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The Lipid Organisation in the Skin Barrier

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The main function of the skin is to protect the body against exogenous substances. The skin barrier is located in the outermost layer of the skin, the stratum corneum. This layer consists of keratin enriched cells embedded in lipid lamellae. These lamellae form the main barrier for diffusion of substances through the skin. In diseased skin the barrier function is often impaired. For a full understanding of the properties of the human skin barrier, insight in the stratum corneum lipid organisation is of great importance. In this paper a short description of the lipid organisation in normal human stratum corneum will be given, after which the role the main lipid classes play in the stratum corneum lipid organisation will be described. In addition the effect of cholesterol sulfate and calcium on the lipid organisation will be discussed. Finally a new model, the "sandwich model", will be proposed that describe the localisation of the fluid phases in the stratum corneum. ***Key words: stratum corneum; lipid organisation; X-ray diffraction; phases.***

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ABBREVIATIONS

SC	stratum corneum
FFA	free fatty acids
CHOL	cholesterol
CER	ceramides
CSO_4	cholesterol sulfate

INTRODUCTION

The natural function of the skin is the protection of the body against exogenous substances from the environment. This means that the skin acts as a barrier for diffusion of substances through the skin. The main barrier for most substances is located in the upper layer of the skin, the stratum corneum (SC). The SC consists of keratin enriched dead cells, surrounded by crystalline intercellular lipid domains. These domains are the only continuous structure in the SC and are required for a competent skin barrier.

The main lipid classes in the SC are ceramides (CER), cholesterol (CHOL) and free fatty acids (FFA). At least 6 different subclasses of CER have been identified, referred to as CER 1, CER 2...CER 6. The individual CER differ in their head-group architecture and chain length distribution (1). The dominant chain length of the FFA is 22 and 24 C atoms. The chain length of the fatty acids linked to the (phyto)sphingosine backbone is approximately C24 and C26. In pig SC, the exceptions are CER 1 and CER 5. The former contains a linoleic acid chemically bound to the C31/33 ω-hydroxy fatty acid, while the latter contains a fatty acyl chain length of approximately 16 C atoms. In human SC the structure of the CER differs slightly. Both, CER 1 and CER 4 contain the linoleic acid bound to the ω-hydroxy fatty acid, while CER 5 has a fatty acid chain length of approximately 24 C atoms.

Next to CHOL, CER and FFA, SC contains also other lipid classes, such as the glucosylceramides and cholesterol sulfate. However, they are present in much smaller quantities. Glucosylceramides are the precursors of the ceramides, while cholesterol sulfate has been suggested to be very important for the desquamation process in the skin.

Electron microscopic studies performed in the seventies and eighties revealed that the lipids are organised in lamellar phases and that these lamellae are oriented approximately parallel to the surface of the keratin enriched cells (2, 3, 4, 5, 6, 7). In recent years the lipid organisation in the SC has been studied using small and wide angle X-ray diffraction (8, 9, 10, 11). These studies revealed that the lipids in SC are organised in two crystalline lamellar phases with periodicities of approximately 6 and 13 nm.

In diseased skin the barrier function is often impaired (12), which might be due to a deviation in lipid composition. Examples of modulated lipid composition in diseased skin are i) a significant change in CER profile in atopic dermatitis and psoriatic scales (13, 14), ii) reduced FFA/CHOL and FFA/CER ratios in recessive lamellar ichthyosis patients (15) and, iii) three to four fold elevated levels of CSO_4 in recessive X-linked ichthyosis patients (16). To understand why the barrier function in diseased skin is impaired, it is necessary to obtain insight in the role individual lipid classes play in SC lipid phase behaviour. Studies with native tissue are hampered by the low availability of the required material from the diseased skin. To mimic diseased SC, one can consider to modulate experimentally lipid composition in SC isolated from normal skin. However, since it is impossible to selectively extract certain lipid classes from the SC, systematic modulation of SC lipid composition cannot be achieved in this way. Therefore, another approach, like the use of mixtures composed of isolated SC lipids can be chosen.

In this paper first a brief description of the small angle X-ray diffraction method will be given, after which the diffraction patterns of the intact human SC as function of hydration level will be explained. Finally recent findings on the lipid phase behaviour of isolated SC lipid mixtures will be presented.

THE X-RAY DIFFRACTION TECHNIQUE

All measurements were carried out at the Synchrotron Radiation Source at Daresbury Laboratory using station 8.2. The samples were put in a special designed sample holder

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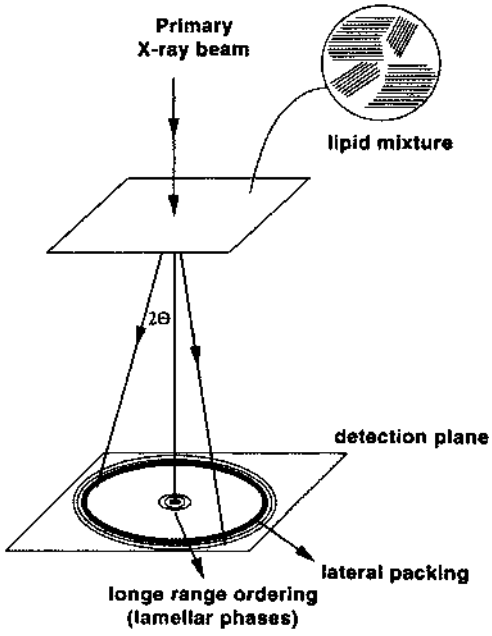

