with those of the surrounding DPPC molecules.

The results achieved here show the importance of considering interactions between the head-groups of the stratum corneum lipids and any polar groupings on the potential enhancer. They also show that these interactions can be important in their own right in the abolition of the LE/LC phase transition and that it is possible that prostaglandins of the 2-series can, to some extent, ease their own penetration through human skin.

Conclusion

Results from the liposome work suggested that the 2-series compounds disrupt the structure of DPPC multilamellar vesicles to a much greater extent than the 1-series and Langmuir film techniques indicate that this disruption is due mainly to head-group interactions.

It therefore seems reasonable to suggest that the main barrier to the percutaneous penetration of prostaglandins resides in the lipid channels of the stratum corneum and that the 2-series prostaglandins may interact with polar head-groups in this region in such a way as to enhance their own permeation. The reason why the 1-series prostaglandins are absorbed comparatively poorly probably lies in their lack of compatibility with the skin membrane constituents and possibly their inability to interact with and destabilise the head-group regions of the stratum corneum lipid bilayers.

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