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The influence of Azone on monomolecular films of some stratum corneum lipids

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Summary

The effects of the lipophilic permeation enhancer Azone on the compressional behaviour of monomolecular films of cholesterol, ceramide, and a mixture of six fatty acids have been investigated using a Langmuir trough. These lipids are those which build the backbone of the lamellar, bilayer structure postulated to exist within the lipid fraction of stratum corneum. Azone changed the solid film of cholesterol and the liquid condensed film of ceramide in a concentration-dependent manner to liquid expanded films of high compressibility. A mixture of the three lipids in proportions corresponding to that which exists in human stratum corneum produced a highly condensed liquid condensed film, which was also changed to liquid expanded in the presence of Azone. These results indicate that Azone reduces the state of condensation of monomolecular films of these lipids, corresponding to increased fluidity within the monolayers. It was also found that Azone alters the rate of loss of water through the monomolecular lipid films, although this effect was very slight. Its effects on the passage of acetone through the monolayers were negligible.

Introduction

It appears that the permeation enhancer Azone exerts strong effects on the lipid fraction of the stratum corneum, reducing its resistence to the passage of drug molecules. An intuitively attractive hypothysis for this action has been presented by Barry (1987), who attempted to identify on the molecular level the possible locations of the Azone molecules within the lipids. Corroboration of these ideas is not yet complete. Indeed, study of the lipid structure within stratum corneum is complicated by its relative inaccessibility to direct experiment, being present only in small amounts between compressed layers of corneocytes. Despite this difficulty experiments have been successfully conducted using thermographic and spectroscopic techniques applied to excised stratum corneum (Walters, 1989). As a complement to such studies it may be profitable to examine the lipid fraction of stratum corneum in the isolated state. In this case the lipid structure would be open to more direct experiment. Such studies require knowledge of its exact composition and the spatial organisation of the lipids within it. This knowledge is indeed available from the work of Elias (Lampe et al., 1983), who identified the

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individual lipids present in the stratum corneum, and Friberg (Osbourne and Friberg, 1987), who has studied extensively the in vitro, lamellar structure built up by these lipids.

Taking this knowledge as our source, we decided to examine the influence of Azone on the structural behaviour of isolated stratum corneum lipids. The lipids chosen were those identified as comprising the lipid fraction of human stratum corneum (Lampe et al., 1983), and were examined in a systematic manner both singly and in combination with one another. In the first part of the work, presented in this paper, we examine the suitability of a simple model for studying the effects of Azone on the lipids, namely that of a two-dimensional, monomolecular film. Measurements of the lateral compressibility and permeability of such films can be used to examine the effects of lipophilic permeation enhancers on film behaviour. Although strictly limited to the two-dimensional case of a monolayer, this model is at first easier to examine experimentally than the three-dimensional, lamellar lipid structure. Indeed, the results obtained provide an interesting insight into the ability of Azone to alter the degree of lateral condensation and, to some extent the permeability, of certain of the lipids examined.

TABLE 1

Composition of human stratum corneum lipids (Elias, 1990)

Substance	wt%
1. Polar lipids	
Phosphatidylethanolamine [a]	trace
Cholesterol sulphate [b]	4
2. Neutral lipids	
Cholesterol [a]	20
Free fatty acids	25
Stearic acid [a] 9.9%	
Palmitic acid [a] 36.8%	
Myristic acid [a] 3.8%	
Oleic acid [a] 33.1%	
Linoleic acid [a] 12.5%	
Palmitoleic acid [a] 3.6%	
Triglycerides [c] (triolein)	trace
Sterol/wax ester [b] (oleic acid palmityl ester)	5
Squalene [c]	4
n-Alkanes [c] (pristane)	4
3. Sphingolipids	
Ceramides [a]	35

[a] Build stable monomolecular films. The water soluble [b] and lipophilic [c] components were not investigated in this study.

Materials and Methods

Table 1 summarises the composition of human stratum corneum lipids discussed recently by Elias (1990). Of note are the virtual absence of phospholipids and the enrichment with sterols, free fatty acids, and ceramides, which exist together with smaller quantities of cholesterol sulphate, triglycerides, sterol esters, and hydrocarbons. Those required for this study were all used as received from Sigma Chemicals (Deisenhofen, Germany) with a stated purity of $\geq 99\%$. Sodium hydroxide pellets (Merck, Darmstadt, Germany) were pA grade. Water was double-distilled from an all-glass apparatus. The permeation enhancer

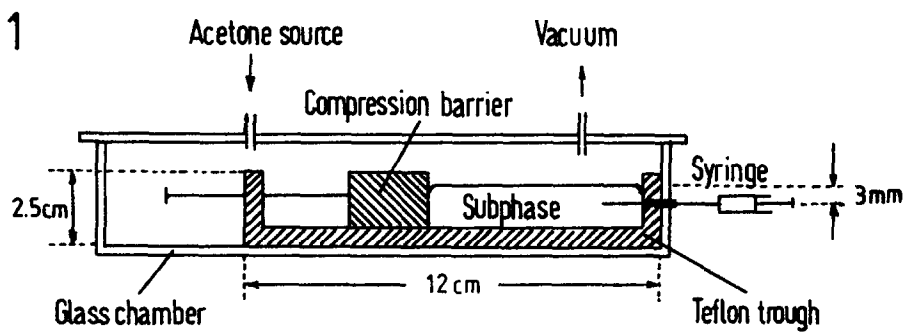

Fig. 1. Schematic diagram of the micro Langmuir trough used to measure the uptake of acetone vapour through monomolecular lipid films.