Fig. 1. The X-ray diffraction technique. The X-rays produced by a source are scattered by the sample and the scattered intensity is measured as a function of the scattering angle. This intensity provides information on the electron density differences in the sample. The scattered intensity at small angle provides information about the periodicity of the lamellar phases in the sample, while the scattering at wide angle provides information on the lateral packing of the lipids in the lamellae.

with two mica windows. A detailed description of the equipment has been given elsewhere (10). Using the X-ray diffraction technique, the scattered intensities are measured as a function of θ, the scattering angle, see figure 1. The intensity of the scattered X-rays as function of θ is directly related to the electron density differences in the sample. If the electron density differences have a well defined repeating pattern the diffraction pattern is characterised by a series of peaks (intensity maxima of scattered X-rays). For a one dimensional structure, as for example a lamellar phase, the relationship between the periodicity (the distance of which the structure is repeated), and the peak positions of the scattered X-rays is given by Bragg's Law: $2\,d\,\sin(\theta) = n\lambda$. In this equation n is the order of the diffraction peak and λ is the wavelength of the X-rays and d the periodicity. Frequently the scattered intensity is plotted as a function of Q, the scattering vector, being defined as $Q = 4\pi \sin(\theta)/\lambda$. This relationship implies that a lamellar phase is characterised by a series of peaks related to the periodicity of the lamellar phase by $d = 2n\pi/Q_n$, in which Q_n is the position of the n^{th} order diffraction peak.

RESULTS

The phase behaviour of the intact stratum corneum

In figure 2A the small angle X-ray diffraction curves of human SC measured at room temperature are plotted as a function of Q. The diffraction curve is characterised by a strong and a weak diffraction peak, both peaks having a shoulder on the right-hand side. Since the number of peaks are limited, very broad and partly overlap each other,

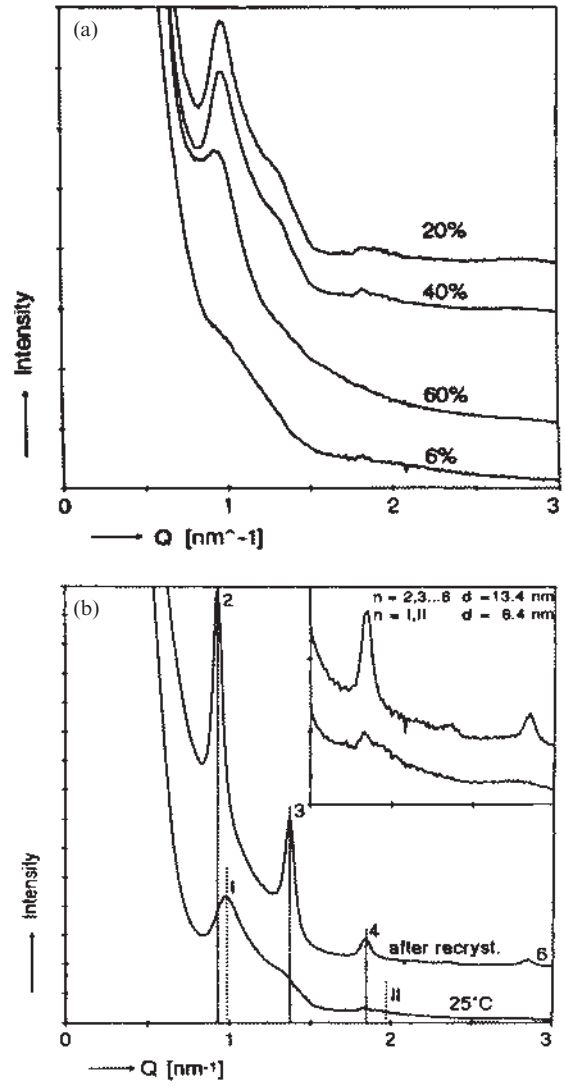

Fig. 2. The diffraction curve of human SC (A) as a function of hydration level varying between 6 and 60%, (B) prior and after recrystallisation.

interpretation of this diffraction pattern is quite complicated. However, from this curve it is obvious that an increase in the SC content from 20 to 60% w/w does not lead to a change in the peak positions, and therefore not to a change in periodicity of the lamellar phases. From this observation it was concluded that addition of water to the SC does not lead to a swelling of the lipid lamellae. To analyse the diffraction curves in more detail additional information was required. For this purpose, the SC lipids were crystallised from 120°C to room temperature, after which the X-ray pattern was monitored. As shown in figure 2B, the diffraction curves revealed a series of peaks that were located at the same inter peak distance. Such a diffraction profile is characteristic for a lamellar phase. From the positions of the peaks, the periodicity of the lamellar phase (d) was calculated. These calculations revealed that after recrystallisation the lipids in SC were organised in a lamellar phase with a periodicity of 13.4 nm. Comparing the peak positions of the diffraction pattern prior and after recrystallisation (figure 2B) revealed that in untreated SC two lamellar phases are present, one with