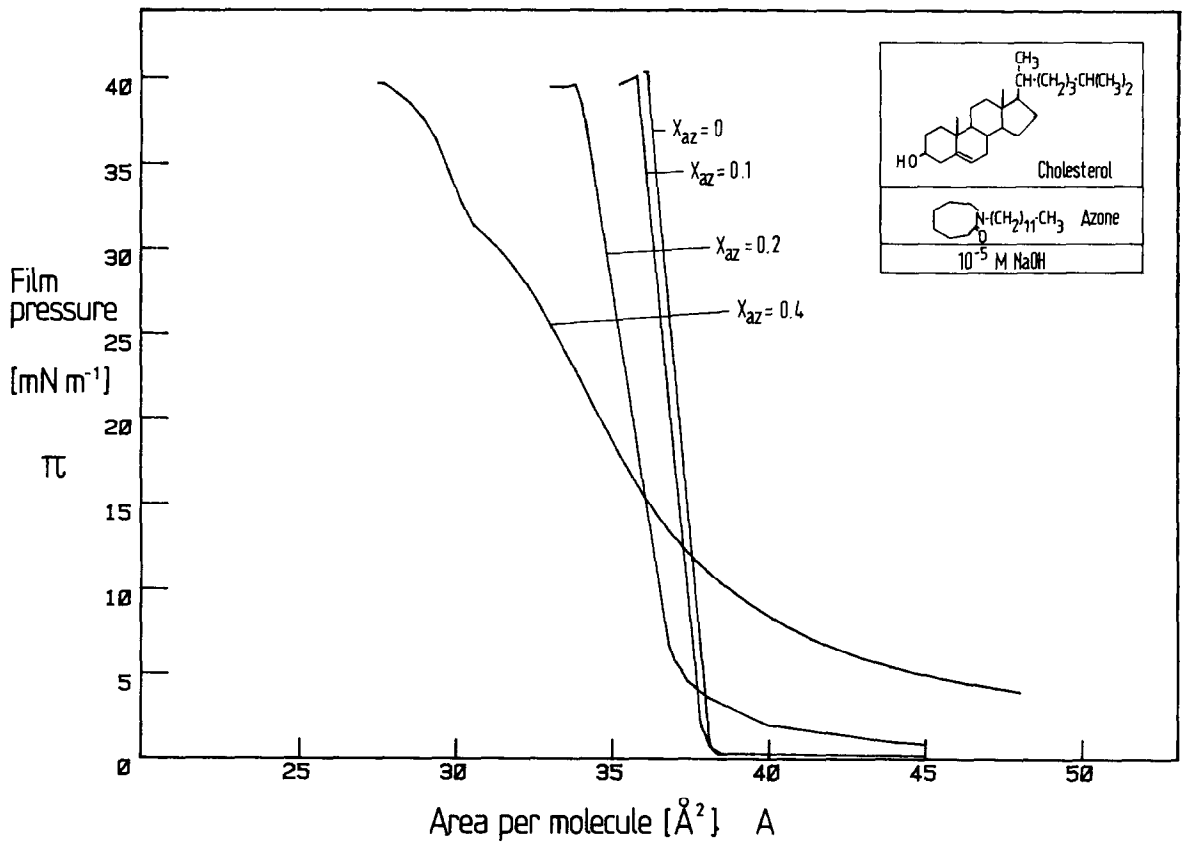

Fig. 2. π-A diagrams for individual lipid films in the presence of increasing mole fraction of Azone (X_{az}). (a) Cholesterol; (b) ceramide; (c) pure Azone; (d) fatty acid mixture.

Azone was obtained from Nelson Research (Irvine, CA, U.S.A.) and used as received.

Measurement of film compressibility

Monomolecular lipid films were examined using a 70 × 15 cm teflon Langmuir trough fitted with a motorised compression barrier (Messgeräte-Werk Lauda, Königshofen, Germany). It was operated exactly as described in detail in Gaines' (1966) standard work, a barrier speed of 5 cm min^{-1} being used. Cleanliness was scrupulously observed to avoid contamination of the monomolecular film. pA grade chloroform (Ferka, Berlin, Germany) was used as a spreading solvent for all lipids. Film pressure, $\pi(A,T)$, and area available per molecule, A, could be measured at a temperature, T, of $35 \pm 0.2°C$ with accuracies of 0.1 mN m^{-1} and 0.2 $Å^2$, respectively. A subphase of aqueous 10^{-5} M NaOH was found to give closely reproducible results. Higher concentrations led to erratic behaviour, certainly due to movement of saponified molecules from the surface into the aqueous subphase (Goddard and Ackilli, 1963).

This method is only suitable for the examination of those stratum corneum lipids that build stable monomolecular films. These proved to be: cholesterol, ceramide, the six fatty acids, and phosphatidyl ethanolamine. Since the latter is not a significant component of the stratum corneum (cf. Table 1), we were most interested in cholesterol, the fatty acids, and ceramide. Indeed, it must be just these three constituents which build

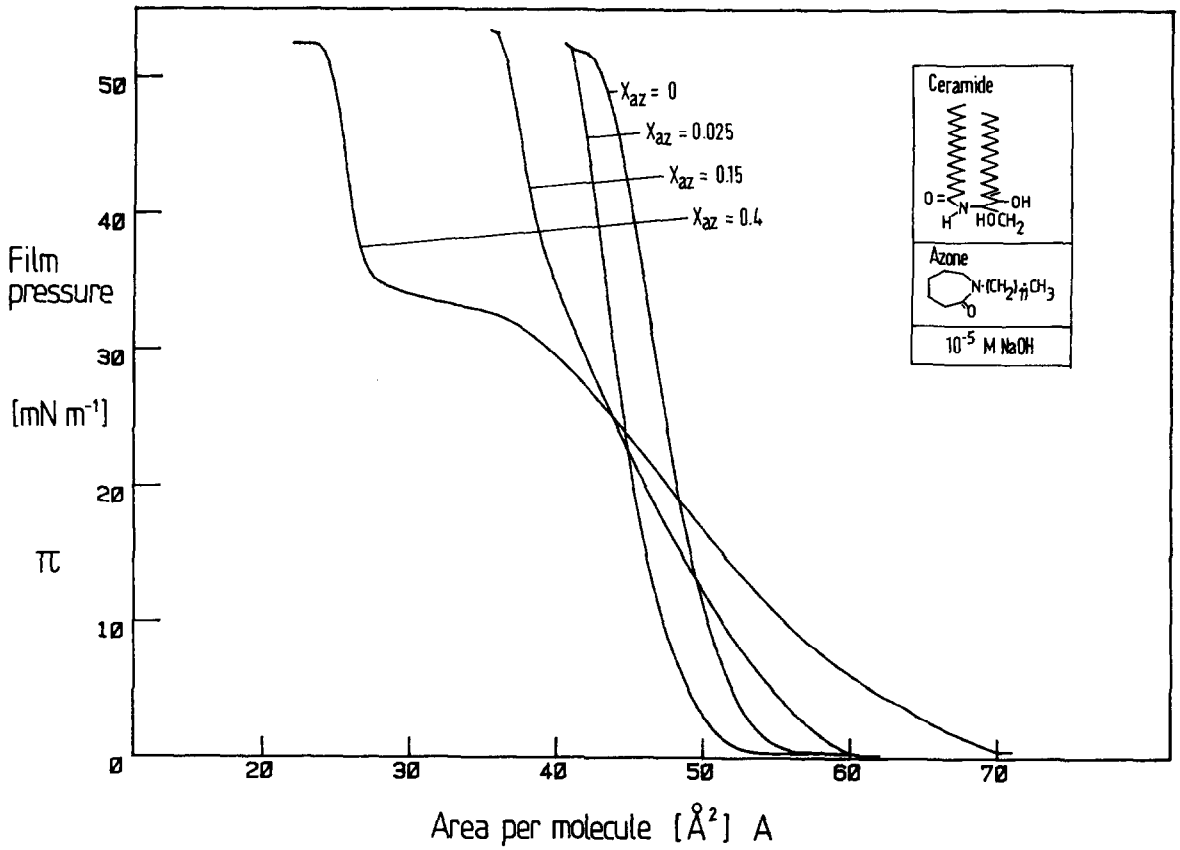

Fig. 2 (b).

extensive bilayers within the lipid fraction of the stratum corneum (Elias, 1990). The remaining components are probably located within the central region of the bilayers (Friberg and Osbourne, 1987). Accordingly, monomolecular films of these three lipids singly or in combination with one another were examined in the presence of increasing mole fraction of Azone (X_{az}) within the film. Azone has low aqueous solubility and partial vapour pressure, and would, therefore, have remained located within the monomolecular film. Each experiment was performed six times on freshly-spread monolayers, and plots of π vs A drawn using an Epson PC AX computer (80 386 processor with 80 387 coprocessor).