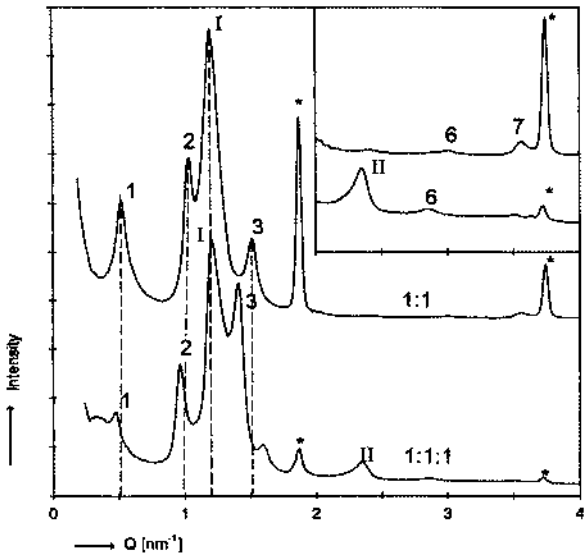

Fig. 3. The small angle X-ray diffraction curves of the 1:1 CHOL:CER and 1:1:1 CHOL:CER:FFA lipid mixtures. The arabic numbers indicate the diffraction orders of the long periodicity phase (repeat distance of 12.2 and 12.8 nm for the equimolar CHOL:CER and CHOL:CER:FFA mixtures, respectively). The roman numbers indicate the diffraction orders of the short periodicity phase (repeat distance between 5.2 and 5.5 nm).

a periodicity of approximately 6.4 nm, and the other with a periodicity of approximately 13.4 nm, respectively (10). Similarly two lamellar phases with periodicities of 6 and 13.2 nm were present in porcine SC (17), while in murine SC the approximate 13 nm lamellar phase was the prominent one (9). Since the 13 nm phase is always present in all the species studied so far, and this phase is very characteristic for the SC lipid phase behaviour, this phase is most probably very important for the skin barrier function (18).

Mixtures of CHOL/CER/FFA

In order to understand the SC lipid phase behaviour in normal and diseased skin in more detail, knowledge on the role the various lipid classes and subclasses play in the SC lipid organisation is required. Since it is impossible to extract selectively lipid components from the SC, the role the various lipid classes play in SC lipid organisation have been studied with ceramides isolated from pig SC. Since the lipid phase behaviour in pig SC is similar to that in human SC and pig SC is available in large quantities we chose for pig ceramides.

In our studies an equimolar mixture of CHOL and CER was prepared at a pH of 5 and examined (19) using small angle X-ray diffraction. In the X-ray pattern the presence of a large number of sharp peaks was noticed (figure 3). The peaks indicated by I and II have been assigned to a lamellar phase with a periodicity of 5.2 nm, and peaks indicated by 1, 2, 3, 5 and 7 to a lamellar phase with a repeat distance of 12.2 nm. Furthermore, two additional peaks at 3.35 nm and 1.68 nm have been detected. These can be assigned to crystalline CHOL, which was not dissolved in the lamellar phases, but phase separated and formed domains of CHOL. Reducing the CHOL/CER molar ratio to 0.4 did not change the phase behaviour, except that a smaller amount of CHOL phase separated in crystalline domains. Only a further reduction to a molar ratio of 0.2 weakened the 12.2 nm phase, while the periodicity of the 5.2 nm lamellar phase increased to 5.6 nm, see figure 4. Similar observations have been made at high CHOL/CER molar ratio and only after increasing the CHOL:CER ratio to 2 the 12.2 nm phase weakened. At this high CHOL content no change in peak positions of the 5.2 nm phase was observed. From these observations it was concluded that over a wide range the phase behaviour of the CHOL/CER mixtures is remarkably insensitive to changes in the CHOL/CER molar ratio.

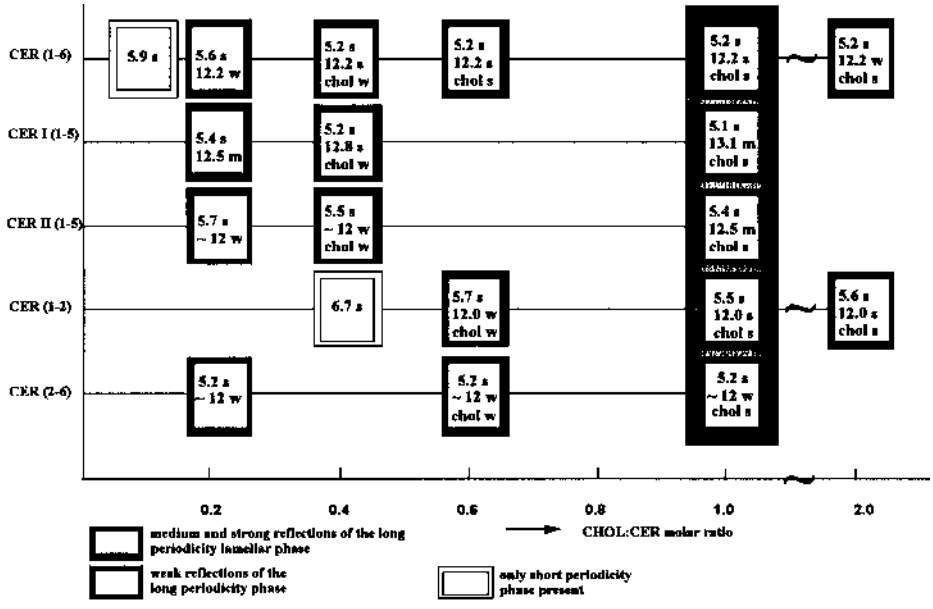

Fig. 4. A schematic overview of the lamellar phases of several CHOL:CER mixtures as function of the molar ratio.* data obtained from (38). Note the similarity in the lamellar phases of the various CHOL:CER mixtures at an equimolar ratio. The indication weak (w), medium (m) and strong (s) of the 12–13 nm phase and CHOL denote the presence of these phases compared to the 5–5.5 nm phase.

The role the individual CER subclasses play in the SC lipid phase behaviour

Since in diseased skin often a deviation in CER composition has been found [13, 14, 15, 16], not only insight in the role of the CHOL/CER molar ratio, but also the role of CER subclasses in SC lipid phase behaviour is of great importance. To examine in more detail this problem, mixtures prepared from CHOL and CER with varying CER composition were examined. For this purpose, mixtures prepared with CER I (1 – 5), CER II (1 – 5), CER (2 – 6) or CER (1 – 2) have been used. The two CER (1 – 5) mixtures differed in relative amounts of CER 2, CER 3 and CER 4, in which the CER II (1 – 5) mixture contained an increased amount of CER 2 and decreased amounts of CER 3 and CER 4 (20).

Since in native SC, CHOL and CER are present in an approximately equimolar ratio, we first examined the phase behaviour of the equimolar mixtures. These studies revealed that in these mixtures the lipids were organised in two lamellar phases with periodicities of approximately 12 – 13 nm and 5 – 6 nm, similarly as observed in intact SC (figure 4). The exception was found with an equimolar CHOL/CER mixture in which the CER 1 was absent. In this mixture, the 12 nm phase was only weakly present (21), indicating that in equimolar CHOL/CER mixtures CER 1 plays a crucial role in the formation of the 12 – 13 nm lamellar phase. Similar observations have been made when the third main class of SC lipids, the long-chained FFA, was incorporated into CHOL/CER mixtures at an equimolar ratio. As can be noticed from figure 5, FFA hardly affected the lamellar lipid organisation. Again, only CER 1 has been found to play a crucial role in the formation of the 12 – 13 nm phase.

In contrast to the situation with equimolar CHOL/CER mixtures, in which only CER 1 profoundly affected the lipid phase behaviour, individual CER became more important when the CHOL/CER molar ratio was reduced. From all lipid mixtures tested only in the CHOL/CER I(1 – 5) and the CHOL/CER(1 – 6) mixtures the 12 nm phase was formed (figure 4) at a molar ratio of 0.2. In the CHOL/CER II(1 – 5) mixture this phase was formed at a molar ratio of 0.4, while in CHOL/CER(1,2) it was formed only when the CHOL content has been increased further to a CHOL/CER molar ratio of 0.6. These findings clearly indicate that the formation of the 12 – 13 nm phase was more susceptible to changes in CER composition at CHOL/CER molar ratio's lower than 1.0. In addition, in the absence of CER 6 the periodicities of the

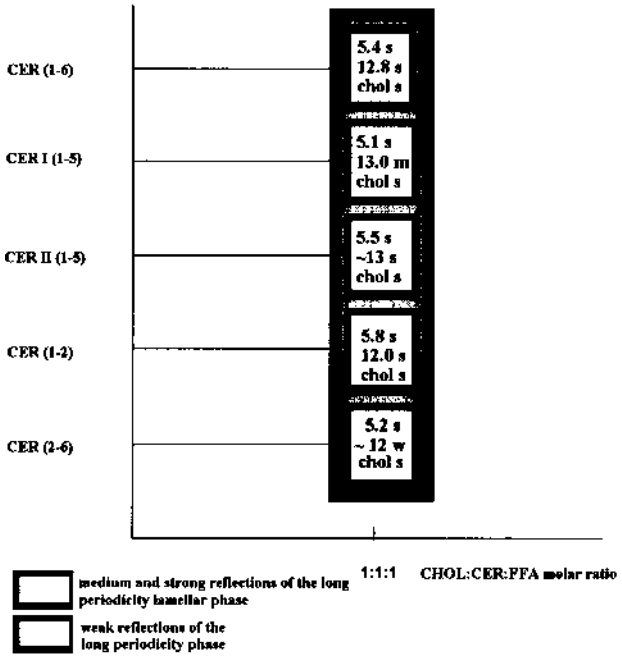

Fig. 5. A schematic overview of the lamellar phases in the equimolar CHOL:CER:FFA mixtures. Note the similarity in the lamellar phases. See for the indication weak (w), medium (m) and strong (s) figure 4.