Measurement of film permeability

Our objective was to determine if the presence of Azone in a monomolecular lipid film had a measurable effect on the movement of foreign molecules through the film. We attempted two experiments on monomolecular films present at the water/air interface.

The passage of water through monomolecular films was examined using the method developed by Archer and LaMer (1954) for use with the Langmuir trough. The rate of water loss from the aqueous subphase through the film (compressed to a particular film pressure) was measured from the mass, $m(t)$, of water sorbed by LiCl held in a shallow box with a permeable bottom suspended 2

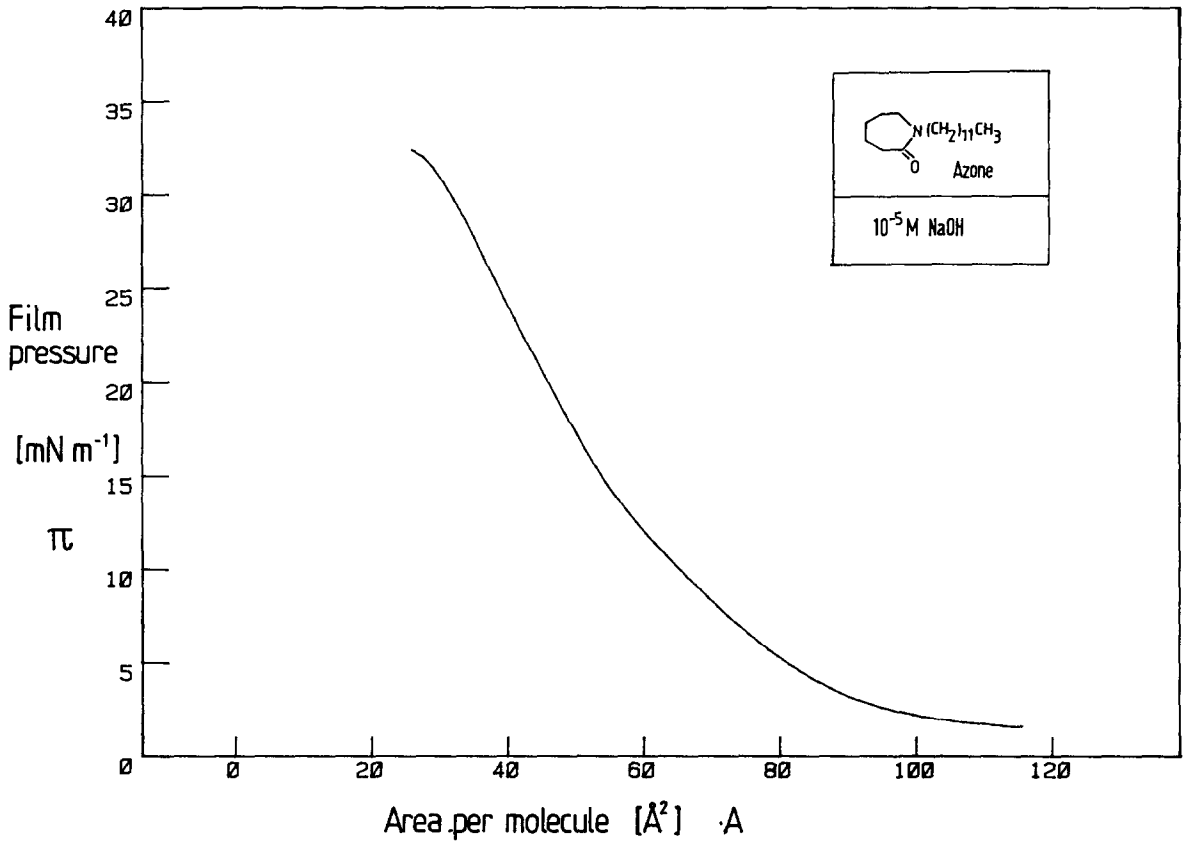

Fig. 2 (c).

mm above the water surface. We followed exactly the method given by Costin and Barnes (1980), each experiment being performed five times on freshly spread monolayers. Plots of $m(t)$ vs t yielded the rates of water sorption in the LiCl (mg s^{-1}) in the absence and presence of a monomolecular film, V_m and V_w, respectively. The permeation resistance of the monomolecular film, r, was then determined from (Costin and Barnes, 1980):

$$r = A \cdot (C_w - C_d) \cdot \left[\frac{1}{V_m} - \frac{1}{V_w} \right] \quad (\text{s cm}^{-1}) \qquad (1)$$

where C_w and C_d are the respective equilibrium water vapour concentrations for the subphase and LiCl, and A is the surface area of the box.

We also measured the passage of acetone through monomolecular films of the lipids. Its uptake from the gas phase into the aqueous subphase was determined using a specially constructed micro Langmuir trough contained within an air-tight, glass chamber (Fig. 1). A lipid film was first spread onto the water surface and compressed to the required film pressure. The chamber was evacuated down to 500 mPa and then restored to atmospheric pressure by allowing air