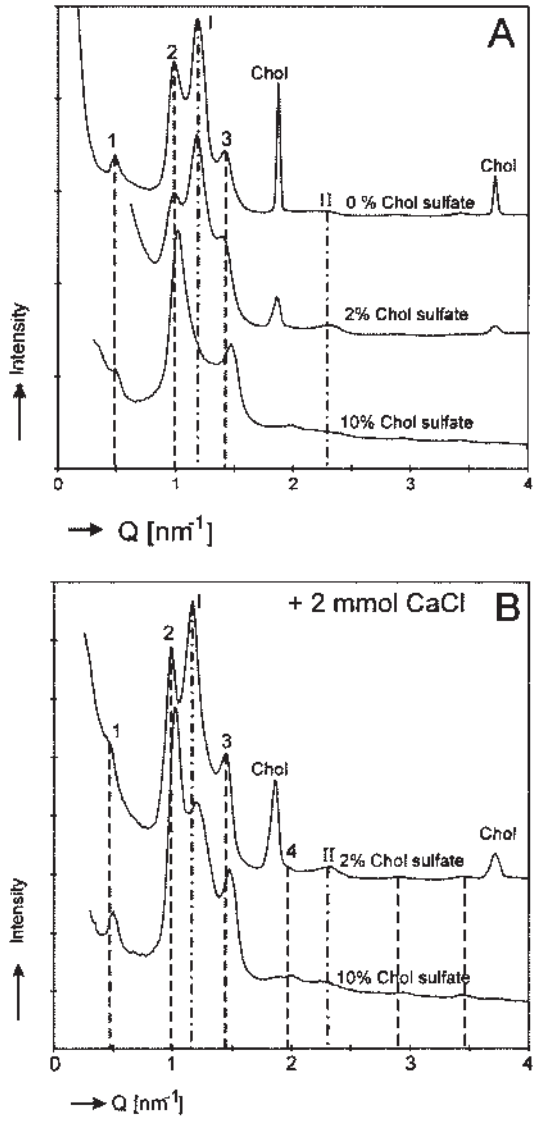

Fig. 6. Diffraction patterns of equimolar CHOL:CER:FFA mixtures. The arabic numbers indicate the diffraction orders of the long periodicity phase (repeat distance between 12 and 13 nm). The roman numbers indicate the diffraction orders of the short periodicity phase (repeat distance between 5.3 and 5.5 nm). The diffraction patterns of the CHOL:CER:FFA:CSO_4 mixtures in molar ratios of 1:1:1:0 (a), 1:1:1:0.06 (b) and 1:1:1:0.1 (c) at pH 5. The diffraction patterns of the CHOL:CER:FFA:CSO_4 mixtures in molar ratios of 1:1:1:0.06 (a) and 1:1:1:0.1 (b) in the presence of 2 mmol Ca^{2+} at pH 5.

12–13 and 5–6 nm lamellar phases changed gradually when decreasing the CHOL/CER(1–5) molar ratio. This was not observed in the CHOL/CER(1–6) mixtures.

Cholesterol sulfate and calcium affect the SC lipid organisation

Next to the major SC lipids - CHOL, CER and FFA - small quantities of cholesterol sulfate (CSO_4) are also present. CSO_4 has been suggested to play an important role in the desquamation process of the skin. It has been suggested that in SC gradients of pH (22, 23, 24) and of CSO_4 (25, 26) exist. In addition, most probably Ca^{2+} is also present (27) at the SC-stratum granulosum interface and in the lower layers of the SC. Previous studies with membranes prepared from either phospholipids or sphingomyelin (28, 29, 30) revealed that CSO_4 most probably stabilises the bilayer organisation. This conclusion was drawn from the observation that in the presence of CSO_4 the transition from the lamellar to reversed hexagonal phase shifted to higher temperatures. Furthermore, in phospholipid and sphingomyelin membranes Ca^{2+}induces crystallisation of the bilayers by dehydration of the lipid head groups (31, 32, 33).

Information about the role of CSO_4 and Ca^{2+} in SC lipid organisation is limited. Therefore the effect of CSO_4 and Ca^{2+} on the lipid phase behaviour of lipid mixtures composed of CHOL, FFA and CER isolated from pig SC has been examined. Two CSO_4 levels were chosen, a 2 % m/m and a 10 % m/m content, that mimic the situation in normal and recessive x-linked ichthyosis stratum corneum, respectively. The measurements were carried out at a pH of 5, the approximate pH value at the skin surface.

The diffraction curve of the equimolar CHOL:CER:FFA mixture is presented in figure 6A. The corresponding periodicities are summarised in figure 7. As already explained above, the phase behaviour of this mixture is similar to that of the CHOL:CER mixture. In addition 3.36 and 1.69 nm diffraction peaks are observed that can be attributed to CHOL that phase separates in crystalline domains.

Addition of only 2% m/m CSO_4 to the equimolar mixture (CHOL:CER:FFA:CSO_4 molar ratio 1:1:1:0.06) did not change the lipid phase behaviour. Two lamellar phases with periodicities of 12.8 and 5.4 nm, respectively, were present. However, the intensity of peaks attributed to crystalline CHOL decreased. A further increase in CSO_4 content to 10% m/m (molar ratio:1:1:1:0.3) induced a pronounced change in the lamellar phase behaviour: the diffraction peaks attributed to the 5.4 nm phase and the peaks assigned to crystalline CHOL disappeared (figure 6A).

Addition of 2 mmol $CaCl_2$ to a CHOL:CER:FFA:CSO_4 mixture in a molar ratio of 1:1:1:0.06 did not affect the peak intensities attributed to the 5.4 and 12.8 nm lamellar phases, but slightly increases the intensity of the CHOL reflections, see figure 6B. This indicates that at low CSO_4 content Ca^{2+} decreases the CHOL solubility in the lamellar phases. In contrast to that, addition of Ca^{2+} to 1:1:1:0.3 CHOL:CER:FFA:CSO_4 mixture does not result in the reappearance of the CHOL reflections. However, a reappearance of the 5.4 nm peak was observed. The results obviously show that Ca^{2+} balances at least partly the lipid phase changes induced by CSO_4. A summary of the results of the phase behaviour studies in the presence and absence of CSO_4 and/or Ca^{2+} is provided in figure 7.

MOLECULAR MODEL FOR THE 12.2 NM LAMELLAR PHASE

In a recent study we have proposed a model for the molecular organisation of the 12.2 nm phase in the CHOL/CER mixtures, in which the repeating unit consists of three lipid layers. In this model the ceramides are either partly interdigitating (the broad low-electron density layers) or fully interdigitating (the narrow low-electron density layers), see figure 8. The latter occurs in the centre of the lamellae. The two broad low-electron density regions are formed by

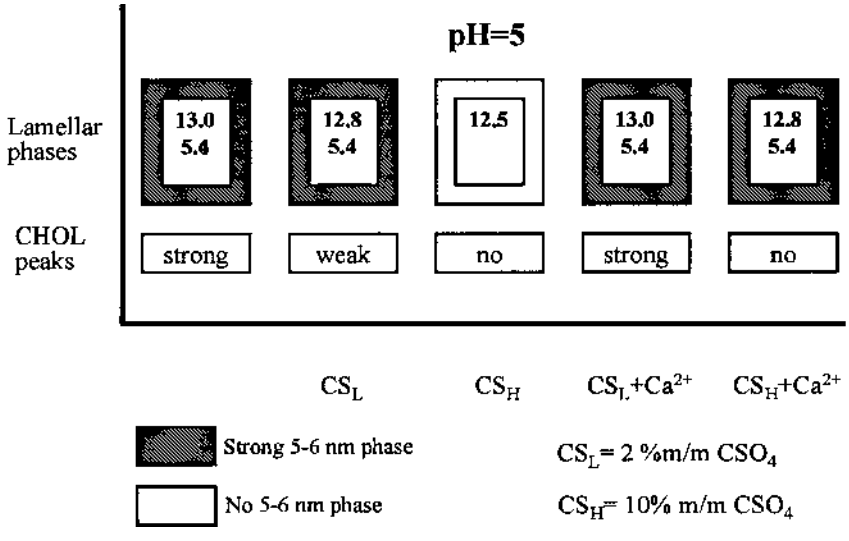

Fig. 7. A schematic presentation of lamellar organisation in equimolar CHOL:CER:FFA mixtures in dependence of CSO_4 (2 and 10% m/m) in the presence and absence of Ca^{2+}. The long periodicity phase (the repeat distance varies between 12.5 and 13 nm) was always prominently present, while the 1st order diffraction peak of the short periodicity phase (repeat distance 5.4) was not present at 10% m/m CSO_4 level in the absence of Ca^{2+}.

ceramides with the long-chain fatty acids (predominantly C24 to C26) linked to the (phyto)sphingosine backbone and by CHOL, while the narrow low-electron density region is formed by the short-chain ceramides (predominantly C16). The proposal of the molecular model is based on the following findings. a) The electron density profile that consists of one narrow and two broad low-electron density regions [20] that has been calculated from the peak intensities of the diffraction pattern of the equimolar CHOL:CER mixture. b) CER 1 has been found to play a crucial role in the formation of the long periodicity phase and [21]. c) The fatty acid chain-length distribution of the ceramides is bimodal [1]. d) In a mixture of ceramides with long chain fatty acids and ceramides with short chain fatty acids phase separation occurs (34), and e) no swelling of the SC lamellar phases has been observed upon increasing the water content [3, 10]. Of particular importance is the role of CER 1 in the formation of the long periodicity phase since this phase is also formed in the CHOL/CER (1, 2) at higher molar ratios than 0.6.

CONCLUSION

When extrapolating the results obtained in the phase behaviour studies with SC lipid mixtures to the in vivo situation, the following conclusions can be drawn. The phase behaviour of equimolar CHOL/CER and CHOL/CER/FFA mixtures, which approximate the in vivo situation, closely mimics the lipid organisation in native stratum corneum at room temperature. At this molar ratio changes in CER distribution do not induce any significant changes in lipid phase behaviour. Only small changes in the periodicities of the two lamellar phases have been observed. It seems that in the equimolar mixtures the lipid organisation is insensitive towards a change in CER. Therefore, one can expect that in intact healthy SC a change in CER composition would not modulate lamellar lipid phase behaviour. The only exception is the situation when CER 1 content is markedly reduced. In this case the formation of the 12–13 nm lamellar phase will be reduced. Although, addition of FFA to the equimolar CHOL/CER mixture does not change the lamellar organisation dramatically, recently it has been reported [18] that incorporation of FFA in the lipid mixtures induces a change in the lateral packing from a hexagonal lateral packing to an orthorhombic one. This means that FFA increase the lipid lattice density, which might be extremely important for creating an competent skin barrier.