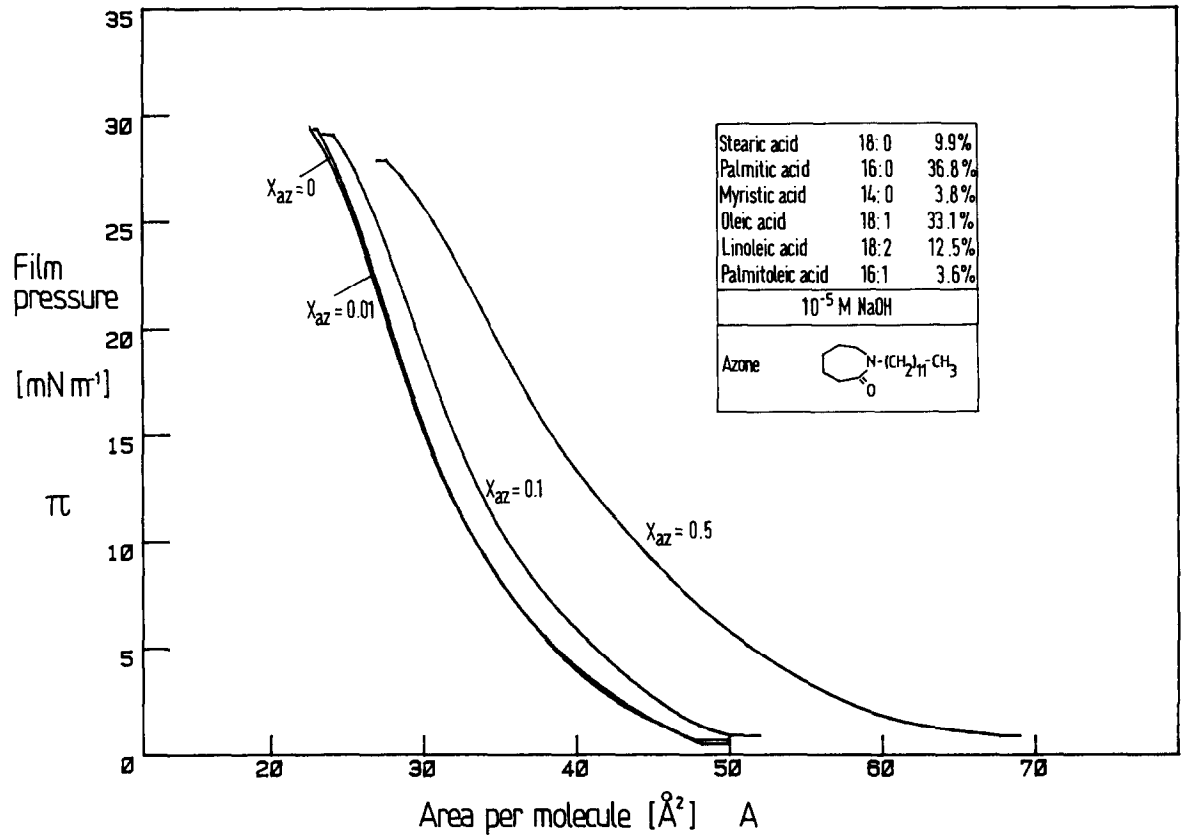

Fig. 2 (d).

saturated with a measured amount of acetone vapour, M_∞, to enter freely into the chamber. Samples of the subphase were removed at various times and their acetone content determined by HPLC analysis. From each experiment a plot of $M(t)/M_\infty$ vs t was constructed, where $M(t)$ is the mass of acetone present in the subphase at time t.

Results and Discussion

Film compressibility

At the most direct level π-A curves can be classified inter alia as solid [S], liquid condensed [L2], liquid expanded [L1], and gaseous [G]. The different curve shapes reflect the compressibility, C^s, of the monomolecular film (Gaines, 1966):

$$C^s = -\frac{1}{A} \cdot \left[\frac{\partial A}{\partial \pi(A, T)} \right]_T \qquad (2)$$

which is a measure of the extent to which the lateral force applied to the film by the compression barrier is dissipated within the film. As such, it reflects the degree of lateral condensation and fluidity within the two-dimensional film. When examining π-A diagrams it is helpful to remember that C^s is inversely proportional to the negative, infinitesimal slope.

The π-A curve shown in Fig. 2a for pure

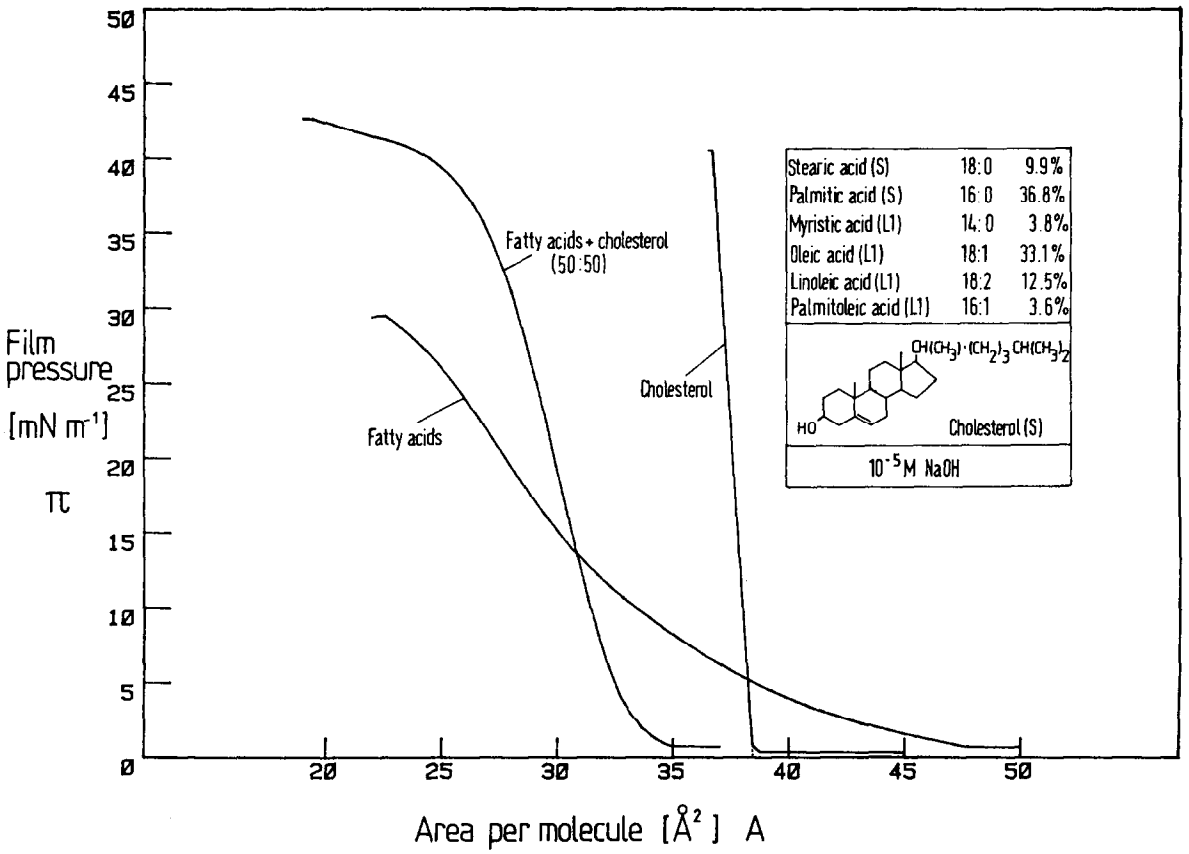

Fig. 3. π-A diagrams for mixed lipid films. (a) Cholesterol + fatty acid mixture; (b) ceramide + fatty acid mixture; (c) cholesterol + ceramide + fatty acid mixture in the presence of increasing mole fraction of Azone (X_{az}); (d) cholesterol + ceramide + phosphatidylethanolamine + fatty acid mixture in the presence of increasing mole fraction of Azone (X_{az}).

cholesterol is identical to that found in the literature (Ries, 1976), being of the S type with very low compressibility indicative of the highest possible condensation within the monolayer. With the lowest mole fraction of Azone shown ($X_{az} = 0.1$) a dilution effect is observed, with a shift of the whole π-A curve to smaller areas per molecule without any appreciable change in compressibility. With $X_{az} = 0.2$ the π-A curve shifts slightly at low pressures to greater areas per molecule, the first sign of a change to L1 behaviour. Film compressibility in the linear part of the plot remains low. At $X_{az} = 0.4$ the transition to an L1 curve is complete; the film is now much more compressible than the original S film of pure cholesterol. The typical, smooth L1 curve shows, however, a slight kink at $\pi \approx 32$ mN m^{-1}, above which a smaller than expected compressibility is evident.