Importantly, when the CHOL/CER molar ratio changes from unity, the lipid phase behaviour becomes more sensitive toward CER composition. In our studies we observed that a change in CER composition often results in a reduction in the formation of the long periodicity phase. This situation may occur in diseased skin, as for example in atopic dermatitis and psoriatic scales, in which a significant change in CER profile has been found compared to normal skin. Simultaneously an increase in the CHOL/CER ratio has been observed.

Since changes in Ca^{2+} and CSO_4 content have been reported in the SC, the effect CSO_4 and Ca^{2+} content on the SC lipid organisation is also of great interest. In the lower layers of the SC and at the stratum granulosum-stratum corneum interface a high Ca^{2+} and CSO_4 content have been reported. However close to the SC surface the Ca^{2+} and CSO_4 content is low. It has also been suggested that a drop in Ca^{2+} concentration already occurs in the lower layers of the SC, while a decrease in CSO_4 content occurs in the superficial layers. We chose to incorporate either 2% m/m or 10% m/m CSO_4 into equimolar CHOL:CER:FFA mixture, to approximate the CSO_4 levels observed in normal and in recessive X-linked ichthyosis skin, respectively [16]. Our studies show that in the presence of CSO_4 the solubility of CHOL in the lipid mixtures increases. This can most likely be ascribed to the presence of the charged sulfate group, which reduces the lattice density in the bilayers. This proposed decrease in lattice density is in agreement with previous results [18], which showed that in the presence of CSO_4 next to an orthorhombic phase, also a liquid lateral packing appeared. The changes induced by CSO_4 can be partly counterbalanced by the presence of 2 mmol Ca^{2+}. Namely Ca^{2+} promotes the formation of crystalline CHOL domains in the 2 % m/m CSO_4 containing mixtures and induces the reappearance of the 5.3 nm reflection in the presence of 10 % m/m CSO_4.

When extrapolating these findings to the situation in intact SC, the changes induced in lipid organisation by CSO_4 are balanced by the presence of Ca^{2+}. However, since Ca^{2+} content already drops in the lower layers of the SC, it is only expected that Ca^{2+} balances the effect of CSO_4 during the formation of the crystalline lamellae and in the lower layers of

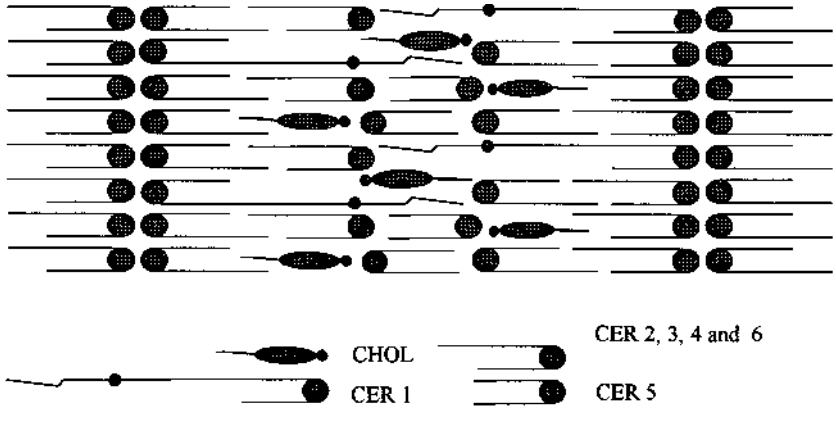

Fig. 8. The proposed molecular arrangement of the 12.2 nm phase in CHOL:CER mixtures. The 12.2 nm phase consist of three layers, two layers contain CHOL and long-chain CER, while the central narrow layer contains the linoleic acid linked to long chain fatty acid of CER 1, CHOL and CER 5.