Pure ceramide shows a typical L2 film (Fig. 2b), of somewhat greater compressibility than that for pure cholesterol. It is more sensitive to the presence of Azone than is cholesterol. Although the π-A curve shifts to lower areas per molecule with $X_{az} = 0.025$, a distinct change to L1 behaviour is evident with $X_{az} = 0.15$. This transition to an L1 film is accompanied by increased compressibility only at film pressures $\leq$ approx. 35 mN m^{-1}; at higher film pressures the compressibility is not changed from the original L2 film of pure ceramide. Indeed, with $X_{az} = 0.4$ this results in a distinct expanded-condensed [I] transi-

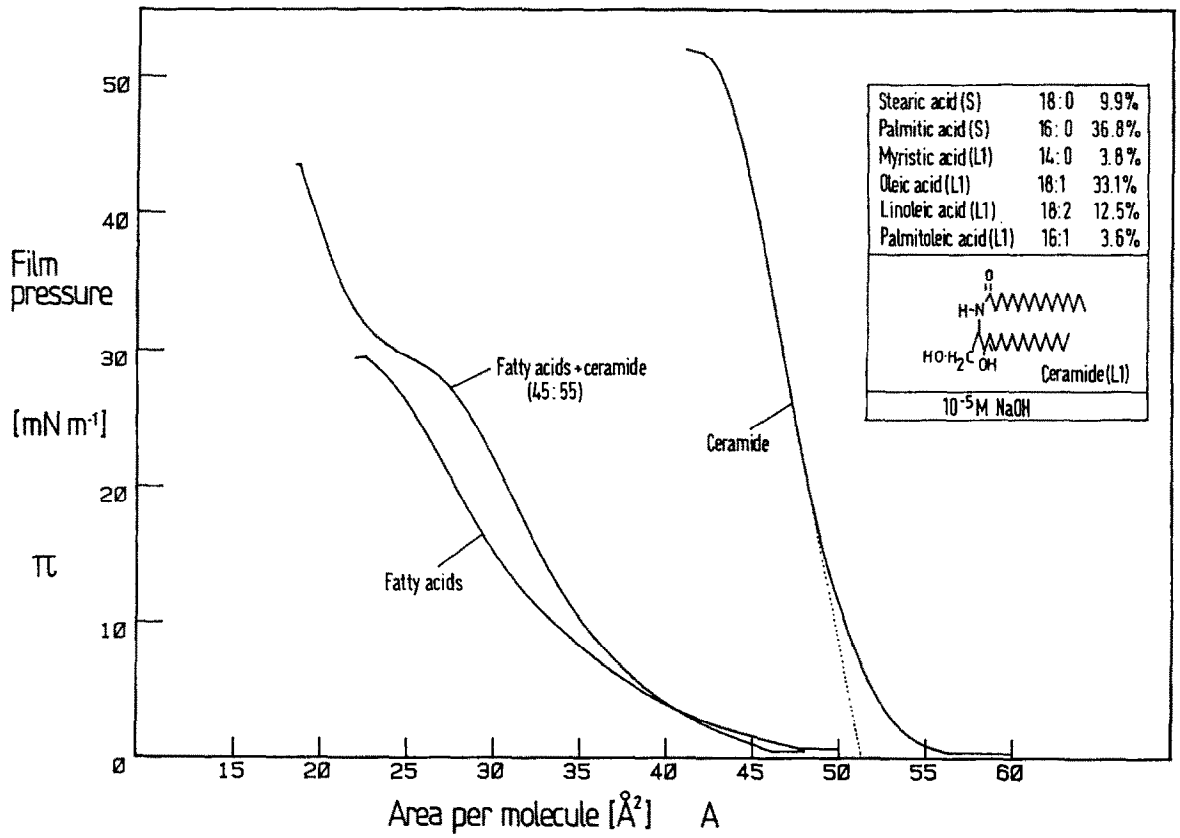

Fig. 3 (b).

tion at the same film pressure where the kink in the L1 curve for cholesterol with $X_{az} = 0.4$ was seen (cf. Fig. 2a).

The presence of Azone in these monomolecular lipid films thus produces a concentration-dependent transition of S or L2 behaviour to L1 behaviour, with a corresponding increase in compressibility. This arises from a decreased degree of lateral condensation of the lipid molecules within the film and corresponds to increased fluidity of the two-dimensional lipid structure. The kink or the I transition which occur in the L1 curves at film pressures of approx. 32–35 mN m^{-1} can be explained by considering the π-A curve obtained for pure Azone (Fig. 2c). Since the Azone molecule is almost purely lipophilic, only a typical gaseous [G] film of very high compressibility is seen (note expanded scale on the x-axis), which collapses at $\pi \approx 32$ mN m^{-1}. This point of maximum compression thus occurs at the same film pressure where the kink or I transition in the L1 curves for films containing both Azone and lipid are seen. It would appear, therefore, that the mixed films are destabilised above this film pressure, possibly by Azone molecules being squeezed out of the film. What remains then behaves similarly to the original film of pure lipid.

The combined, pure fatty acids show a quite different behaviour (Fig. 2d). Although each of the stearic and palmitic acids form S-type films alone, the branched, L1-film forming oleic, linoleic and palmitoleic acids are present in sufficient

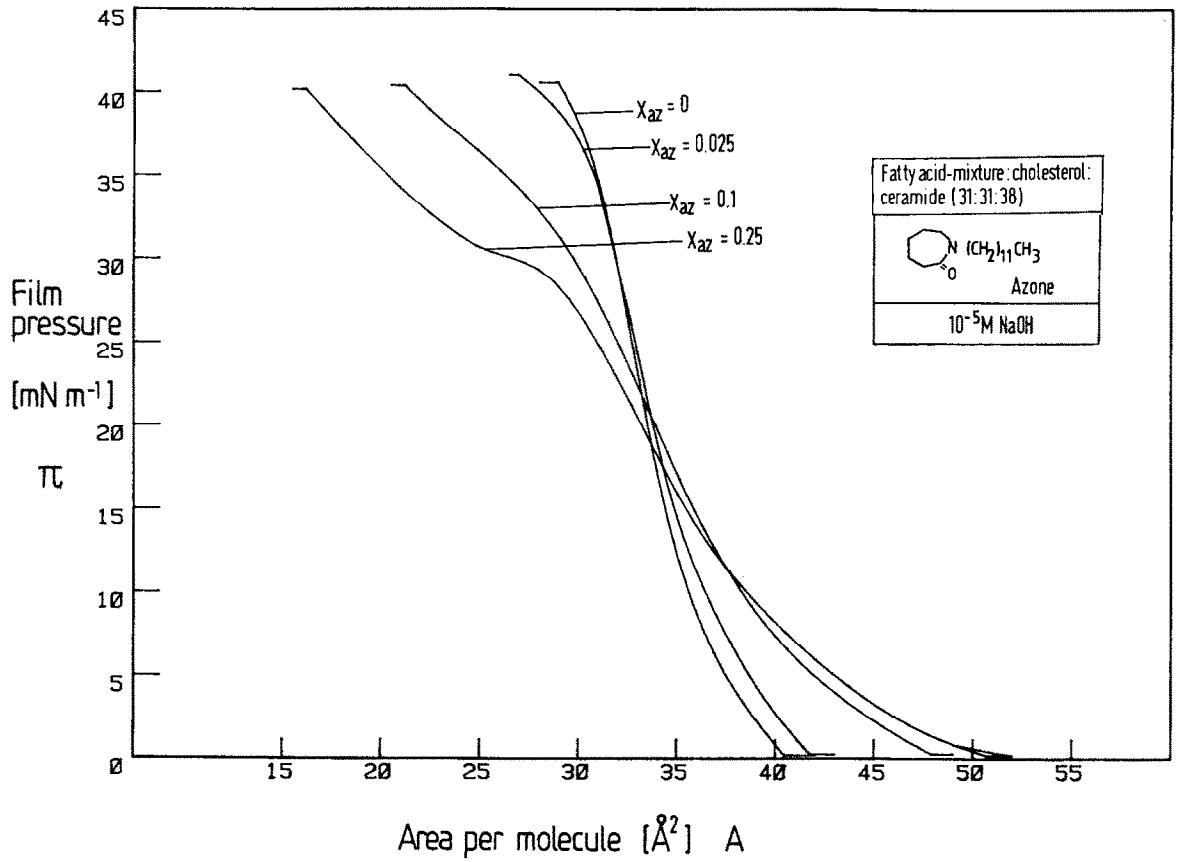

Fig. 3 (c).

amounts within the mixed film to produce an L1-type curve of high compressibility. With increasing mole fraction of Azone the π-A curve is not altered substantially in shape and remains of the L1 type, but is shifted to higher areas per molecule. No change in compressibility and degree of condensation is evident. This dilution effect is clearly in contrast to the effects of Azone on the more condensed films of cholesterol and ceramide described above. There are also no kinks in the curves, since their points of maximum compression lie below the point of collapse of the pure Azone film.

Combination of the three lipids illustrates some interesting effects concerning mixed monomolecular films. Thus the addition of S-film forming cholesterol to the L1-type film of the combined fatty acids (Fig. 3a) in the ratio 50 : 50 decreases the latter's compressibility. The weaker, L2-film forming ceramide has much less condensing effect on the combined fatty acids when present in the proportion 55 : 45 (Fig. 3b). The combination of all three lipids (free fatty acids/cholesterol/ceramide; 31 : 31 : 38) produces an L2 film of low-to-moderate compressibility (Fig. 3c). The condensed nature of this mixed film must, therefore, result largely from the presence of the S-film forming cholesterol. In the presence of Azone an immediate change to L1 behaviour occurs at $X_{az} = 0.025$, with the usual kink being seen at $\pi \approx 30$ mN m^{-1}. In contrast to the individual lipids, compressibility is also increased above the kink,

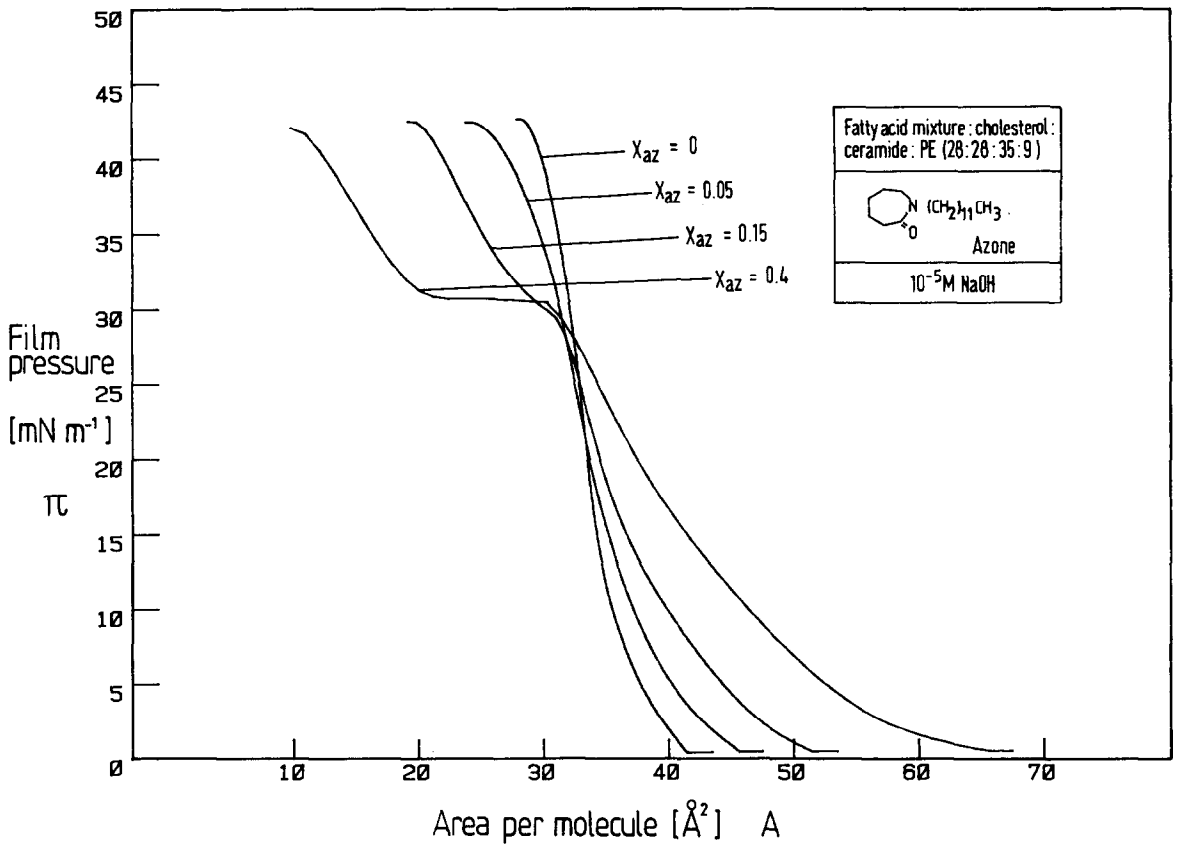

Fig. 3 (d).

suggesting that Azone may be better retained within the mixed film. Azone thus reduces the degree of condensation of the film over the entire π-A curve.

At this point we note that the published combination of epidermal lipids used by Friberg et al. (1990) includes a substantial proportion of phosphatidyl ethanolamine. This is usually considered to exist only in trace amounts within the lipid fraction of stratum corneum (Elias, 1990). Fig. 3d illustrates, however, that its presence does not alter the π-A behaviour of the mixed film of free fatty acids, cholesterol, and ceramide used in the present study (cf. Fig. 3c). It has also little effect on the influence of Azone on the film, the I transition being somewhat more pronounced and the L1 curves tailing away more at higher areas per molecule.

Film permeability

As defined in Eqn 1, the permeation resistance of a monomolecular film, r, is a measure of the reduction it causes in the rate of water loss from the subphase. The phenomenon being measured is thus the evaporation and condensation of water molecules through the interstices of the monomolecular film. As such, r should be inversely proportional to the available area per molecule, A (Barnes, 1978), and directly proportional to film pressure, π.