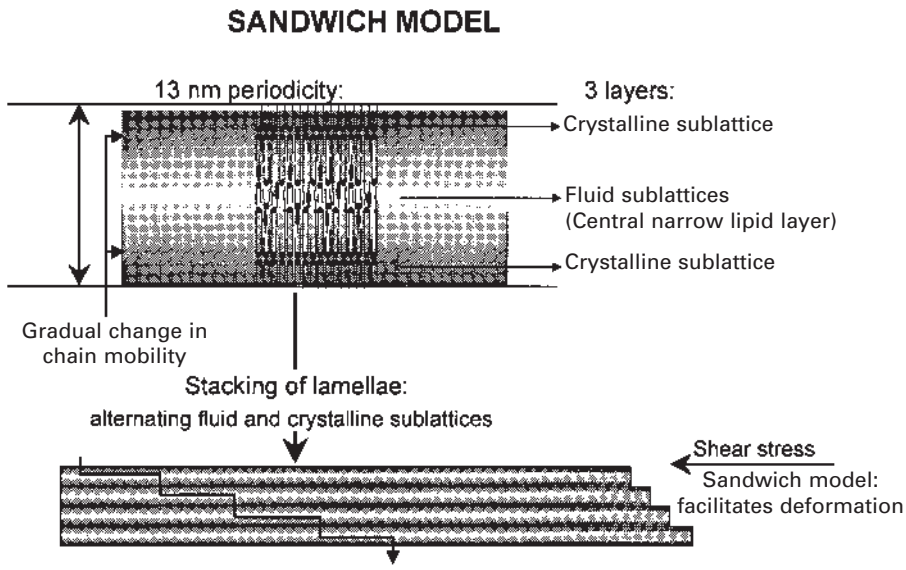

Fig. 9. A schematic presentation of the proposed "sandwich model", that describes the presence of fluid phase in the SC. The fluid phase is mainly present in the narrow layer located in the centre of the 12.2 nm repeating unit. Most probably the lipid packing in this central layer gradually changes into more densely packed lipid layers on both sides of the central layer. The latter contain less mobile hydrocarbon chains. This gradual change in packing avoids the formation of new interfaces. Since substances always have to cross these densely packed lipid layers even when the diffusion pathway is situated mainly parallel to the basal plane of the lamellae, the skin barrier is retained. The presence and localisation of fluid domains facilitates deformation of the lamellae especially perpendicularly to the stacking direction.

the SC. The CSO_4 level in SC does not change until the superficial SC cell layers are reached. In these layers the CSO_4 level drops, which might induce a crystallisation of CHOL and decrease the cohesion between the lipid lamellae.

Since at high CSO_4 levels the presence of only the 12–13 nm phase is observed in the lipid mixtures, in recessive x-linked ichthyosis skin, in which the CSO_4 level is increased from 3.4% w/w to 11.2% w/w [16], a change in the lipid phase behaviour can be expected. However, the consequences of the presence of only this phase for the skin barrier function is yet unknown. Since in a previous study [18] it was shown that CSO_4 induces a liquid packing, one can speculate that this increase in CSO_4 level might reduce the diffusional resistance of the SC by reducing the lattice density. In contrast, the presence of a small amount of lipids in a liquid phase might be very important for maintaining the elasticity of the SC (35). Therefore not only for inhibiting certain enzyme activity in the lower layers in SC, but also for maintaining the elasticity of the skin, moderate levels of CSO_4 in the intercellular regions might be required.

The localisation and presence of a liquid subphase in the SC is still a matter of extensive discussions. In the previously published domain mosaic model (35) it has been proposed that the liquid phase is a narrow continuous phase from the superficial SC layers down to the stratum granulosum-stratum corneum interface. This model is the first and intriguing attempt to explain the contrast between the presence of mainly crystalline lamellae, while the skin elasticity and the skin barrier also demand for lamellae being elastic and able to follow the sharp edges of the cell boundaries. The domain mosaic model initiated extensive discussions in the field. However, this model requires the creation of new interfaces throughout the SC, which might be energetically unfavourable. Furthermore, since this liquid phase forms a tortuous continuous pathway through the SC, substances would be able to diffuse only through this liquid phase. This might reduce the diffusional resistance of the skin. We therefore like to propose an alternative model, referred to as the "sandwich model", which is based on the molecular arrangement of the lipids in the 12–13 nm phase. We propose that the liquid sublattice is located in the central part of the repeating unit presented in figure 8. In this central part mainly unsaturated linoleic acid, CER 5 and cholesterol are present instead of the long saturated hydrocarbon chains that are present in the adjacent layers. In fact, the molecular models of Swarzendruber et al (36) and Kuempel et al (37) for the 13 nm phase propose a similar narrow central lipid layer. Most probably the liquid sublattice in this central layer gradually changes into a sublattice containing less mobile hydrocarbon chains (see figure 9). This results in densely packed lipid layers on both sides of the central layer and avoids the formation of new interfaces. Since the fraction of lipids forming a fluid phase in the SC is very limited, most probably this central lipid layer is not a continuous fluid phase, but only contains fluid domains distributed throughout this layer. Ruthenium tetraoxide stained SC revealed that the lamellae are mainly oriented parallel to the surface of the cells. Therefore substances passing the SC lipid regions, although partly diffusing through the less densely packed lipid regions parallel to the basal planes, also have to pass the crystalline lipid lamellae in the direction perpendicular to the basal plane (see figure 9). In this way substances have to permeate alternatively through two densely packed layers and one less densely packed layer. Therefore, the creation of liquid sublattices in the central layer of the 13 nm phase maintains the skin barrier. Furthermore since the orientation of the fluid region in the central layer parallels the basal planes of the lamellae, the fluid regions might facilitate the deformation of the lipid lamellae in the SC especially in case of shear stresses (see figure 9) perpendicularly to the stacking direction.

Although in the past 15 years many studies have been undertaken to obtain more detailed insight in the SC lipid organisation, basic questions about the lipid organisation cannot be answered yet. This is illustrated by the lack of knowledge about the localisation of the liquid sublattices in SC. Therefore, more studies are needed to unravel the lipid organisation in normal and diseased skin in order to understand barrier abnormalities.

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