Thus Fig. 4a illustrates how r is indeed directly proportional to π in the linear section of the S curve for pure cholesterol (cf. Fig. 2a). The values of r lie within the range found for other lipids (Archer and LaMer, 1954), albeit at the bottom end. The effect on r of a large proportion of Azone within the cholesterol film ($X_{az} = 0.4$) is

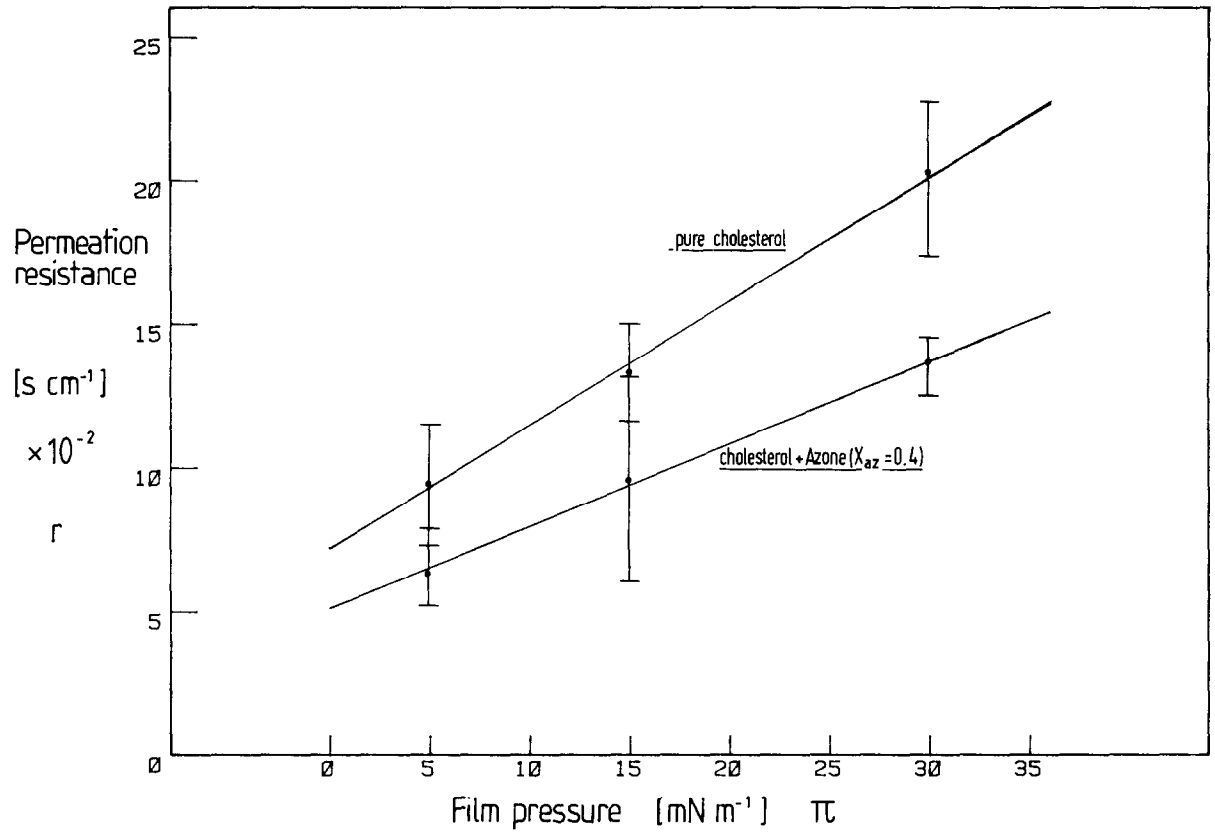

Fig. 4. (a) Plot of permeation resistance (r) vs film pressure (π) for pure cholesterol with or without Azone ($X_{az} = 0.4$) ($n = 5$); (b) plot of permeation resistance (r) vs film pressure (π) for lipid mixture of cholesterol + ceramide + phosphatidylethanolamine + fatty acid mixture with or without Azone ($X_{az} = 0.4$) ($n = 5$); (c) plot of relative mass of acetone taken up into subphase ($M(t)/M_{\infty}$) vs time for mixed film of cholesterol + ceramide + phosphatidylethanolamine + fatty acid mixture with or without Azone ($X_{az} = 0.15$) ($\pi = 5$ mN m^{-1}; $n = 5$).

also illustrated in Fig. 4a for the same three film pressures. Although the scatter in the data is substantial, we note that r was always reduced in the presence of Azone for each individual experiment. There is no direct correlation between r and the change in A occasioned by the presence of Azone: only at film pressures of 5 and 10 mN m^{-1} is A increased with Azone (cf. Fig. 2a); at $\pi = 30$ mN m^{-1} A is decreased, due to the change in shape of the π-A curve from S to L2.

The permeation resistance of the L2 film of combined lipids including phosphatidylethanolamine (Fig. 4b) is somewhat less than that for cholesterol. It is also not so strongly influenced by increasing film pressure, the slope of the line being less for the mixed-lipid film than for pure cholesterol. As seen with cholesterol the data show substantial scatter, although a reduction in r always took place in the presence of Azone for each individual experiment.

Although these changes in r occasioned by Azone are slight, it is noteworthy that this effect could be detected at all for a single, compressed monolayer of lipid. It is also of consequence that the presence of the extremely lipophilic Azone influences the loss of water through lipid mono-

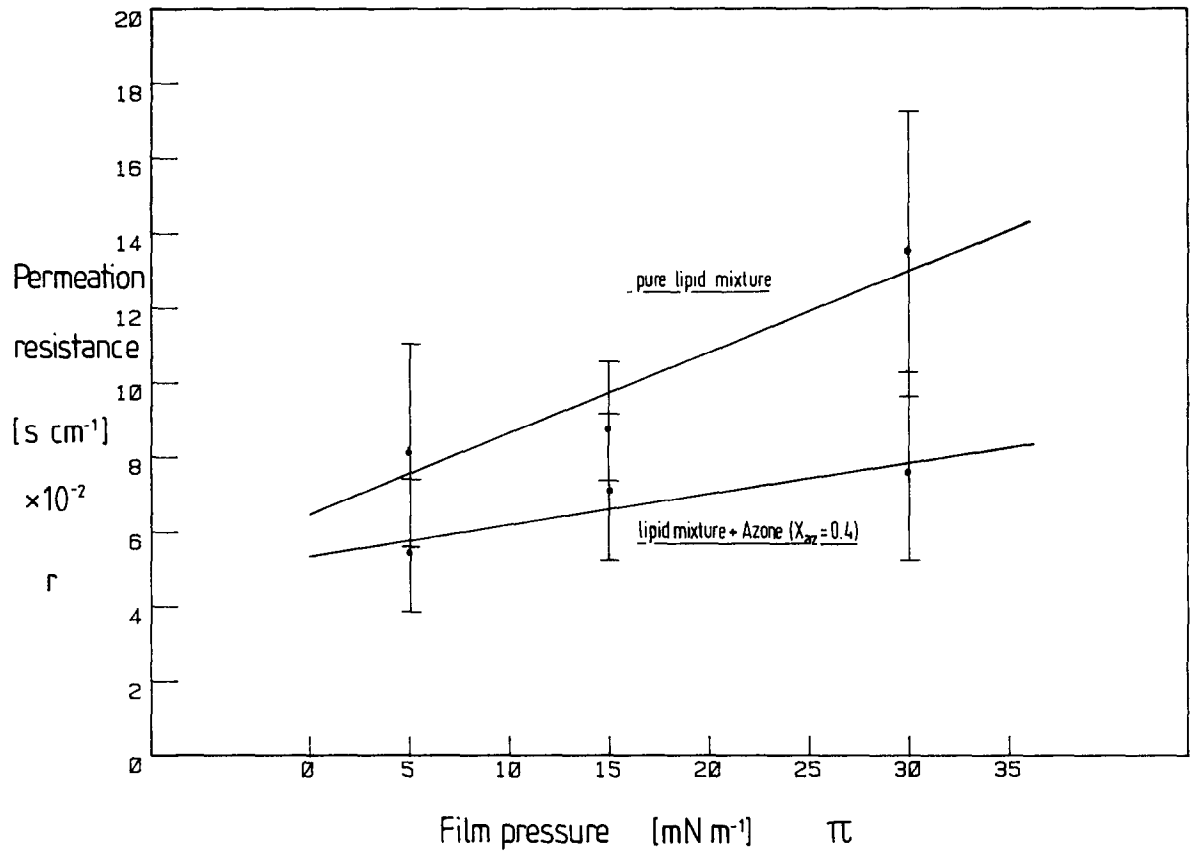

Fig. 4 (b).

layers. Since water is insoluble in Azone, this effect must arise from an alteration in the resistance of the whole monolayer. The results for the permeation of acetone vapour (semi-polar) through the monolayers may now be of interest. Bearing in mind the crudity of the experimental design used for this experiment, the curves shown in Fig. 4c were surprisingly reproducible. The uptake of acetone into the aqueous phase is clearly substantially reduced by the pure mixed-lipid film. The presence of Azone within the film does not, however, alter this uptake for mole fractions of up to 0.4 (only $X_{ax} = 0.15$ shown). This may be due to the substantial solution of the acetone vapour within the monomolecular film.

Conclusions

Azone causes a concentration-dependent reduction in the compressibility of monolayers of the three lipids which form the backbone of the lamellar, bilayer structure of the lipid fraction of stratum corneum. The fluidity of the two-dimensional lipidic structure is increased. The slight changes observed in the permeability of the monolayers to water may be related to these effects. Indeed, it is known that Azone increases water uptake into bilayers of a non-ionic surfactant (Ward and Tallon, 1988) and brings about alterations in phase behaviour (Ward and Tallon, 1990). The two-dimensional monomolecular lipid films examined in

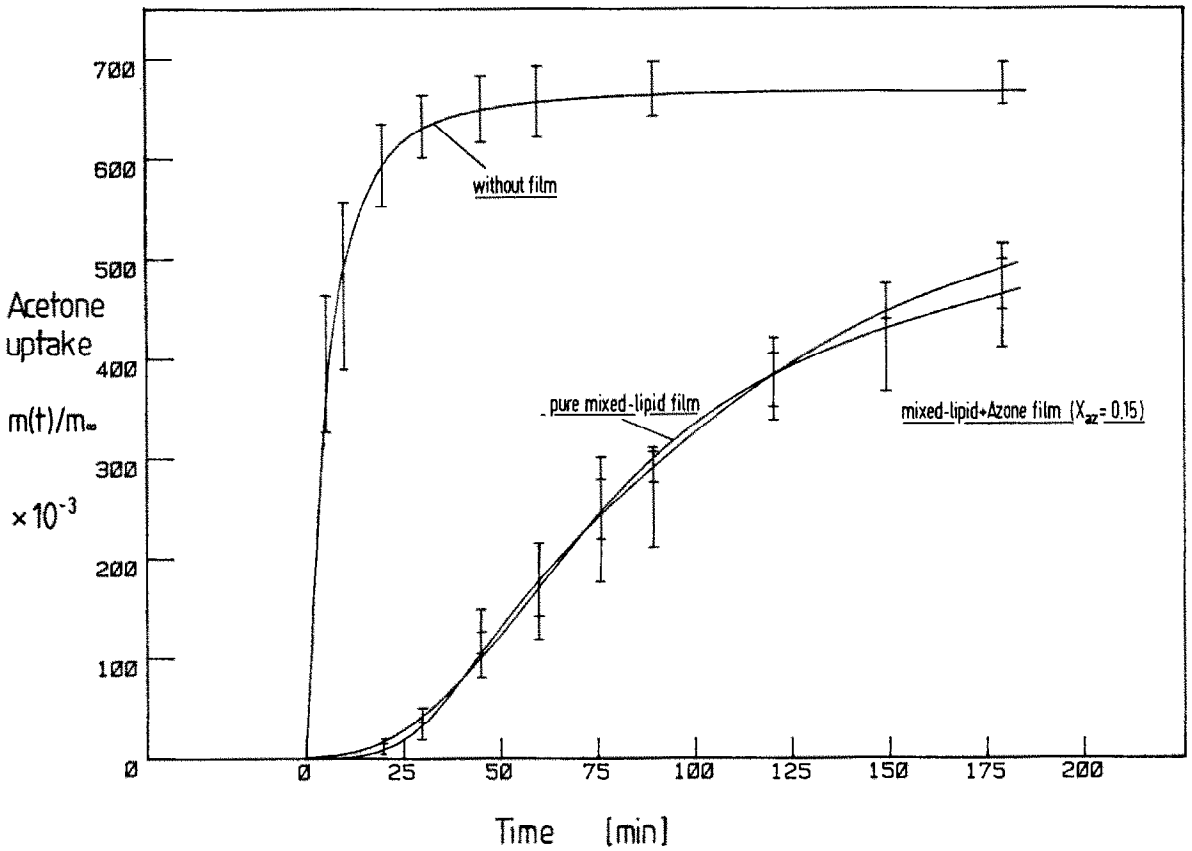

Fig. 4 (c).

this study are a useful, if restricted, model for examining the effects of lipophilic permeation enhancers on stratum corneum lipids. Any comparison of the findings presented here with the three-dimensional, bilayer lipid structure of stratum corneum should at present be viewed with circumspection. The important differences between the two systems should not be overlooked. For example, the existence of a bilayer structure requires a specific degree of saponification of the fatty acids (Osbourne and Friberg, 1985), which is not possible with a monomolecular film. It is also not possible to consider the location of Azone molecules between the parallel monolayers of the lipid bilayer structure, which may indeed be their prefered site of location (Barry, 1987). Studies of the effects of Azone on the three-dimensional stratum corneum lipid structure will, however, be presented in due course.

Acknowledgments

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